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POLICY BRIEF

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Using problem formulation for an efficient, fit-for-purpose risk assessment of microbial plant protection products

Anne K. Steenbergh[1*], Gertie Arts[2], Peter A. H. M. Bakker[3], Wietse de Boer[4,5], Debora C. M. Glandorf[6], Mechteld M. S. ter Horst[2], Rob de Jonge[7], Willem Jan de Kogel[8] and Jürgen Köhl[8]

Abstract

The safety of the use of microbial plant protection products (PPP) is assessed before the products can be placed on the market. Due to large differences amongst microbial PPP a case-by-case approach is needed for this assessment. We propose to use the problem formulation approach based on pathways to harm to tailor the assessment to individual microbial PPP and to harmonise this approach when possible. The steps in problem formulation are described and examples are given of how the approach can be used for case-by-case assessments of microbial PPP. We also describe which other elements are needed to fully optimise the risk assessment of microbial PPP and how our approach fits in with the current EU regulatory framework and ongoing activities.

Keywords Risk assessment, Microbial plant protection products, Problem formulation

Introduction

Biological plant protection products (PPP) are important building blocks for integrated pest management and can contribute to more sustainable agriculture. Examples of biological PPP are microbial PPP, which are PPP based on living microorganisms, such as bacteria, fungi, and viruses (see [1]). Microbial PPP are referred to as PPP containing an active substance that is a microorganism in Commission Regulation (EU) No 284/2013. However, like conventional PPP, which are based on chemicals, the use of microbial PPP may have negative effects on humans and the environment. Therefore, just as for chemical PPP, the safety of using a microbial PPP is assessed before the product can be placed on the market. In the European Union (EU), both chemical and microbial PPP are regulated by the PPP regulation (Regulation (EC) No 1107/2009) and separate data requirements and assessment criteria for microbial PPP are available. These requirements and criteria have recently been revised to acknowledge that a different approach is needed for the risk assessment of microbial PPP; one which takes the biology of the microorganism into account (see

Debora C. M. Glandorf; Retired.

*Correspondence:
Anne K. Steenbergh
anne.steenbergh@ctgb.nl
[1] Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), Bennekomseweg 41, 6717 LL Ede, The Netherlands
[2] Wageningen Environmental Research, Wageningen University and Research, Droevendaalsesteeg 3, 6708 PB Wageningen, The Netherlands
[3] Plant-Microbe Interactions, Institute of Environmental Biology, Department of Biology, Utrecht University, Utrecht, The Netherlands
[4] Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands
[5] Soil Biology Group, Wageningen University & Research, Wageningen, The Netherlands
[6] GMO Office, National Institute of Public Health and the Environment, Bilthoven, The Netherlands
[7] Center for Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands
[8] Wageningen Plant Research, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Commission Regulation (EU) 2022/1441). However, making optimal use of these new requirements and criteria requires that the overall approach to the risk assessment should also be adapted so as to make it suitable for living organisms. Due to the large differences amongst microbial PPP a case-by-case approach to meet the data requirements and assessment criteria is deemed most suitable for a fit-for-purpose risk assessment of microbial PPP.

One method for focusing the risk assessment of living organisms on individual cases is the use of problem formulation as a first step. Problem formulation can identify plausible potential adverse effects of a specific organism which need to be addressed in subsequent steps of the risk assessment. This scoping step tailors the risk assessment to the specific case to be assessed (e.g., type of microorganism, mode of action, type of application) as well as to the most important plausible adverse effects that may result from its use. Problem formulation can help to streamline and structure the risk assessment process, and makes this process transparent to stakeholders by detailing which potential hazards were taken into account, which ones were discarded and how a risk conclusion was reached.

A drawback of any case-by-case approach is that it is not feasible to draft a harmonised guidance document that spells out how the risk assessment should be performed in each case. The lack of harmonisation of the risk assessment (between applicant and risk assessor as well as between risk assessors) can reduce the efficiency of the risk assessment process and lead to inconsistent outcomes of the evaluation process. In turn, uncertainty about the outcome of the assessment can hamper decision-making by risk managers. This uncertainty can increase the time to market of new microbial PPP, can lead to disproportionate risk mitigation measures, and can even prevent microbial products from reaching the market [2, 3], thereby contributing to a continued use of less sustainable pesticides (see [4]).

In this paper, we propose to use the problem formulation approach to arrive at a fit-for-purpose risk assessment of microbial PPP (i.e., both the active substance and the product in the context of the European PPP regulation), by tailoring the risk assessment to the individual microbial PPP and harmonising this approach when possible. We describe the steps in problem formulation for microbial PPP and propose to draft generic pathways to harm which can be used to characterise risks for all microbial PPP. Examples are given that show how these pathways to harm can be used for a case-by-case risk assessment for two specific microorganisms. We also describe which other elements are needed to fully optimise the risk assessment of microbial PPP and how our approach fits in with the current EU regulatory framework and ongoing activities.

Need for case-by-case risk assessment of microbial PPP

In view of the similarities regarding hazards and exposure routes for chemical active substances, the risk assessment of chemical pesticides follows a relatively uniform approach and can be performed by adhering to the existing data requirements and guidance documents [2, 5]. For example, which exposure model should be used depends on the relevant exposure routes, and whether particular data on breakdown products of the chemical active substances is required depends on the formation fraction relative to the parent substance.

This relatively uniform approach to risk assessment is not possible for microbial PPP, which consist of living organisms. The use of these microbial PPP involves different hazards (apart from toxicity also, e.g., pathogenicity), their characteristics vary widely, and standard testing cannot be used as the starting point for hazard characterisation (see inset 'hazard characterisation'). Furthermore, the microorganisms used as microbial PPP originate from the environment; endogenous populations may already exist in the environment as a natural background (see inset 'natural background'), so information on the natural background should be used to inform the risk assessment. As a result of these differences, separate data requirements have been developed for chemical and microbial PPP [e.g., Commission Regulation (EU) No 283/2013]. However, as testing cannot be used as a starting point for hazard characterisation, the most appropriate approach to the risk assessment of individual microbial PPP does not follow automatically from these data requirements; the approach needs to be adapted to each microbial PPP.

Hazard characterisation

The assessment of chemical PPP involves using screening tests as a first step in hazard identification and characterisation. Guideline tests are available for the assessment of effects on both humans and the environment. As a rule, several tests of increasing complexity are available to characterise a particular hazard. As a first step, the tests with the lowest complexity (the 'tier 1 tests') are performed. If the outcome of these tests shows an effect, further testing may be necessary. In this way, chemical substances can be screened for their potential hazards. In addition, to assess their effects on human health, the toxic effects observed in test animals can be extrapolated to the expected effects in humans.

This approach is not possible for microbial PPP, which is not only due to a lack of test guidelines for microbial PPP. Using test animals to characterise potential pathogenicity is often not possible: whether or not a microorganism can cause pathogenic effects in an organism depends on the host range of the microorganism. In addition, test animals such as rats have a higher immune response, making them in general less susceptible to pathogens than humans. Therefore, in contrast to toxicity testing for chemical PPP, an animal model may not be appropriate for the characterisation of hazards related to the pathogenicity of microbial PPP.

While animal models are appropriate to test for the toxicity of secondary metabolites produced by microorganisms, in practice toxicity testing for the metabolites of a microorganism is mostly not feasible, as the metabolites/compounds cannot be produced in sufficient quantities. In addition, determining toxicity endpoints is only useful when they can be related to the exposure to a metabolite. For metabolites produced by a microorganism after application of the product (so-called in situ production), it may not be possible to determine the quantities of these metabolites which humans and the environment are exposed to. Furthermore, as most bacteria and fungi can produce many different metabolites [6], which may be produced locally only under certain conditions in the environment and frequently have relatively short half-lives of a few days [7], assessing the effects and exposure for all these metabolites is not possible.

Since screening tests cannot be used for hazard identification and characterisation in microbial PPP, a different approach is needed. Such an approach uses all information available for a microbial PPP, and is a part of the problem formulation approach.

Natural background

Microorganisms naturally occur in every habitat. For example, natural soils on average harbour a billion microorganisms per gram [8], with microbial densities increasing more than 100-fold in the rhizosphere [9, 10]. On leaf surfaces one to ten million microorganisms are present per cm^2 [11]. Furthermore, the average level of diversity of microorganisms per unit of soil is spectacular, with thousands of species of bacteria per gram of soil [12]. When these microorganisms are active, they produce many different metabolites, depending on the specific biotic and abiotic conditions in their immediate surroundings [6]. As a result, humans and the environment are continuously exposed to huge numbers of microorganisms and their metabolites.

Most microorganisms are not known to cause negative effects. In addition, when negative effects do occur, these are as a rule caused by a relatively small number of well-known microbial culprits. In the context of food safety, for example, only a small number of microorganisms and their microbial metabolites are included in EU legislation for food safety (Commission Regulation (EC) No 2073/2005 and (EU) 2023/915). This concerns human pathogens (such as *Listeria* or *Salmonella*) and nine (sub)groups of mycotoxins such as aflatoxins. This means that even though humans are experiencing a high and continuous exposure to microorganisms, no negative effects have been observed for the vast majority of the microbial diversity.

This combination of known exposure and absence of negative effects is an example of data which should be used in the problem formulation step of the risk assessment.

Case-by-case risk assessment using problem formulation

Since its initial development and adoption by the US EPA in 1998 [13], problem formulation has gained support around the world and is now more widely used [5]. In the EU, Directive (EU) 2018/350, which amends Directive 2001/18/EC on the deliberate release of GMOs, including plants, in the environment, formally introduces problem formulation as a key first step and requirement for the environmental risk assessment of GM (genetically modified) plants [14]. Like microbial PPP, GMOs such as GM plants and GM animals can be highly diverse, and a risk assessment is carried out before a GMO can be released into the environment. For this risk assessment, the European Food Safety Authority (EFSA) has proposed the explicit use of problem formulation [15, 16], and assessments are conducted on a case-by-case basis.

Problem formulation has also gained attention in the context of plant protection products. Although not explicitly included, Regulation (EC) No 1107/2009 allows the use of problem formulation as a first step in the environmental risk assessment. Moreover, a guidance document has recently been endorsed in the EU which describes how problem formulation can be used to justify not submitting the information which is needed according to the (legally binding) data requirements [i.e., 'waiving' of data following article 1.5 of the Annexes of Regulations (EU) No 283/2013 and No 284/2013: problem formulation for environmental risk assessment in the context of Regulation (EC) No 1107/2009] ([17]).

We propose to apply problem formulation not only to justify data waiving, but to use it as the basis for fit-for-purpose, case-by-case risk assessments of microbial active substances and PPP. In this way, problem formulation helps not only to structure the risk assessment, but also to interpret the results and thereby make informed risk management decisions.

Although a case-by-case risk assessment is most appropriate for microbial PPP, the case-by-case approach also has the drawback that opinions can differ on what is the best strategy for an assessment. As a result, it may be challenging to align the approach for such case-by-case assessments between the applicant (who needs to determine the risk assessment approach to be used in the application dossier) and the risk assessor, as well as between all risk assessors involved in a single risk assessment. For example, the EU peer review process of risk assessments involves risk assessors from EFSA and all 27 member states. Therefore, making the best use of problem formulation for case-by-case risk assessments of microbial PPP requires guidance to align the approach as much as possible. To this end, we propose to set up generic pathways to harm, which can be used for each individual microbial PPP. These generic pathways can also contribute to acceptance of the chosen approach to risk assessments by regulators globally.

Below, we describe how the problem formulation approach based on generic pathways to harm can be applied to the risk assessment of microbial PPP (Sect. "Problem formulation approach by means of

pathways to harm and its steps"). To further illustrate this approach, we provide examples concerning two microorganisms in Sect. "Examples of pathways to harm for in-soil arthropods, aquatic invertebrates and food safety".

Problem formulation approach by means of pathways to harm and its steps

Problem formulation tailors the risk assessment to each individual case [18]. The problem formulation approach is designed to identify potential adverse effects resulting from the use of a stressor (such as a microbial PPP) and potential pathways to such harm. Furthermore, the approach aims to guide the collection of information needed to assess the likelihood that adverse effects can occur and their severity. The approach helps to focus the risk assessment on those aspects that are important for the risk assessment, while steering it away from those aspects that are less important or irrelevant [14].

Here, we apply the problem formulation approach using pathways to harm (Fig. 1). A pathway to harm is a conceptual model that sets out the events that must occur if the intended activity (e.g., the use of a microbial PPP) is to cause harm [2]. Therefore, if one of the events in the pathway to harm does not take place, harm does not occur. Although pathways to harm include the prerequisites for harm to take place, they do not necessarily consist of chronological events and do not necessarily give a mechanistic description of the biological processes. Therefore, pathways to harm are more loosely defined than adverse outcome pathways [2], which mechanistically describe biological processes in toxicological risk assessments.

*Steps in the problem formulation approach (*Fig. 2*)*: A prerequisite to perform risk assessments, and, therefore, to apply problem formulation, is to *identify protection goals* (what should not be harmed by the release of a microbial PPP) which are defined in legislation. Examples of protection goals include human and animal health and the environment. These protection goals need to be made operational so they can be used in risk assessment (*operational or specific protection goals*; for example, the use of PPP must not have non-negligible effects on the functioning of off-field soil arthropods). Next, *pathways to harm* regarding the specific protection goals are established. To maximise efficiency and harmonisation of the risk assessment, we propose to develop generic pathways to harm which can be used for the risk assessment of any microbial PPP. For example, the release of a microbial PPP can cause non-negligible effects on soil arthropods (harm) via a causal chain of events (see Figs. 1 and 2). Each of the events in a pathway has to take place for harm to occur. Subsequently, *testable hypotheses* are formulated for each of the events in the causal pathway to harm (for example, the microorganism will not become active in the soil environment). For each testable hypothesis, the *information is identified* which is needed to test it. For example, information may be needed on the potential exposure of soil arthropods, such as springtails to the microbial PPP, on the potential production of metabolites of concern, or on the potential toxicity of metabolites of concern for springtails. The next step is to obtain this information *via analysis plans*. For example, the potential for exposure of springtails to the microbial PPP will depend on the method used to apply the microbial PPP, while the potential for the production of a metabolite of concern can be determined by whole genome sequencing and searching for the relevant gene cluster. Similarly, information on the toxicity of metabolites for springtails may be found in the available body of knowledge, or experimental data may need to be obtained.

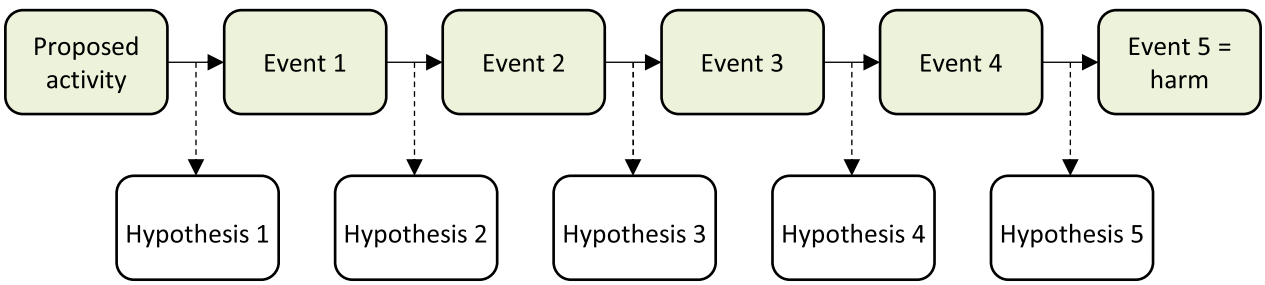

Fig. 1 Example of a pathway to harm. The pathway to harm is depicted by the green shapes. The proposed activity, such as the application of a microbial PPP on a crop, may lead to a potential adverse effect (harm) through a causal chain of events (green shapes; top row). Each specific step (solid arrows) in this causal chain of events leads to a testable hypothesis that may be challenged by, for example, the available body of knowledge or by experimental data. If one of the steps does not occur (for example, due to a lack of exposure), the causal chain of events is broken and the harm cannot take place. As pathways can be developed for each combination of a hazard (e.g., toxicity and pathogenicity) and a protection goal (e.g., for non-target arthropods), the use of a microbial PPP leads to multiple pathways to harm. Figure adapted from Devos et al. [5]

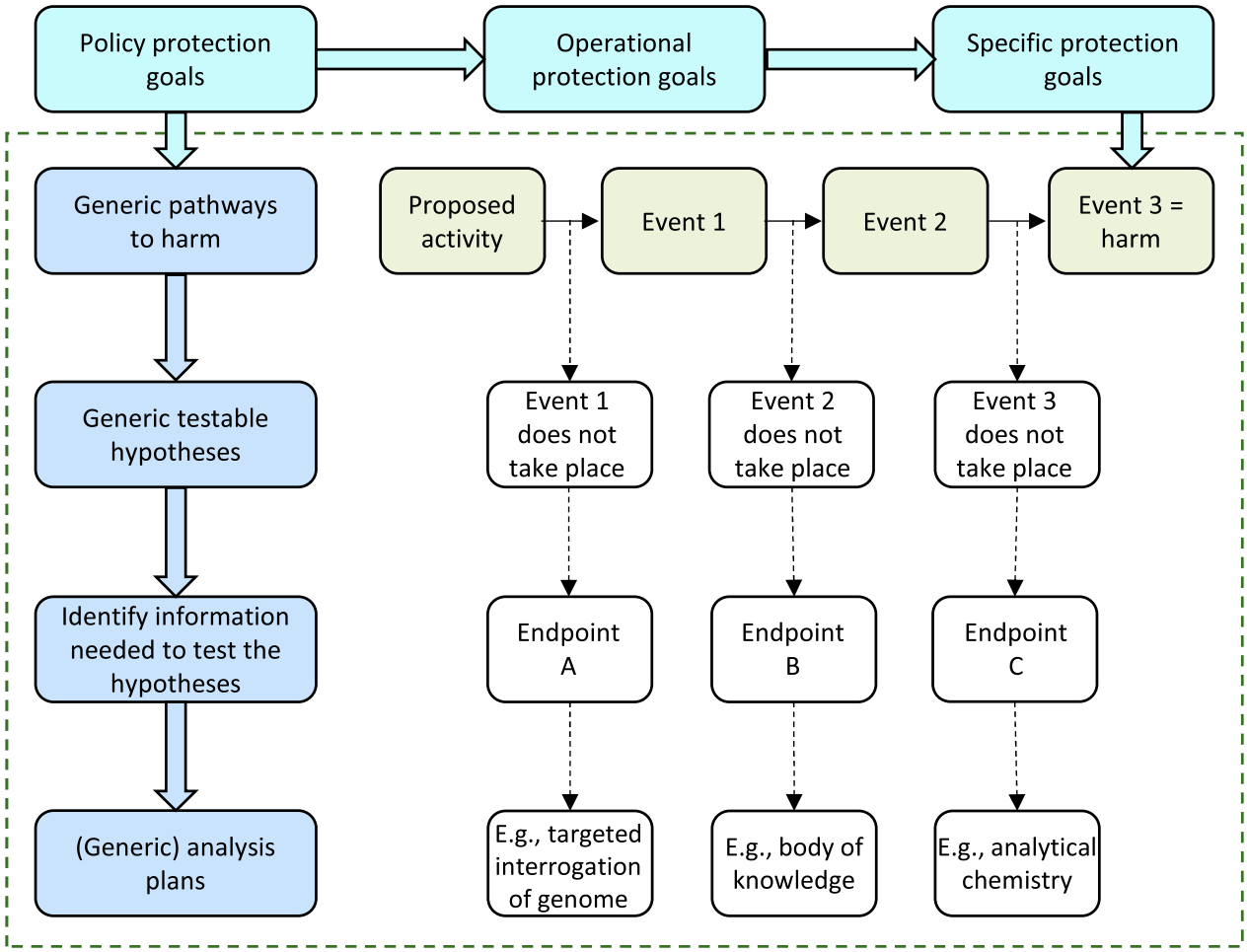

Fig. 2 Schematic overview of the proposed elaboration of problem formulation (within green dashed lines). Generic pathways to harm (green shapes) are developed based on the specific protection goals (which in turn have been derived from the policy protection goals; turquoise shapes). Analysis plans for the assessment are developed by identifying which information is needed to test the hypotheses for each of the steps in the pathway to harm. Where appropriate, these analysis plans can follow a tiered approach, in which the available, mostly qualitative information is used in the first tier. The resulting information on the likelihood and uncertainty of each relevant event in the pathway taking place is combined to characterise the likelihood of harm occurring (i.e., not meeting the protection goal; the full pathway occurring) and the uncertainty in the assessment. Figure adapted from Devos et al. [19]

Applying problem formulation to the risk assessment of microbial PPP

Below, we describe in more detail how problem formulation including the use of pathways to harm can be applied in the risk assessment of microbial PPP in the EU (see Figs. 2 and 3 for a schematic overview).

Definition of harm: protection goals

The purpose of the risk assessment is to provide information to risk managers on the risks resulting from the use of a PPP and on whether human health and the environment are sufficiently protected as defined by the protection goals (see Fig. 3). Using a problem formulation approach for the risk assessment, therefore, requires a clear definition of these protection goals (Fig. 2). In the context of the risk assessment of PPP in the EU, the protection goal for human health is defined as 'having no negative effects' and that for the environment as 'having no unacceptable effects'. The latter is further specified by the specific protection goals, which clarify which ecosystem services need to be protected where and when [38, 39, 40]. Supplementary information 1 presents more information on protection goals.

Pathways to harm

A pathway to harm describes the events which need to occur for harm to arise. As the risk assessment needs to provide information on the likelihood that the use of a

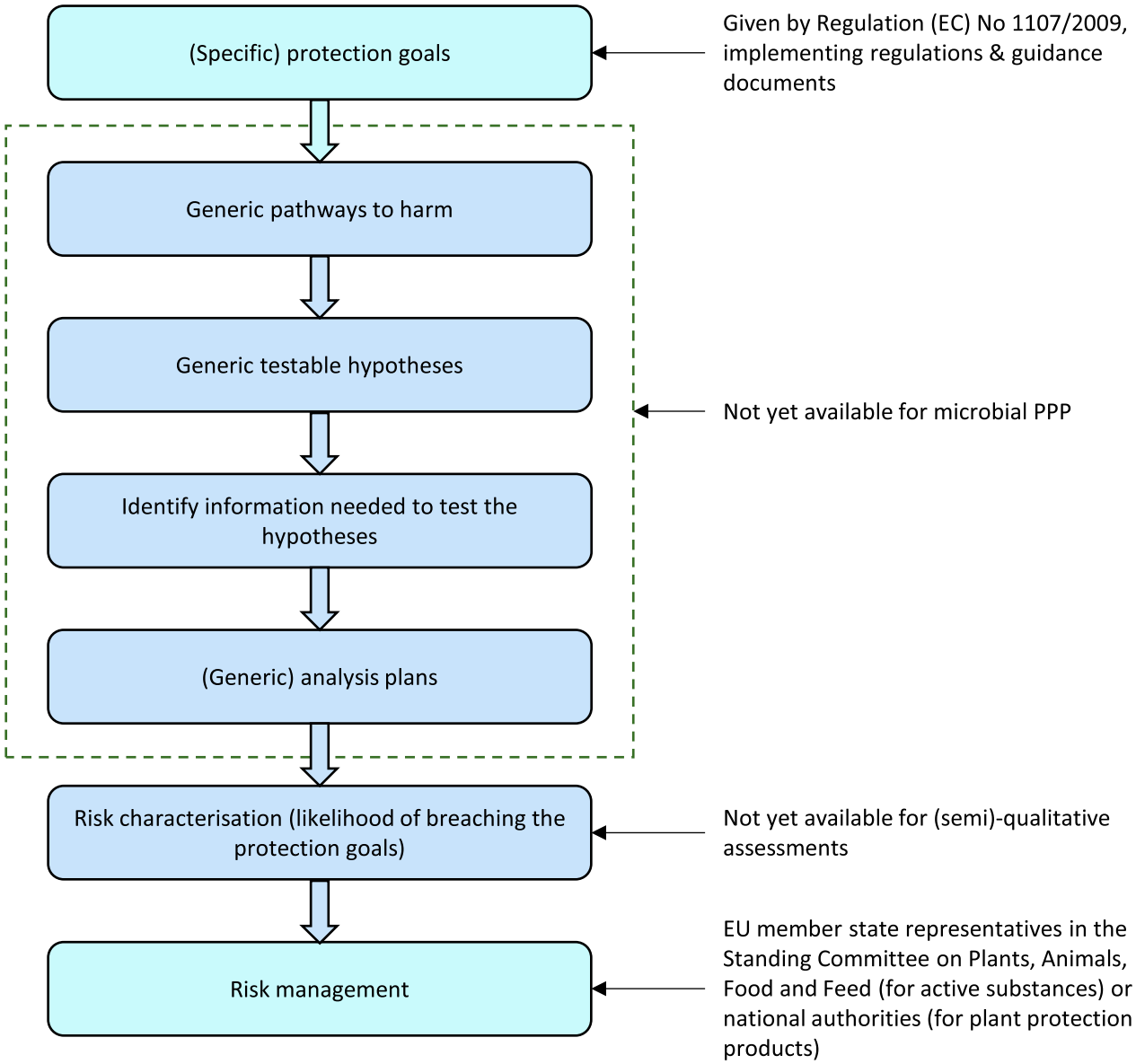

Fig. 3 Schematic overview of the framework for risk assessment and risk management of microbial PPP. The protection goals and decisions on approval or authorisation are part of risk management (turquoise shapes). Risk assessment (blue shapes) includes the problem formulation phase of the assessment (within the green dashed lines). By combining the information for all of the pathways to harm for all protection goals, the risk resulting from the use of a microbial PPP is characterised. The outcome of risk characterisation is the input for risk management

microbial PPP will not meet the protection goals, we propose to define harm (i.e., the last event in a pathway to harm) as not meeting a protection goal. Harm is thereby defined as having consequences which are severe enough to have negative effects on human health or unacceptable effects on the environment.

Harm always results from the combination of hazard and exposure. Harm due to the use of a microbial PPP can result from different hazards relating to microorganisms. These hazards include pathogenicity and toxicity, but also, for example, the presence of genes that encode resistance against medically important antimicrobials (see [20]). Therefore, pathways to harm need to be constructed for each of these hazards. Similarly, all exposure routes should be included in pathways to harm. These exposure routes should include both direct and indirect exposure. For metabolites produced by microorganisms in particular, the exposure routes should include exposure to metabolites which are present in the product at the time of application, as well as metabolites which may

be produced by the microorganism after application (i.e., in situ production).

To harmonise the approach as much as possible, we propose to set up a framework of generic pathways to harm (e.g., as part of a guidance document for the risk assessment of microbial PPP). These generic pathways should be appropriate to most microbial PPP, and can serve as a flexible basis which can be adapted to the particularities of individual microbial PPP and their uses.

The pathway to harm approach enables the likelihood of harm occurring (i.e., not meeting the protection goals) to be assessed in a structured way by gathering information on the severity and likelihood of negative effects. Since all the events in the pathway to harm need to occur if the use of the microbial PPP is to result in harm, demonstrating that any single event does not occur is sufficient to conclude that the likelihood of harm occurring is negligible (i.e., not distinguishable from zero; [21]). Alternatively, if none of the events can be demonstrated not to occur, the likelihood of harm occurring can be assessed by determining the likelihood of the individual events in the pathway. The likelihood of these individual events occurring can be assessed using a testable hypothesis and an accompanying analysis plan (see Fig. 2).

Since all events in a pathway need to occur for harm to take place, the assessment can start with the event in the pathway which is easiest to assess. If it is demonstrated that a particular event does not occur, in principle no further information is needed about the other events in the pathway. This means that the approach is flexible and can be adapted to the microorganism in question or the use which is being assessed.

Testable hypotheses

Obviously, hypotheses should be drafted in such a way that they can be tested and that the outcome is relevant for the risk assessment. Testable hypotheses can be formulated for all the events in a pathway to harm. As a result, some testable hypotheses will be relevant for hazard characterisation, others for exposure assessment and others for risk characterisation (i.e., combining information on hazard characterisation and exposure assessment).

The aim of testable hypotheses is to provide information for decision making [2]. Therefore, it should be clear beforehand how the outcome of testing these hypotheses will affect the outcome of the risk assessment.

An example of a testable hypothesis which is relevant to assess the exposure of consumers to metabolites due to the treatment of flowering fruit trees with a microbial PPP against aphids is: 'The microbial strain present in the product cannot be detected in or on the fruit at the time of harvest.' This hypothesis is testable, and in case the microbial strain is not detected, it is unlikely that the microorganism will produce metabolites in or on the fruit after harvest. In contrast, a hypothesis such as 'The microbiome on the fruit at the time of harvest is affected by the use of the microbial PPP' is testable, but its relevance for risk assessment is unclear.

By default, the testable hypothesis for the final event in the generic pathway to harm is formulated in accordance with the (specific) protection goal. For example, for the assessment of pathogenic effects on off-field non-target soil arthropods, the resulting testable hypothesis is that 'the use of the product does not lead to non-negligible effects on the abundance of non-target soil arthropods.'

Analysis plans

For each of the testable hypotheses, a generic analysis plan should be developed to enable the hypothesis to be tested. An analysis plan to test a hypothesis describes which information is needed and how the information should be assessed to test the hypothesis. Ideally, the generic analysis plans are formulated in such a way that the data required for these plans is part of the current legal data requirements. The information needed can come, for example, from literature searches or databases (including information on the Qualified Presumption of Safety (QPS) status of the microorganism [22], or from analyses performed as part of the dossier preparation (e.g., guideline studies or other analytical studies, such as whole genome sequencing analyses).

For chemical PPP, harmonised analysis plans are available, for example, as part of risk assessment schemes [23]. These schemes for the environmental risk assessment are based on tests using vulnerable species to determine if the specific protection goals are met. They generally follow a tiered approach, where simpler tests or models provide a more conservative result for the risk assessment (i.e., more worst-case). An example of a well-developed tiered risk assessment scheme is the aquatic risk assessment (EFSA PPR, 2013), where the first tier includes standard tests with standard test organisms and the higher tiers include modified exposure tests with standard organisms (2nd tier), microcosm and mesocosm studies (3rd tier), ecological modelling (4th tier) and field approaches.

In contrast to chemical PPP, such risk assessment schemes for microbial PPP are not available. Furthermore, due to the inherent differences between chemical and microbial PPP, it is impossible to develop similar risk assessment schemes for microbial PPP based on guideline tests for hazard characterisation and exposure models (see inset 'hazard characterisation'). Therefore, the microbial counterpart of the chemical risk assessment schemes—the microbial analysis plans—should take into

account these inherent differences and be specifically developed for microbial PPP. We propose to use a qualitative assessment as a first step in the microbial analysis plans where appropriate (see, e.g., [21]). If this qualitative assessment does not provide the necessary information, a quantitative or semi-quantitative approach may be used in the second tier.

Risk characterisation

Risk characterisation is the final step in a risk assessment (see Fig. 3). At this stage, for each pathway to harm, the available information on the likelihood and uncertainty from each event in the pathway is combined to assess the likelihood and uncertainty of breaching the protection goal (i.e., the full pathway occurring).

The (specific) protection goals describe which level of effects on humans and the environment are considered acceptable. For all effects caused by the use of microbial PPP, there are two outcomes: depending on the severity of the effect, the protection goal is met, or it is breached. In case the effect is sufficiently severe to lead to harm (i.e., to breach the protection goal), the full pathway, including the last event, occurs. In case an effect is not assessed as being sufficiently severe to lead to harm, the likelihood that this effect does lead to harm can be regarded as negligible. In case an effect is assessed as sufficiently severe to lead to harm, the likelihood of not meeting the protection goal should be further described (e.g., negligible, highly unlikely, likely). To assess the likelihood of an event in the pathway occurring, the information from all qualitative and quantitative analyses performed for a particular event in a pathway to harm should be combined and presented in a clear way, including an assessment of uncertainty (see p. 31 of [21], for example).

The combined risk characterisation for all pathways to harm provides the necessary information for risk managers regarding the risk of the use of the microbial PPP. The level of detail provided by risk characterisation should be sufficient to determine which risk management conclusion is most appropriate (for example, approval without restrictions, approval with restrictions or non-approval).

Examples of pathways to harm for in-soil arthropods, aquatic invertebrates and food safety

To illustrate our proposed approach to the risk assessment of microbial PPP, we include a number of examples below. Please note that the purpose of these examples is purely to provide insight into what generic pathway may look like and how the approach can be applied; they are not intended as actual risk assessments.

The examples concern two well-known microorganisms: *Bacillus amyloliquefaciens* and *Pseudomonas chlororaphis*. For the sake of the examples, *B. amyloliquefaciens* is used as a foliar spray against mildew and *P. chlororaphis* as a seed treatment against soil-borne plant diseases.

The examples cover three selected areas of the risk assessment:

- Example 1: Effects on non-target soil arthropods due to pathogenicity of the microorganism;
- Example 2: Effects on aquatic invertebrates due to toxicity of metabolites;
- Example 3: Food safety related to toxicity of metabolites.

The pathways to harm relevant for these examples are shown in Fig. 4. An overview of the testable hypotheses, analysis plans and their hypothetical outcomes based on these pathways to harm is presented in Supplementary information 2. The main steps and results of the proposed approach to the three examples are described below. For more elaborate information, please see Supplementary information 2.

Example 1: Effects on non-target soil arthropods due to pathogenicity of the microorganism

The specific protection goal for in-field non-target soil arthropods defines the maximum acceptable effect as small to medium effects for weeks to months on the abundance/function of terrestrial non-target arthropods at the level of functional groups [23]. This means that in our example, harm occurs if effects are larger or last longer (see the last event in pathway Example 1 in Fig. 4).

Both microorganisms used in our examples are not used as an insecticide. In addition, for both application methods (foliar spray and seed treatment) it is assumed that soil arthropods are exposed. Because of this, event 3 (the microorganism is pathogenic to soil arthropods; see Fig. 4) is a logical next step in the pathway to look at: if the microorganism is not pathogenic, the likelihood of unacceptable effects occurring due to pathogenicity is negligible. This is the case for *B. amyloliquefaciens*; although this species can be used for the production of compounds which have adverse effects on arthropods, the species is not known to cause disease in arthropods. As a result, the pathway to harm for this species can be broken off at this step and the likelihood of not meeting the protection goal due to pathogenic effects is negligible.

For *P. chlororaphis,* the situation is a bit more complex, as this species exhibits insecticidal activity due to the production of metabolites, including metabolites which are only produced inside insects [24]. In addition, the closely related species *P. protegens* is known to be able to cause disease in insects under laboratory conditions, although it is not yet known whether these

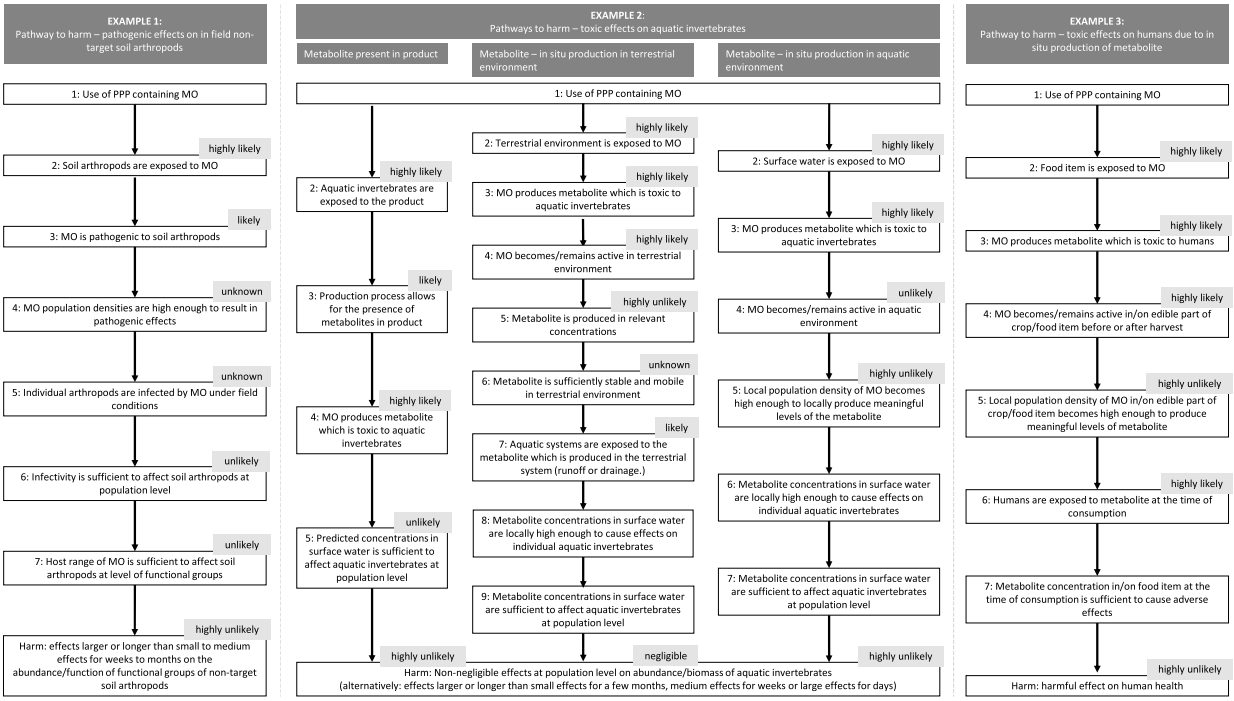

Fig. 4 Examples of pathways to harm for in-soil arthropods, aquatic invertebrates and food safety. Pathways to harm are shown for in field non-target arthropods (due to pathogenic effects of the microbial PPP), for aquatic invertebrates (due to toxic effects resulting from the presence of a metabolite in the product, from production of a metabolite in the terrestrial environment, and from production of a metabolite in the aquatic environment), and for human health (due to toxic effect resulting from the production of a metabolite in or on the crop). Please see the main text for a more elaborate description. The likelihood of events in the pathway and of the full pathway occurring is indicated in the grey boxes for the example of a hypothetical strain of *P. chlororaphis* (see main text). Where the likelihood is not shown, information on the likelihood of the event is not available as it is not a priori known or not needed to determine the overall likelihood of the pathway in this example. (MO: microorganism)

effects are relevant under field conditions [24]. Regarding the pathway to harm, this means that it can be considered likely that event 3 occurs, and that the likelihoods of events 4 and 5 are unknown (see Fig. 4). To assess the likelihood of harm occurring, the likelihoods of events 6 and 7 occurring can be assessed using information on the natural occurrence of the species and the history of its use. *P. chlororaphis* is ubiquitous in soils [25], which results in a continuous exposure of soil arthropods to this species. In addition, this species has been used as a PPP in the EU for decades [25]. In view of this exposure of arthropods in combination with the absence of information on insect–pathogenic effects under field conditions, it is unlikely that events 6 and 7 take place. Due to differences between groups of arthropods in their susceptibility to infections by the closely related species *P. protegens* [24], the likelihood of event 7 taking place is even lower. Based on the above, and on the assumption that the population density of the microorganisms in the soil will return to background values, it is considered highly unlikely that harm (i.e., not meeting the protection goal) occurs due to pathogenic effects.

Example 2: Effects on aquatic invertebrates due to toxic metabolites produced by the microorganism

The specific protection goal for aquatic invertebrates defines the maximum acceptable effect as negligible effects at population level on the abundance and/or biomass of aquatic invertebrates in edge-of-field waters (ecological threshold option), or small effects for a few months, medium effects for weeks and large effects for days on the abundance and/or biomass of vulnerable populations of invertebrates, as long as their reduction does not result in more persistent indirect effects (ecological recovery option; EFSA [26]). For this example, harm (i.e., the last event in the pathway), therefore, occurs if effects are larger or last longer.

Exposure of aquatic invertebrates to metabolites which may be produced by a microorganism can occur by different routes: a metabolite can be present in the formulated product; the microorganism may, upon application,

produce a metabolite in terrestrial systems in sufficient quantities for it to be transported to aquatic systems; or in case the aquatic system is exposed to the microorganism, the microorganism may produce a metabolite in the aquatic system. As a result, assessing the likelihood of harm occurring to aquatic invertebrates due to toxic effects requires these three pathways to harm (see Fig. 4, Example 2). Please note that these pathways are relevant to assess the effects of the active substance; information on the components in the formulated product would be needed to assess its toxic effects.

For both microorganisms used in our examples, exposure of surface water to the product is assumed, based on the application method (foliar spray and seed treatment; in the latter, surface water can be exposed due to dust drift during sowing of the treated seeds). Both *B. amyloliquefaciens* and *P. chlororaphis* are known to be able to produce metabolites which have toxic effects on, for example, arthropods [27, 28]. Absence of hazard or exposure does, therefore, not apply to either of these microorganisms.

Pathway for toxicity due to presence of metabolites in the product

In our hypothetical example of *B. amyloliquefaciens*, the active substance is manufactured in such a way that the presence of toxic metabolites is highly unlikely (see also Supplementary information 2). As a result, the entire pathway is highly unlikely to result in harm, and further information is not needed. Although further information on this pathway may result in the likelihood being assessed as negligible, demonstrating this even lower likelihood is not considered to have consequences for the risk conclusion. Therefore, further information can be considered as 'nice to know' for this pathway, but not needed to conclude on the risk assessment.

For the sake of this example, we assume that the presence of metabolites in the product cannot be excluded for our hypothetical *P. chlororaphis* strain. As a result, event 3 is assessed as likely. Furthermore, although the production of a known insect toxin by our strain can be excluded based on whole-genome sequencing data, it is unknown if the strain can produce other metabolites which are toxic to aquatic invertebrates. As all bacteria and fungi produce numerous metabolites, it is highly likely that some of these metabolites are toxic to aquatic invertebrates in sufficiently high concentrations. For event 5 (see Fig. 4), combined information on toxicity and exposure is assessed. Based on the relatively low exposure of surface water and the available information on the potential toxicity of the metabolites produced by the strain (body of knowledge, including mode of action, absence of known insect toxin and low toxicity to non-target soil arthropods), this event is assessed as unlikely. Based on the above, and since the number of applications of the product for seed treatment is limited to one per crop cycle, the likelihood of harm occurring (e.g., small effects for weeks to months) due to toxic effects resulting from the presence of a toxic metabolite in the product is assessed as highly unlikely.

Pathway for toxicity due to production of a metabolite in the terrestrial system and transportation to the aquatic system:

The second route of exposure of aquatic organisms to microbial metabolites due to the use of a microbial PPP is when the microorganism produces a metabolite in the terrestrial system and this metabolite subsequently reaches aquatic systems. While this exposure route may in theory lead to harmful effects, defining the steps in this pathway to harm immediately makes it clear that this pathway is unlikely to be relevant for the risk assessment of microbial PPP, as event 5 is unlikely to occur. Thus, the use of the proposed problem formulation approach means that the focus of the risk assessment can be shifted away from this pathway, preventing unnecessary effort to further assess this route.

For this pathway to lead to harm, the main prerequisite is that the microorganism produces a toxic metabolite in the terrestrial environment in such amounts, and on a wide spatial and temporal scale, that leaching or run-off to aquatic systems affects populations in these aquatic systems. In addition, the metabolite needs to be stable enough for concentrations to build up in the terrestrial system, and the adsorption needs to be low enough for the metabolite to be mobile. We do not know of any case where the use of a microbial PPP would meet these prerequisites, so we consider this route to be, in principle, irrelevant for microbial PPP. We have included this pathway for the sake of completeness of this example (see also Supplementary information 2), but we consider it irrelevant in the context of generic pathways to harm for microbial PPP.

Pathway for toxicity due to production of a metabolite in the aquatic system:

This pathway is used to assess the likelihood of the microorganisms reaching surface waters and there producing a metabolite which is toxic to invertebrates, resulting in unacceptable effects on the abundance or biomass of aquatic invertebrates. As mentioned above, since both hazard and exposure cannot be excluded for this pathway, further information is needed to assess the likelihood of this pathway leading to harm.

Both *B. amyloliquefaciens* and *P. chlororaphis* can become active in the aquatic environment, where *P.*

chlororaphis can, for example, inhabit the rhizosphere of aquatic plants [29]. However, it is considered unlikely that the use of the microorganism as a seed treatment will result in successful colonisation of the rhizosphere of aquatic plants. Furthermore, based on the body of knowledge for both microorganisms, it is considered highly unlikely that population densities of these microorganisms in aquatic systems will become high enough for them to produce concentrations of metabolites which are relevant at a larger scale than in microsites. As a result, event 5 in this pathway (see Fig. 4) is assessed as highly unlikely for both examples. In addition, as both species are ubiquitous in the environment, surface waters are naturally exposed to these microorganisms. Even if the use of the microorganism as a PPP should increase population densities, these population densities are expected to decrease rapidly back to background levels [10, 30]. As a result, it is highly unlikely that this pathway will lead to harm for both examples.

Combined pathways for effects on aquatic invertebrates due to toxic metabolites

The three pathways leading to toxic effects on aquatic invertebrates are assessed as being highly unlikely or having a negligible likelihood. Combining the information on these three pathways shows that it is highly unlikely that harm due to toxicity will occur to aquatic invertebrates.

Example 3: Effects on human health due to dietary exposure to toxic metabolites

The relevant protection goal for this example is that there should be no harmful effects on human health arising from exposure to the metabolites remaining in or on plants or plant products [Commission Regulation (EU) No 546/2011].

Two exposure routes are relevant to assess the likelihood of harmful effects on human health due to dietary exposure to metabolites produced by a microorganism: exposure due to the presence of the metabolite in the PPP and exposure due to the production of the metabolite by the microorganism after its use as a PPP (i.e., in situ production). As a result, two pathways are needed to assess the likelihood of this harm occurring.

The pathway relevant to human dietary exposure to metabolites present in the product broadly follows the same steps as the first pathway of example 2 (toxic effects on aquatic invertebrates due to metabolites present in the product). For this example, we have, therefore, focused on the second exposure route: dietary exposure due to in situ production of metabolites.

For both our examples, we assume that they are used in edible crops and that food items can be exposed to the microorganism. Furthermore, we assume that the whole-genome sequencing information of our hypothetical microorganisms has been screened for genes encoding known food toxins and for toxins which are known to be relevant within the genus. In addition, for *B. amyloliquefaciens*, information on toxicity is available for a number of metabolites belonging to the lipopeptides.

Looking at the events in the pathway to harm (see Fig. 4, Example 3), it is clear that it is likely or highly likely that most of the events of this pathway will occur for most microorganisms. For example, bacteria and fungi all produce metabolites which may have harmful effects on humans at high exposure levels. In this context, it is important to note that toxicity in itself is not sufficient to lead to harmful effects on human health; it is always the combination of toxicity and the dose that determines the effect (e.g., many common edible plants contain low quantities of plant secondary metabolites which would be toxic in high doses). Therefore, it is not the aim of the pathway to exclude the possibility that any potentially harmful metabolites can be produced, but to assess the likelihood of a sufficient concentration of metabolites being present in the food items of treated crops to cause harmful effects.

In our examples, an extensive body of available knowledge shows that both microorganisms are ubiquitous in the environment, including agricultural systems, and both species have been used in agriculture for decades. This, together with the absence of evidence of adverse effects on human health due to in situ production of metabolites, means that event 5 in the pathway (see Fig. 4) is assessed as highly unlikely. As a result, the likelihood of harm occurring is also assessed as 'highly unlikely'.

In the case of less well-described microorganisms, analysis of whole-genome sequencing information in relation to the capacity to produce known food toxins and relevant toxins based on the phylogeny of the microorganisms will be even more important to assess the likelihood of harm occurring. If more information is needed to draw conclusions regarding the likelihood of harm occurring, a subsequent step may be to determine the population density of the microorganism on edible parts of crops and food items before and after harvest (see event 4).

Discussion

In this paper, we propose to use problem formulation based on generic pathways to harm to make the risk assessment of microbial PPP more efficient and fit for purpose. This way we aim to combine the benefits of a case-by-case assessment with the means to harmonise the approach between applicant and risk assessor as well as between all risk assessors involved in a single assessment.

Problem formulation also leads to a more transparent assessment and helps to communicate about risks. Using problem formulation as a first step in a risk assessment focuses the risk assessment on the most important hazards and leads to a more robust risk assessment process, thereby preventing conclusions, such as 'a risk cannot be excluded'. Hence, risk managers are better informed and the resulting risk management decision can be better substantiated.

Problem formulation is not the only approach to adapt risk assessment to individual cases. Another method which has been proposed to increase the efficiency of the risk assessment of biological PPP is the use of decision trees [31]. Using a decision tree, the risk assessor can decide if sufficient information is available for particular elements of the risk assessment, or if more information is needed. While both problem formulation and decision trees make use of the main characteristics of microorganisms, their basis, process and resulting output are different. Decision trees aim to structure the risk assessment using a fixed set of predefined questions in a predefined order. Since decision trees are generally developed based on existing experience with microorganisms which have already been assessed, they may not be suitable for microorganisms which have not been assessed previously. In contrast, the problem formulation approach is inherently flexible. In addition, whereas decision trees can be very efficient for those assessments where it can be concluded that certain hazards do not apply to the microorganisms in question, or where exposure to the microorganism does not occur, decision trees do not aim to assess the likelihood and severity of harm occurring in situations where hazard or exposure is not absent.

To implement the proposed problem formulation approach for microbial PPP, generic pathways to harm should be collated for all combinations of a (specific) protection goal and the relevant hazard(s) and exposure route(s). In addition, to be able to use a qualitative assessment as a first step in the microbial analysis plans, definitions of likelihood and severity of effects need to be agreed on. Information on both generic pathways to harm and definitions could be included in a guidance document; amendment of EU legal requirements for microbial PPP is not considered necessary to already implement this approach.

While we consider that the proposed problem formulation approach can greatly improve the risk assessment of microbial PPP, additional efforts are needed. One example is the development of test guidelines to be used in case testing is needed for a microbial PPP. Efforts to develop guidelines for microbial PPP are being undertaken by OECD.

It should be noted that even with all elements in place for an efficient and effective risk assessment of microbial PPP, the outcome of the risk assessment will likely never be as clear-cut as a quantitative assessment of chemical PPP. Risk assessors and risk managers alike should become familiar with the different format of a qualitative or semi-quantitative risk assessment (see Fig. 3). In this context, it may be helpful to remember that the quantitative thresholds (e.g., toxicity–exposure ratios) which are used in chemical assessment are agreements between risk assessors and risk managers—they are agreed best approximations to determine if effects are acceptable; meeting the thresholds does not mean harm can never occur. Experience may be derived from other regulatory frameworks in which qualitative assessments are used.

Outlook

Currently, several projects are being undertaken which aim to improve the risk assessment of microbial PPP. For example, the development of a pipeline to analyse whole-genome sequencing data by EFSA will lead to a more harmonised and efficient assessment of this data [see Ad hoc meeting with stakeholders—external use of the EFSA MoPs portal | EFSA (europa.eu)]. At OECD level, efforts are ongoing to develop test guidelines for microbial PPP. These guidelines are needed to harmonise the approach in case testing is required for a microbial PPP. In addition, actions at both EU and OECD level are being undertaken to provide reviews of the information on species of microorganisms and the implications for the risk assessment (referred to as 'consensus documents' or 'group reviews'). These reviews have the potential to greatly improve the efficiency of risk assessments for strains belonging to such a microbial species. Currently, all available information on a species is included and assessed for each strain within the species. These consensus documents have the potential to indicate which particular information is needed at strain level for the specific microbial species. In principle, this means that only this particular information needs to be addressed for a strain within the species (e.g., 'the content of a particular metabolite needs to be determined in the active substance as manufactured', thereby making a full assessment of all metabolites superfluous). Another activity at EU level aims to provide an overview of the biogeography of species of microorganisms used as microbial PPP. This information can be used in the assessment of natural exposure of humans and the environment to these microorganisms.

All these ongoing activities are fully compatible with our proposed problem formulation approach. Combined, they can greatly increase the efficiency of the risk

assessment of microbial PPP, thereby removing unnecessary hurdles for microbial PPP to become available to farmers.

Abbreviations

EFSA	European Food Safety Authority
EU	European Union
GM	Genetically modified
GMO	Genetically modified organism
MO	Microorganism
PPP	Plant protection product
QPS	Qualified presumption of safety
US EPA	United States Environmental Protection Agency

Glossary

Biological plant protection products (PPP):	Living organisms as well substances from biological origin used as plant protection products in agriculture. No legal definition of biological PPP is currently available in the EU.
Environmental risk assessment:	The process of assessing potential harm to the environment caused by a substance, activity or natural occurrence. This may include the introduction of GM plants, the use of pesticides, or the spread of plant pests [32].
Genetically modified organism (GMO):	A genetically modified organism (GMO) is an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination ([32]; see Directive 2001/18/EC).
Hazard (harmful characteristics):	The characteristics of a potential stressor that can cause harm to or adverse effects on human health and/or the environment (EFSA Scientific [33]).
Microbial plant protection product (microbial PPP):	PPP based on a microbial active substance.
Microbial active substance:	Active substance that is a microorganism capable of replication or transferring genetic material (i.e., 'living'), either as a single strain or as a qualitatively defined combination of strains and potentially including one or more metabolites produced by the microorganism [Commission Regulation (EU) No 283/2013]. Microorganisms include bacteria, fungi, yeasts and viruses.
Non-target organism (NTO):	An organism that is not intended to be affected by the potential stressor under consideration (EFSA Scientific [33]).
Pathway to harm:	A causal chain of events that need to occur for a harm to arise [34, 35]. The steps in the pathway enable the formulation of testable hypotheses that can then be tested to characterise risk.
Population:	Community of humans, animals or plants from the same species [32].
Problem formulation:	A method that enables identification of potential harms deriving from the deployment of a regulated stressor and potential pathway(s) to such harm, and defining the information needed to assess the likelihood of the harm occurring and its seriousness. This helps to focus the risk assessment on those phenomena that are important for decision-makers and shift it away from those that are less important or irrelevant (e.g., [36]).
Protection goals;	Comprise the objectives of environmental policies, typically defined in laws or regulations [37].
Regulation (EC) No 1107/2009:	An EU-wide regulation concerning the placing of plant protection products on the market, laying down the rules both for the authorisation of plant protection products and for the approval of active substances.
Risk:	The likelihood of consequences (of specified type, magnitude and duration) arising if an ecological entity is exposed to a specified stressor (EFSA Scientific [33]).
Specific protection goal (SPG):	An explicit expression of the environmental components that need protection, the maximum impacts that are predicted or can be tolerated, where and over what time period and with what degree of certainty. In EFSA Scientific Committee [33], the concept of SPG is considered consistent with that of 'assessment endpoint'.

Supplementary Information

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Supplementary Material 1. Protection goals.

Supplementary Material 2. Generic pathways to harm, testable hypotheses and analysis plans, including examples.

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