Swedish Chemicals Agency

Methods for assessing the effects of plant protection products on biodiversity

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Swedish Chemicals Agency

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Preface

Lund University was commissioned by the Swedish Chemicals Agency to map and describe approaches to develop methodologies for assessing the impact of plant protection products on biodiversity. The impacts to be considered were i) to what extent there are emerging methodological approaches to assess the indirect effect of plant protection products on individuals or populations, and ii) to what extent current risk assessment is sufficient to evaluate the direct effect of individual plant protection products on biodiversity. The commission also included proposing suitable methodology for assessing impacts of plant protection products on biodiversity. The relevant literature was mapped using a systematic search for literature to avoid bias in the selection of literature, and an inventory of emerging methods to assess indirect effects in other countries.

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Glossary

Biodiversity is the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this variability may include diversity within species, between species and of ecosystems (Regulation (EC) No. 1107/2009, Article 3 (29)).

Community is an association of interacting populations, usually defined by the nature of their interactions or by the place in which they live (EFSA, 2016a).

A **direct effect** is mediated solely by the interaction between the specified receptor/target and the environmental stressor, i.e. when the receptor/target is exposed directly to the stressor and as a result the receptor/target exhibits a response or an ecological effect (EFSA, 2016a).

European Food Safety Authority (EFSA) is the responsible authority risk assessment of active ingredients in plant protection products in Europe.

Environmental risk assessment (ERA) is the evaluation of the probability and seriousness of harmful (or adverse) effects to human health and the environment, whether direct or indirect, immediate or delayed, following exposure to a potential stressor (EFSA, 2016a).

An **indirect effect** is involving effects being transmitted to the specified receptor through an indirect route involving one or more other, intermediary, receptor/s. A predatory non-target organism, for example, could be affected indirectly by a stressor in several ways, including effects of the stressor reducing the abundance of its prey species, its intra-specific or inter specific competitors, its pathogens or its parasites. (EFSA, 2016a).

Non-target organism (NTO) is an organism that is not intended to be affected by the potential stressor under consideration (EFSA, 2016a).

Plant protection product (PPP) is a substance (or device) used to protect (crop) plants from damage by killing or reducing pest organisms or by mitigating its effects (EFSA, 2016a).

Population is a group of individuals of the same species (EFSA, 2016a).

Sammanfattning

Lunds universitet fick i uppdrag av Kemikalieinspektionen att kartlägga och beskriva ansatser till metoder som bedömer indirekta effekter av växtskyddsmedel på individer och populationer och studier som utvärderar om nuvarande riskbedömningsmetoder är tillräckliga för att bedöma direkta effekter av växtskyddsmedel på biologisk mångfald. Uppdraget utfördes i två delar. För det första inventerade vi metoder för att bedöma indirekta effekter av växtskyddsmedel bland riskbedömningsmyndigheter i nio länder. Parallellt granskade vi den vetenskapliga litteraturen genom att genomföra en systematisk sökning efter litteratur i vetenskapliga databaser för att kartlägga relevant forskning om indirekta effekter av växtskyddsmedel på individer och populationer och direkta effekter av växtskyddsmedel på biologisk mångfald.

Inventeringen visade att det finns få pågående ansatser att bedöma indirekta effekter av växtskyddsmedel mot bakgrund av miljöriskbedömningssystem bland de länder vi tillfrågade. I Tyskland infördes år 2018 krav på att bedöma indirekta effekter av växtskyddsmedel via trofiska interaktioner vid registrering av nya växtskyddsmedel, men dessa krav återtogs hösten 2019 eftersom de saknade rättslig grund för att implementeras.

Vår litteraturöversikt visade att metoder för att bedöma indirekta effekter på individer eller populationer av växtskyddsmedel i en riskbedömningskontext involverar både modellekosystem (cosmer), fältstudier och matematiska, huvudsakligen mekanistiska modeller. Kunskap om interaktioner mellan arter, som är grunden till potentiella indirekta effekter av växtskyddsmedel, är en nyckel för att förstå mekanismerna som ligger bakom växtskyddsmedels påverkan på den biologiska mångfalden.

Medan många studier hävdar att nuvarande riskbedömningsmetoder är otillräckliga för att skydda biologisk mångfald, har få studier jämfört hur väl olika metoder för miljöriskbedömningar skyddar biologisk mångfald. De nuvarande metoderna utgörs till stor del av laboratoriestudier på enstaka testarter, eller experiment med förenklade samhällen i cosmer, som ger information om akut toxicitet. Validiteten och användbarheten av sådana laboratoriestudier har kritiserats för att inte inkludera variation i rum och tid, interaktioner med andra stressfaktorer och indirekta effekter som uppkommer genom konkurrens eller trofiska interaktioner mellan populationer. Detta begränsar möjligheten att använda flera av de nuvarande metoderna till att bedöma effekter på biologisk mångfald i fältsituationer. Vidare föreslås att nuvarande riskbedömning av växtskyddsmedel kan förbättras genom att utöka representationen av testarter, inkludering av tidigare försummade taxonomiska grupper, såsom mikroorganismer och svampar, och bedömning av effekter på den genetiska variationen inom arter och populationer.

Framtida miljöbedömningsmetoder bör enligt flera studier i större utsträckning än idag kombinera laboratoriestudier, semifält- och fältstudier och matematiska modeller för att fånga indirekta effekter och direkta effekter på biologisk mångfald. Andra förslag är att komplettera de bottom-up-metoder som används i nuvarande miljöriskbedömningar, vilket i stor utsträckning är beroende av extrapolering av effekter på individer som bedöms i standardiserade laboratorietester till samhällen, med top-down-metoder såsom miljöövervakning av landskap och samhällen, och att kombinera bottom-up och top-down metoder, för att göra mer exakta bedömningar av riskerna för växtskyddsmedel med biologisk mångfald.

Summary

Lund University was commissioned by the Swedish Chemicals Agency to map and describe emerging methodologies that assess the indirect impact of plant protection products on non-target organism individuals and populations, and studies that evaluates if current risk assessment methodologies are sufficient to assess direct effects of plant protection products on biodiversity. The commission was performed in two parts. First, we made an inventory of emerging methods to assess indirect effects of plant protection products on non-target organisms among risk assessment authorities in nine countries. Second, we reviewed the scientific literature by performing a systematic search of scientific databases and mapped research discussing method development to assess indirect effects of plant protection products on non-target individuals and populations, and direct effects of plant protection products on biodiversity.

The inventory shows that there are few ongoing attempts to assess indirect effects of plant protection products in the light of environmental risk assessment schemes among the countries we asked. In Germany, requirements were introduced in 2018 to assess the indirect effects of plant protection products via trophic interactions when registering new plant protection products, but these requirements were withdrawn in the autumn of 2019 due to lack of legal basis for their implementation.

Our literature review showed that approaches to assess indirect effects on individuals or populations of plant protection products in a risk assessment context involves both model ecosystems (cosms), field studies, and mathematical, mainly mechanistic effects models. Knowledge of species interactions is a key to understand the underlying mechanisms that shape how plant protection products impact biodiversity.

While plenty of papers suggest that current risk assessment methodologies are insufficient to safeguard biodiversity, few papers have actually compared how well different environmental risk methodologies protect biodiversity. The current risk assessment methods are based short-term laboratory studies on single test species, or on simplified communities in mesocosm experiments, which provide information on acute toxicity. The validity and usefulness of such laboratory studies have been criticized for not including variation in space and time, interactions with other stressors and indirect effects caused by competition and trophic interactions between populations. This hampers the possibility of using them to assess effects on biodiversity in field situations. Furthermore, it is proposed that the current risk assessment of plant protection products can be improved by increasing the representation of test species, including previously neglected taxonomic groups, such as microorganisms and fungi.

According to several studies, future environmental risk assessment methods should to a larger extent than today combine laboratory, field and semi-field studies and mathematical models to capture indirect effects and direct effects on biodiversity. Other proposals are to complement and combine the bottom-up approaches of the current environmental risk assessment, that largely relies on extrapolation of effects on individuals assessed in standard laboratory tests to communities, with top-down approaches such as monitoring of landscape and communities, as well as combine bottom-up and top-down methods, to make accurate assessments of the risks plant protection products poses to biodiversity.

1 Introduction

Within the European Union, the approval of active substances in PPPs are regulated by Regulation (EC) No. 1107/2009 (EU, 2009) which in Article 4 covers the criteria active substances must be assessed against. For most of the areas mentioned in Article 4, well-developed methods for assessing active substances against the criteria are specified in supporting guidelines. However, for point 4.3.e.iii, concerning assessing impact on biodiversity and the ecosystem, methods are lacking. Article 4(3) states that:

> *3. A plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use, shall meet the following requirements:*
>
> *[...]*
>
> *(e) it shall have no unacceptable effects on the environment, having particular regard to the following considerations where the scientific methods accepted by the Authority to assess such effects are available:*
>
> *[...]*
>
> *(iii) its impact on biodiversity and the ecosystem.*

Current risk assessment methods focus mainly on assessing direct effects on individuals and populations of non-target organisms. With a few exceptions, indirect effects are not considered in current guidelines (Topping et al., 2020). Effects of plant protection products on "biodiversity and the ecosystem" are not per se evaluated, and it is questioned if current methods for risk assessments are sufficient to assess effects on biodiversity (Brühl and Zaller, 2019).

The aim of this report was to examine the following questions:

1) Are there any emerging method developments to assess the indirect impact of individual plant protection products on individuals or populations? If so, where are the difficulties or the development potential?
2) Are there reliable studies on the extent to which current methodology is sufficient to assess the direct impact of individual plant protection products on biodiversity? If such studies exist, have methodological shortcomings been identified, and are there suggestions for improvements?
3) How would a method be designed to assess the impact of the use of individual plant protection products on biodiversity?

2 Background

Biodiversity – the diversity within and among species, and of ecosystems (UN Secretariat, 1992) – declines at a historically unprecedented pace due to human activities (IPBES, 2019). In 2010, the United Nations agreed on a strategy to address biodiversity decline, including targets to halt and reverse the loss of biodiversity over a decade. In 2020, most of the set targets are not met (Secretariat of the Convention on Biological Diversity, 2020), including those of sustainable agriculture, aquaculture, and forestry and reduced pollution.

In agricultural landscapes, use of PPPs is considered to be one of the drivers of biodiversity decline (Potts et al., 2010; van Lexmond et al., 2015; IPBES, 2019). Use of PPPs has been associated with reduced or low diversity and abundance of both terrestrial and aquatic organisms from a large range of taxa including plants, arthropods, and vertebrates (e.g. Bünemann et al., 2006; Geiger et al., 2010; Mineau and Whiteside, 2013; Stehle and Schulz, 2015a; Tassin de Montaigu and Goulson, 2020; Woodcock et al., 2016). Hence, reliable methods to assess the biodiversity risk of PPPs are important to enable informed decisions about their use in agriculture (Topping et al., 2020).

In the current ERA, the consequences of PPP use on NTOs are initially evaluated based on tests of the impacts on individuals of model species under laboratory conditions (EU, 2009; SANCO, 2002), with the assumption that capturing effects on the most sensitive species will protect biodiversity (Brock et al., 2006). Even in higher tiers of ERA, mainly direct effects are measured, and indirect effects are seldom included (EFSA, 2016b). While this approach may reveal many of the potentially detrimental consequences of PPPs on biodiversity, it also has important limitations. First, PPPs may not only affect NTOs through direct effects, but also via indirect effects through competitive or trophic interactions with organisms that in turn are directly or indirectly affected by the PPP (Hoffman, 2003). Second, laboratory tests of PPP impacts on single organisms do not allow the diversity of reactions to PPPs by wild organisms in general, such as long-term effects and interactions with abiotic context and other driving forces (also called drivers) of biodiversity declines. Thus, assessment methodologies where the effects of PPPs on biodiversity *per se* are evaluated, using either empirical or modelling approaches, may be necessary to capture the full range of consequences.

EFSAs approach to focus on ecosystem services in future ERA (EFSA, 2016c) moves the focus from structural endpoints (e.g. abundance and species richness) to ecological processes. However, this does not necessarily invalidate a focus on biodiversity *per se*. Protecting structural endpoints such as different aspects of biodiversity may protect ecosystem service delivery if the former is equally or more sensitive than functional endpoints, but not necessarily so (Maltby et al., 2018; Rohr et al., 2006a). On the other hand, protection of ecosystem services does not necessarily safeguard biodiversity and future ERA therefore still will require methodologies that assess effects of PPPs on biodiversity *per se* (Maltby et al., 2018).

In this report we have summarised the scientific literature for evaluation of methods able to overcome these shortcomings, as well as investigated the occurrence of emerging methods.

2.1 Effects of PPPs on non-target organisms

PPPs target pests and pathogens, but in addition may have unintended consequences for non-target individuals, populations, communities, and ecosystems. Direct effects of PPPs arise when an ecological receptor, e.g. an individual, a population or a community, is exposed to a PPP and exhibits a response (EFSA, 2016a). Thus, direct effects can be observed on all levels of biological organization. Direct effects fundamentally occur because fitness components of NTOs are affected by PPPs. Such effects could be increased mortality or reduced fecundity, which in turn can be caused by physiological effects or by behavioural changes with secondary fitness consequences (Köhler and Triebskorn, 2013). Effects of PPPs may be immediate such as increased mortality or delayed because sub-lethal effects have consequences for fitness-components (Hoffman, 2003). Direct effects act through a cascade of individual effects, translating into population effects and finally into community and ecosystem consequences (Köhler and Triebskorn, 2013). However, any direct effect of a PPP on a NTO may translate into an effect of another NTO through interactions such as competition and predation (see section 2.2). Consequently, direct effects on higher levels of biological organisation (communities, ecosystem) may occur because of a combination of direct and indirect effects at lower levels of biological organisation (individuals, populations). The risk of effects on biodiversity by use of PPPs is therefore related to a complex interaction between the PPPs mode of action, the risks of exposure, interaction with other drivers and the occurrence of indirect effects (Hoffman, 2003).

PPPs can have different modes of action in the target organisms, which is useful knowledge when managing pests (Kopit and Pitts-Singer, 2018; IRAC, 2020). Less is known about mode of action and ensuing consequences for NTOs, although close phylogenetic relationships between pests and NTOs suggest that there may be parallels between how they react (Köhler and Triebskorn, 2013). In particular, results from a limited set of model organisms may not capture the array of responses by wild organisms (Rohr et al., 2016) limiting the ability to assess risks for biodiversity.

The degree of harm caused by a PPP on an individual or population may be affected by the effect of other stressors (Goulson et al., 2015; De Castro-Català et al., 2020), such as land-use change or climate change. As a result, subtle effects on performance such as behavioural changes (sub-lethal effects) may only translate into fitness consequences under field conditions (Desneux et al., 2007; Saaristo et al., 2018). Potential context dependencies that seldom are considered could relate to abiotic conditions such as temperature and moisture (Kimberly and Salice, 2014; Laskowski et al., 2010), or to biotic conditions such as recolonization potential within the ecosystem (Kattwinkel et al., 2015). Context dependency is one of several reasons to why it is difficult to generalize consequences of PPPs from laboratory conditions to population consequences in the field (Amossé et al., 2020).

When organisms interact with their environment to fulfil their basic needs of e.g. nutrients, water, shelter, mating, and nesting, they may be exposed to PPPs (Kopit and Pitts-Singer, 2018; Uhl and Brühl, 2019). Thus, organisms' life-history traits and their activity patterns, in combination with PPP use and fate over space and time, results in patterns of PPP exposure and potential for impacts (Sponsler et al., 2019). Many organisms in agricultural landscapes utilize multiple habitats linked by dispersal or foraging movements (Smith et al., 2014), such that their exposure to PPPs may be related to their relative use of habitats. Such movements may be on different time scales, relating to daily foraging movement, over seasonal movement that may involve different life stages, to migratory movement, with implications for PPP exposure (Awkerman and Raimondo, 2018; Centrella et al., 2020; Colwell et al., 2017).

PPPs impact NTOs present in the field of application but may due to drift, runoff, or leakage also have more wide-ranging consequences (Gove et al., 2007; Dupont et al., 2018; Prosser et al., 2016). These consequences may affect NTOs in the surrounding landscape and be transported downstream by surface water (de Jong et al., 2008). They may also be spread by dispersing animals exposed to the PPPs (Schiesari et al., 2018). While most of the PPPs stay in the field of the application, the level of detectable exposure may be surprisingly large in non-target habitats (Krupke et al., 2012; Botías et al., 2015; Krupke et al., 2017; Uhl and Brühl, 2019; Pelosi et al., 2021). Contaminants will also be transported through food webs (Brühl and Zaller, 2019), with particularly severe consequences when they bio-accumulate across food chains (Carson, 1962). Thus, exposure rates to a wide range of NTO during field conditions may be difficult to predict from application rates without detailed knowledge about the environmental fate of PPPs (Bonmatin et al., 2015), hampering risk assessment related to biodiversity.

Consequences of individual fitness components, as estimated in small scale evaluations, may not necessarily translate into population level consequences. This may be the case if for example redistribution of organisms at the landscape level compensate for reduced local fitness (Kattwinkel et al., 2015) or if there is density-dependent compensation in fecundity because of increased mortality (Moe et al., 2002). Such recovery from the PPP exposure is hardly measured, since most individual-level tests prevents colonization and reproduction (Rohr et al., 2016).

2.2 Indirect and direct effects of PPPs on NTOs

Indirect effects on NTOs emerge as a result of direct effects of PPPs on organisms, both those targeted by PPPs and NTOs, with which they in one or the other way interact (EFSA, 2016a). In principle, a direct effect on any level of biological organisation can translate into an indirect effect at a higher level of biological organisation, but in this synthesis, we focus on when direct effect on populations translates into effects in other populations through between-population interactions, resulting in community changes. Both effects of PPPs on mortality and sub-lethal effects such as altered behaviour, can lead to changes in interactions with other organisms, for example through altered competitive or trophic interactions. This in turn translates to changes in community composition and changed ecosystem properties (Halstead et al., 2014). Indirect effects may be spatially displaced from where PPPs are applied, because of the mobility of directly affected organisms (Spromberg et al., 1998; Boatman et al., 2004).

Interactions can be either positive, neutral, or negative for the populations involved (Andrewartha and Birch, 1984). Consequently, indirect effects of PPPs can be either positive or negative for the affected populations. For example, insecticide application led to a decrease in florivorous beetles harming flower buds, resulting in increased availability of flower resources for pollinators (Lindström et al., 2018), while the use of a broad-spectrum herbicide in contrast reduced pollen-and nectar resources for pollinating insects (Dupont et al., 2018). It is thought that broad-spectrum and/or persistent PPPs are more likely to cause indirect effects than highly specific and short-lived PPPs (Cloyd, 2012).

Indirect effects of PPPs on NTOs can occur via relatively short interaction chains, such as when the application of insecticides reduce food resources and thus breeding success of a farmland bird (Hart et al., 2006). However, they can also occur via longer interaction chains, such as when application of herbicides results in reduced food resources for weed seed eating insects which in turn lead to reduced food availability for insectivorous farmland birds (Potts G.R, 1986; Boatman et al., 2004).

According to ecological theory, species interactions and thus the potential for indirect effects, is determined by the density and traits of interacting species (Wootton, 2002; Wootton and Emmerson, 2005), a framework that applies to understanding indirect effects in ecotoxicological contexts (Fleeger, 2020; Relyea and Hoverman, 2006). Indirect effects can be complex, because they may involve both direct and indirect interactions between species and be either density related, or trait mediated when one species affect the functional trait of other species (Wootton, 2002). Both the horizontal (number of species in a trophic level) and vertical (number of trophic levels) affect the complexity of responses (Zhao et al., 2020). When more than two species are involved in an interaction, and in spatially complex environments, the scope for complex and surprising effects increases (Hoffman, 2003; Schiesari et al., 2018; Gutiérrez et al., 2020). Detecting indirect interactions requires studies of communities involving the relevant species.

Indirect effects of PPPs can occur through altered behaviour. Avoidance of contaminated areas by one species (Araújo et al., 2016) or aggravated aggressivity, induced by a PPP leading to increased competition with another species, will alter the behaviour of interacting species (Saaristo et al., 2018). Such cascading effects can occur in a top-down or bottom-up orientation of a food web and will depend on the species composition of the community and their specific reactions to the PPP.

Indirect effects of a PPP can induce prompter and stronger effects in an ecosystem than an assessment of the PPPs direct effects may predict (Sih et al., 2016), but effects at the species level can theoretically also be buffered by compensatory effects resulting in less apparent changes at the community level (cf. Supp and Ernest, 2014). Because indirect effects have the potential to alter entire communities or even ecosystems, they are important to understand and assess when evaluating risks of PPPs on NTOs (Saaristo et al., 2018). However, it is generally not known how important indirect effects are compared to direct effects (see overview in EFSA, 2016b), but for at least some organisms such as farmland birds, indirect effects have been suggested to be the more important (Bright et al., 2008).

Tracking the impact of PPPs occurring on cellular level within non-target individuals to indirect effects among interacting species and to biodiversity decline is a challenge (Köhler and Triebskorn, 2013; Saaristo et al., 2018). The complexity of behavioural, competitive and trophic interactions and their importance for individuals, populations and biodiversity and the spatial and temporal scales they are acting on, makes indirect effects hard to predict (Köhler and Triebskorn, 2013; EFSA, 2016b; Saaristo et al., 2018). In ERA, recommended methodologies to capture such impacts needs to be well documented, reproducible, and targeting endpoints verified to be linked to consequences for long term effects for other individuals and populations (EFSA, 2016b). However, although it is challenging to find methods that fulfil all these criteria given the potential importance of indirect effects on NTOs, *there is a need of credible risk assessment methods able to capture indirect effects.*

2.3 Effects of PPPs on dimensions of biodiversity

Biodiversity is defined as “the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems” according to the Convention on Biological Diversity (CBD). Thus, biodiversity if often referred to as being inherently multidimensional, including e.g. different organisational levels (genes, species, communities, ecosystems) and various aspects of diversity (taxonomic, phylogenetic, functional diversity) (e.g. Naeem et al., 2016). As a result, there are many ways to quantify biodiversity, expressed as various biodiversity indices (e.g. Magurran, 2013).

PPPs may affect diversity at all levels of biological organisation. It may affect genetic diversity through induced alterations (induced heritable mutations) or through effects on natural processes (bottlenecks, contaminant-mediated natural selection) (Bickham et al., 2000), species diversity through direct or indirect effects on populations (see below), and diversity of communities and ecosystems through cascading effects of population changes (Freedman, 2013).

The consequences of PPPs on individual populations may translate into consequences for biodiversity at the community level for several reasons. Most trivially, PPPs may result in the loss of individual species or changes in relative abundances, due to chance effects when all organisms are affected or because species are differentially affected (Köhler and Triebskorn, 2013). However, when species interactions are affected, this may also translate into changes in species richness, community composition, and ecosystem properties (Relyea and Hoverman, 2006). It is generally expected that many PPPs effects on biodiversity are the result of indirect effects (Köhler and Triebskorn, 2013; Relyea and Hoverman, 2006; Saaristo et al., 2018; Wootton, 2002) which may involve multiple species and be quite complex (Fleeger, 2020). Thus, the emerging properties of communities, such as various aspects of biodiversity, may be difficult or even impossible to predict from the reactions of individual populations.

Furthermore, effects of PPPs may lead to altered relative abundances (evenness) and to altered species sorting (community composition) (Fischer et al., 2013). Effects of PPPs on individual populations may or may not translate into consequences for specific biodiversity indices, depending on how they treat individual species and their abundances when calculating them. For example, a reduction in population sizes may not translate into consequences for species richness unless species go extinct but may translate into change in the effective number of species which also account for the relative abundance of different species. Biodiversity indices are also scale-dependent because effects of population changes and extinctions on the indices across scales are not additive, e.g. because a species may go extinct locally but not regionally after exposure to PPPs.

Because consequences on multiple aspects of biodiversity cannot easily be deduced from the reaction of individual populations, *there is a need for risk assessment methods that capture the effect of PPPs on biodiversity as such*.

2.4 Current ERA of PPPs

The current ERA approaches are the results of historical uses and regulations and they are dynamically evolving based on revised regulation and emerging needs of their users. The founding principles of ERA came from the field of ecotoxicology, originating in the 1970s from the field of toxicology (Van den Brink, 2008). This heritage is the basis for focusing on evaluating consequences of PPPs on individuals of model species under laboratory conditions and also the reason for the lack of underpinning ecological theory in ERA (Forbes et al., 2009; Van den Brink, 2008).

The requirements of the regulations have evolved and the current EU regulation of PPPs (Regulation (EC) No 1107/2009) specifically mentions the aim of avoiding unacceptable effects on *biodiversity*. Departing from these general protection goals, and also more operable specific protection goals that are under development (EFSA, 2016c, 2010) the EU, presently by EFSA, has developed guidance documents. These documents are intended to guide both applicants of PPP approval, that are responsible for providing the dossiers, and member state authority risk assessors that evaluate the information in the dossiers, so that the European Commission can decide on approval, or not, of PPP active ingredients.

There are currently three implemented guidance documents for ERA of PPPs, which focus on non-target terrestrial organisms (ESCORT 2, 2000; SANCO, 2002), birds and mammals

(EFSA, 2009), and aquatic organisms (EFSA, 2013a). A guidance document for bees has also been developed (EFSA, 2013b), but not implemented, and this is currently under revision (EFSA, 2020). In addition, EFSA has, to prepare for revisions of current and development of new guidance documents, published scientific opinions compiling the state of the art for non-target terrestrial plants (EFSA, 2014a), non-target arthropods (EFSA, 2015), in-soil organisms (EFSA, 2017), and amphibians and reptiles (EFSA, 2018).

The specific scenarios are different among the different guidance documents, but the general principles are fairly similar. In general, the European ERA of PPPs is a tiered process starting with simple and conservative assessments of exposure and effects of PPPs that are combined to assess risks and moving towards more complex and realistic assessments, focusing on specific target groups defined (EFSA, 2013b; SANCO, 2002). The initial step determines if there is any need for further consideration, usually based on worst case assumptions of PPP exposure and model species (EFSA, 2009). In lower tier, exposure is usually based on model predicted environmental concentrations and effects are assessed for acute and chronic exposure in controlled (mostly laboratory) settings using selected representative species (EFSA, 2009, 2013b; SANCO, 2002). Uncertainty factor are used to account for variation among species in their sensitivities (Rohr et al., 2016). The possible risks and uncertainties indicated in the lower tier determines if the PPP should be assessed in higher tiers. The higher tier assessments are usually more adapted case by case and move towards evaluating consequences in semi-field studies, field studies and landscape level models (EFSA, 2009, 2013b).

The current ERA of PPPs has been criticized to not fully protect NTOs and biodiversity (Stehle and Schulz, 2015b; Storck et al., 2017; Streissl et al., 2018; Brühl and Zaller, 2019; Uhl and Brühl, 2019; Topping et al., 2020). The interactions among organisms and their physical environment, *the ecology*, is a fundamental aspect of biodiversity that is rarely included in ERA (Forbes et al., 2009; Van den Brink, 2008). Assessments of indirect effects are only considered in higher-tier or field tests (EFSA, 2016b), but from a scientific perspective, it is challenging to evaluate how well indirect effects are currently assessed in ERA (EFSA, 2016a). Indirect effects are considered in several of the scientific opinions (concerning non-target terrestrial plants (EFSA, 2014a), non-target arthropods (EFSA, 2015), in-soil organisms (EFSA, 2017), and amphibians and reptiles (EFSA, 2018)) that are in preparation for revision of current guidance documents. Given that species interactions are highly likely to influence impacts of PPPs indirectly on NTOs, and thereby affect biodiversity, methods for assessing indirect effects of PPPs on individuals and populations needs to be developed and incorporated in ERA (Uhl and Brühl, 2019). Furthermore, it needs to be evaluated whether direct impacts of PPPs on biodiversity is fully covered by the current ERA methodologies (Brock, 2013; Streissl et al., 2018; Brühl and Zaller, 2019).

2.5 Scope of the study

Here, we aimed to review the scientific literature considering risk assessment methods that assess indirect effects of PPPs on NTOs, and inventory method development initiatives among authorities that handles ERA. Furthermore, we review the scientific literature that evaluate if current methodology is sufficient to assess the direct impact of individual PPPs on biodiversity. Finally, based on this, we summarise how a future method should be designed to assess the impact of the use of individual PPPs on biodiversity.

2.6 Delimitations

In this report we review methods to assess indirect effects of PPPs on individuals and populations, and we do not consider the question if direct effects are or are not sufficiently considered in current ERA. We also review direct impact of PPPs on aspects of biodiversity. In spite of the potential importance of effects on biodiversity of the use of PPPs through the structure of agricultural cropping systems (e.g. Hendlin et al., 2020), this was outside the scope of the assignment and therefore not considered. The assignment was also limited to the assessment of risks of single PPPs and does not take combination effects into account.

3 Methods

The commission was handled in two parallel parts. First, an inventory of approaches to develop the methodology on assessments of indirect effects of PPPs on individuals and populations was conducted to map initiatives taken in other countries. Second, a review of the scientific literature concerning indirect effects of PPPs on individuals and populations and direct effects of PPPs on biodiversity was performed, based on a systematic search for relevant literature in scientific databases.

3.1 Inventory of method development approaches

In order to find approaches to develop current risk assessment methods to assess impacts of individual PPPs, we made an inventory among authorities working with regulations of risk assessments of PPPs. We identified risk assessment authorities in 13 countries and two international organisations and made personal contacts via email to persons in key positions. We asked them to answer key questions (Annex I) on current risk assessment methods and if there were any approaches in development to also include indirect effects on individuals and populations in risk assessments of PPPs. The answers were compiled and discussed in the light of the findings in the literature review.

3.2 Review of the scientific literature

We mapped the scientific literature inspired by the framework for Systematic review and Evidence synthesis (CEE, 2013) to avoid bias and increase transparency of the review process (cf. Haddaway et al., 2015). We defined search terms based on research questions 1 and 2, and combined them into the following search string:

((((plant* OR animal* OR fung* OR bacteri* OR insect* OR arthropod* OR bird* OR fish* OR aquatic* OR terrestrial OR amphib* OR bee*) NEAR/2 (communit* OR populat* OR divers* OR species OR richness)) OR wildlife OR biodivers* OR nontarget* OR “non-target”) AND (pesticid* OR herbicid* OR fungicid* OR insecticid* OR molluscicid* OR "plant protection product*") AND (((indirect* OR direct) NEAR/2 effect*) OR (risk* NEAR/2 assess*)) NOT antibiotic*)

A list of 16 key publications, suggested by eight subject-experts, were compiled and used to quality check and refine our search to capture highly relevant publications. As in any literature study, it is possible that some relevant publications were not captured, but our systematic approach ensured that we avoided bias in the selection of studies to include.

We applied the search string on ISI Web of Science academic database, including Core Collection, BIOSIS Citation Index and Zoological Records, on the 1 October 2020. We limited the search to records that matched the search string in topic (including title, abstract, author keyword and keyword plus) and were written in English.

3.2.1 Study inclusion criteria

We screened titles and abstracts of the papers identified through the search based on a decision tree (Annex II), including criteria for focuses on single PPPs and indirect effects on individuals or populations or direct effects on biodiversity, to identify relevant records. All records that were assessed to be potentially relevant based on title and abstract were forwarded to full-text screening. In addition, records lacking abstract but that were likely to contain relevant information based on the title, were forwarded to full-text screening. Records in other languages than English, duplicates, and those for which full-text papers could not be found, were excluded. In the full-text screening, we also excluded non-peer reviewed publications and book-chapters. The screening was performed by four persons. We calibrated our assessments by comparing individually performed assessments of a subset of 50 abstracts and 30 full-text papers.

During the full-text screening, relevance was assessed against the same inclusion criteria as title and abstract screening. Records that were assessed to fulfil all criteria were categorised (Annex II) based on publication type, continent where the study was performed, PPP-type, study environment, and study method (Table 1). We also noted which organism(s) and which habitat(s) the studies focussed on. The studies were categorised with regard to EFSA's risk assessment groups, based on the organism groups in the guidance documents and in EFSA scientific opinion documents; birds and mammals, terrestrial organisms, aquatic organisms, bees, non-target terrestrial plants, non-target arthropods, in-soil organisms, and amphibians and reptiles.

Furthermore, we assessed if each record dealt with risk assessment methods to assess indirect impacts of individual PPPs on individuals or populations and contained approaches to develop such methods. If so, we summarised what kind of species interactions (Table 2) that were studied and which response variables that were measured. We also assessed if each record described difficulties or development potential of such methods. Furthermore, we assessed if the study evaluated whether current methodology is sufficient to assess the direct impacts of individual PPPs on biodiversity or not. If they did, we noted the measured aspects of biodiversity (e.g. genetic variation, species richness, evenness, diversity, phylogenetic diversity, community composition). We also noted what level of biodiversity the study focussed on (genes, species, or ecosystems). Studies on the species level of biodiversity were separated into two groups, a) studies measuring species richness and b) studies including more complex aspects of community composition (e.g. indices accounting for relative abundances, food web structure). Finally, we noted if the study had identified shortcomings in the current methodology and suggested improvement of the same.

Table 1. Levels applied to categorise search records in the full-text article screening.

Type of publication	Continent	PPP-type	Environment	Study method
Review, empirical, theoretical, policy brief, opinion article, or other	Africa, Asia, Australia, Europe, North America, South America, or a combination	Herbicide, insecticide, fungicide, nematicide, rodenticide, molluscicide, or general	Agriculture, forest, urban, marine, limnic, or general	Lab, cosm, greenhouse, field, landscape, modelling, or other

Table 2. List of species interactions.

Term	The effect of one species on the other, positive (+), neutral (0), or negative (-)
Mutualism or protocooperation	+ +
Commensalism	+ 0
Predation/herbivory/parasitism/parasitoidism	+ -
Amensalism	0 -
Competition or mutual predation	- -
Neutralism	0 0

We defined relevant types of outcome to facilitate the screening process. For indirect effects on individuals or populations, we expected that the studies concerned effects on mortality, fecundity, abundance/biomass/growth, population density, population persistence, behaviour, populations and meta-populations on all spatial and temporal scales through feed/prey diversity and abundance, altered intra or interspecific competition, reduced or increased predation-pressure, parasite and pathogen prevalence. For studies concerning direct effects, we expected focus on ecological entities other than individuals or populations: alpha-, beta-, gamma- species diversity, species richness, evenness, genetic diversity, abundance of functional/taxonomic groups of organisms, species diversity, phylogenetic diversity, functional diversity, community composition/structure, trophic interactions, and food webs on all temporal and spatial scales.

4 Results

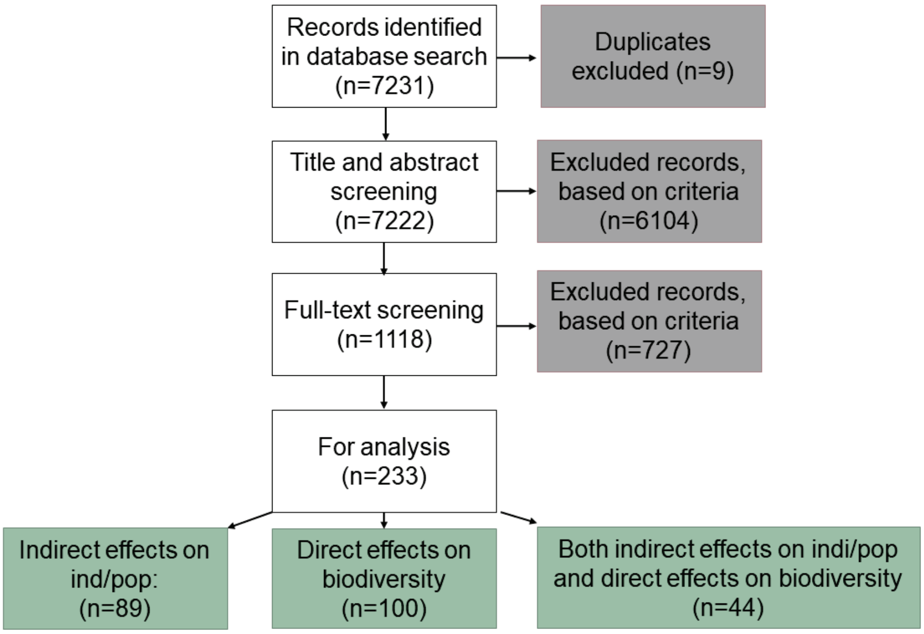
4.1 Inventory of approaches for method development to assess indirect effects

We received answers from authorities in nine of the 13 consulted countries: Austria, Australia, Belgium, Brazil, Canada, Denmark, Germany, the Netherlands, and the USA. We also received answers from the EFSA and the OECD. Their responses are compiled in the discussion (section 5.1).

4.2 Literature map

The search in academic databases resulted in 7231 papers, with 7222 papers remaining after removal of duplicates (Fig. 1). After exclusion of 6104 papers in the title and abstract screening, 1118 papers were retained for full-text screening. The full-text screening identified 233 papers as relevant for either or both of the questions, with 89 papers about indirect effects of PPPs on individuals and populations, 100 papers about direct effects of PPPs on biodiversity, and 44 papers about both indirect effects of individuals and populations and direct effects on biodiversity (Fig. 1). Among the papers concerning indirect effects of PPPs on individuals or populations, 31 papers discussed both risk assessment methods, method development, and difficulties or potentials of such methods. Among the papers identified to consider direct effects of PPPs on biodiversity, we found that 47 papers discussed both evaluation, shortcomings, and improvements of methods.

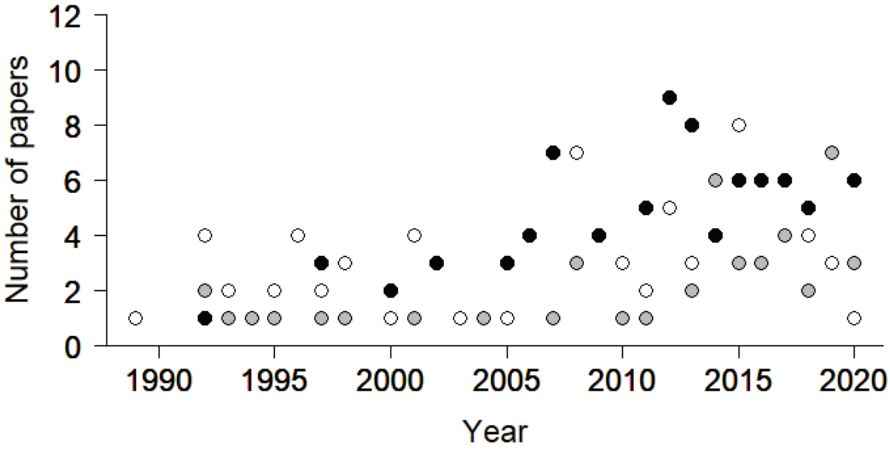
Figure 1. Results from the literature database search. Numbers within parentheses indicate number of papers.



4.2.1 Publication year, origin, and type

Papers discussing methods to assess indirect effects of PPPs on individuals or populations in the light of risk assessment were published from the late 1980's and showed modest increase in frequency from year 2005. Papers about ERA methods to assess direct effects of PPPs on biodiversity were less represented in the 1990s than papers about indirect effects on individuals and populations but increased in frequency from the millennium shift. Papers discussing both topics showed similar tendencies to increase in frequency after year 2010 (Fig. 2).

Figure 2. Number of relevant papers per publication year concerning either methods to assess indirect effects of PPPs on individuals or populations (open dots) or evaluation of direct effects of PPPs on biodiversity (black dots), in a risk assessment c



Most of the studies in the relevant papers were performed in Europe (138 studies) and North America (54), followed by studies performed in two or more continents (14), Asia (12), Australia (8), and South America (6). Only one study performed in Africa was included.

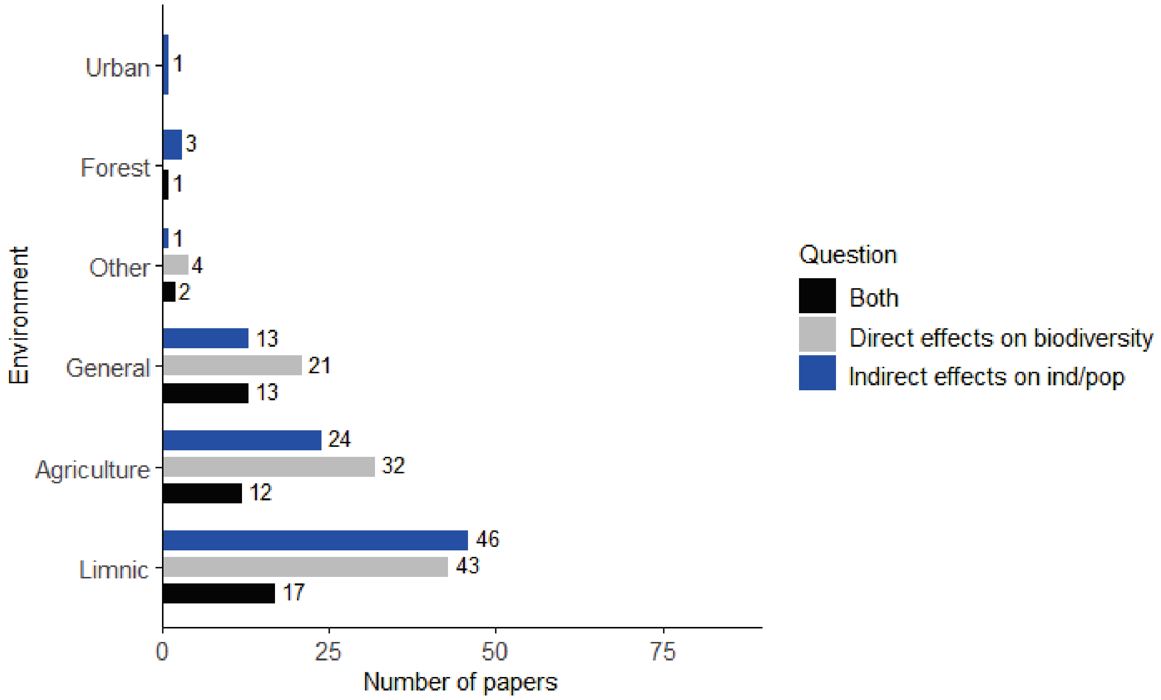
Most of the studies were empirical (126), but review papers (40) were also common. Some opinion papers (21) and theoretical papers (24) were also included, along with a few other types of papers (22); these consisted of policy briefs, editorials, or combinations of several publication types (for example theoretical and empirical).

4.2.2 PPP types and study environments

Papers discussing PPPs in general were highly abundant (84 papers) in the review category. Papers focussing on insecticides (67) and herbicides (54) were somewhat more abundant than those with a fungicide focus (20). Papers focussing on two or more PPP types were fewer (8). Only one paper with focus on a molluscicide was included.

According to the review, most studies were done in limnic environments, followed by agricultural environments (Fig. 3). A large share of the papers in the review had a general focus across different environments. Studies focussing on forests and urban areas were rare and dealt with only indirect effects on individuals and populations, with one exception, and no study focused on effects on biodiversity (Fig. 3). Remaining studies focused on deserts, subalpine-arctic, and marine environments, or a combination of several environments.

Figure 3. Number of papers concerning direct effects on biodiversity (black bars) versus indirect effects on individuals or populations (grey bars) distributed over study environments (N=233). The number next to each bar represents the number of papers



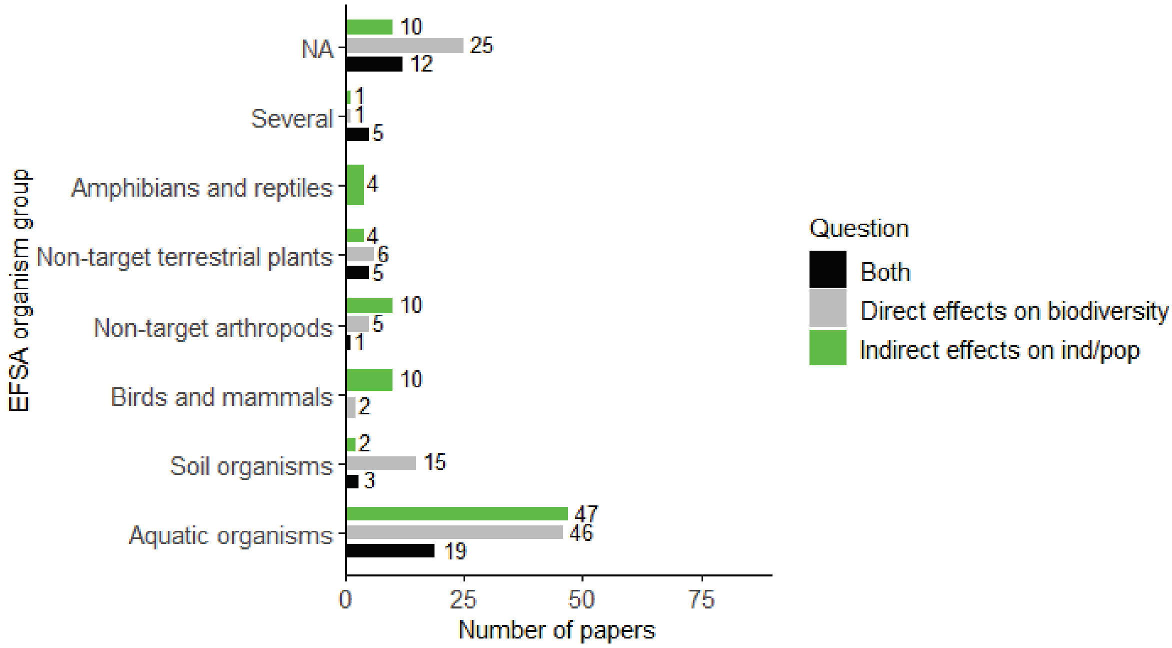
The papers focusing on limnic environments studied a range of limnic habitats, from ground water to streams, ponds, ditches, wetlands, rivers, lakes, and benthic habitats. Within agricultural settings, farmland in general, semi-natural habitats, grasslands, soil, and crop-habitats were represented.

4.2.3 Studied organisms

The included papers were categorised across the different organism groups defined in the ERA guidance documents and scientific opinions by EFSA. Papers studying aquatic organisms were most common (112 papers). These were represented by for example fish, invertebrates, amphipods, diatoms, and algae. Soil organisms were studied in 20 papers, with focus on for example earthworms, fungi, nematodes, microorganisms, rhizospheric bacteria, and arthropods. Non-target arthropods were the focus for 16 papers, represented by for example lady beetles, spiders, butterflies, social Hymenoptera, spider mites, and carabids. For the birds and mammals' group, only birds were represented, with 12 studies. Non-target terrestrial plants and amphibians and reptiles were studied in 15 and four papers, respectively.

Of the 112 papers about aquatic organisms, 47 studied indirect effects on individuals or populations, while 46 studied direct effects of PPPs on biodiversity, and 19 studied both indirect effects on individuals or populations or direct effects on biodiversity (Fig. 4). Most of the papers on birds considered indirect effects on individuals or populations. Among the papers with a focus on soil organisms, most studied direct effect on biodiversity (Fig. 4). For the papers on non-target arthropods, five considered direct effects of biodiversity and ten considered indirect effects of PPPs on individuals or populations and one study included both aspects.

Figure 4. Number of papers concerning direct effects on biodiversity, indirect effects on individuals or populations, or both, distributed over organism groups represented by EFSA's environmental risk assessment guidance documents or scientific opinion



Seven papers included organisms from more than one of the organism groups. For example, a few papers concerning pollinators or flower-visiting insects studied both bees and other non-target arthropods. A large share of the papers (47) discussed biodiversity or indirect effects in general terms, without specific organism groups in focus.

Few papers studied effects of PPPs on genetic diversity; only one covered genetic level biodiversity solely, and one genetic and species diversity. The species level of diversity was studied in 109 papers, of which 43 contained simple species richness-measures, 42 measured more complex community aspects, and 24 both of these. None of the studies considered the ecosystem-level of diversity.

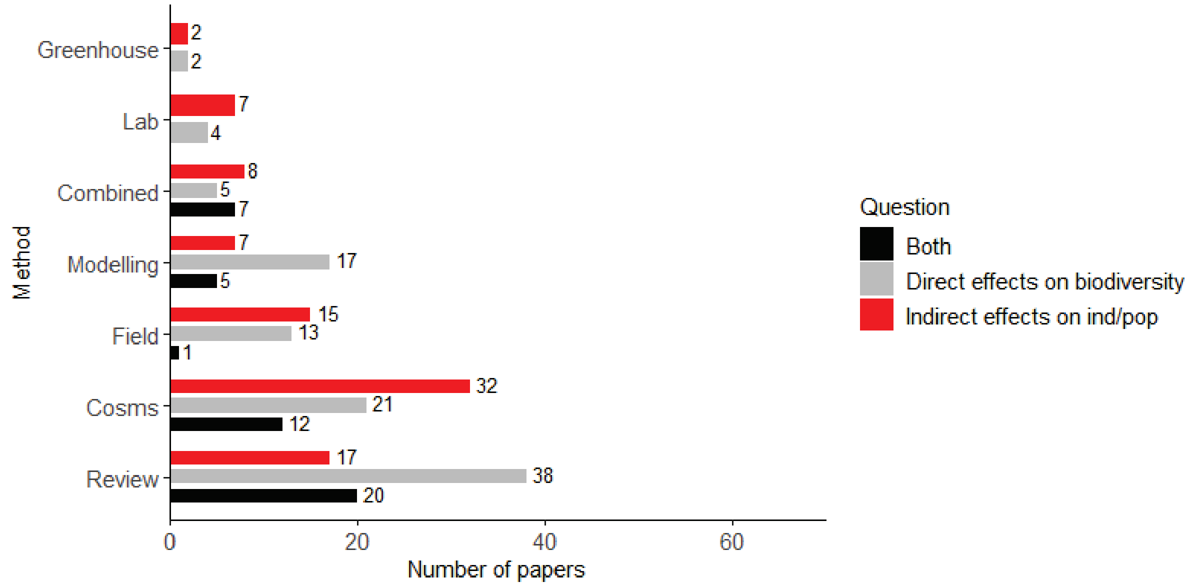
The papers about indirect effects of PPPs concerned species interactions such as competition, consumer-resource interactions, mutualism (plant-mycorrhiza interactions, pollination), trophic cascades, and even interactions between microbiota, immune system, and disease. The measured endpoints ranged from abundance and growth (e.g. plant coverage, biomass, biovolume, population density) and behaviour (e.g. flower visitation, attack rate) to fecundity (e.g. egg size, flower number, seed production) and mortality. Community-level endpoints measured ranged from richness on genetic, species, or higher taxa-level and, diversity indices such as Shannon's H', to community composition.

4.2.4 Study methods

Literature review was the most common study method among the papers identified in the review (75 papers). Various kinds of cosm-experiments (microcosms, mesocosms) were used in 60 papers, while field-level studies were less common (29 papers). 17 of the papers were lab-studies, and greenhouse studies were found in four of the papers. Modelling was the method in 29 papers, and 19 papers combined methods (for example field-scale observations and modelling or field scale and mesocosm-studies).

Of the papers that used field studies to assess effects of PPPs, 15 dealt with indirect effects on individuals and populations and 13 with direct effects of biodiversity, and one paper studied both indirect effects on individuals or populations and direct effects on biodiversity (Fig. 5). Almost as many papers studied indirect effects of PPPs on individuals and populations as direct effects on biodiversity using lab-methods or combined several methods. Of the papers about modelling, 17 focussed on direct effects of biodiversity, and seven on indirect effects on individuals or populations (Fig. 5). More of the papers that built their discussions on reviews concerned direct effects on biodiversity than on indirect effects on individuals or populations, 38 and 17 papers, respectively, while 20 papers discussed both topics.

Figure 5. Frequency of study methods appearing in the review, and the distribution between direct effects on biodiversity versus indirect effects on individuals or populations (N=233). The number next to each bar represents the number of papers per EFS



5 Literature summary

The task of this review was to investigate if there are emerging *method developments on how to assess the indirect impact* of individual PPPs on individuals or populations, and if so *what the difficulties or development potential of these methods* are. In addition, we should investigate if there are *studies on the extent to which current methodology is sufficient to assess the direct impact of individual plant protection products on biodiversity*, and if there are any *studies identifying shortcomings of these methodologies*. In this section we provide a narrative summary of the papers retrieved by the systematic literature search. Occasionally we also refer to papers that do not stem from the literature search, e.g. to provide necessary background; to separate these papers from those from the systematic research we consequently refer to them by “see” or “see also” the particular reference.

Methods to capture indirect effects, and by extension effects on biodiversity, require that approaches to identify risks of direct effects on individuals or populations are scaled up to communities in which indirect effects are either directly studied or captured as emergent properties by using community-related endpoint such as biodiversity indices. Thus, the challenge of assessing risks of PPPs related to indirect risks and biodiversity is to *upscale studies* on individuals and populations to communities and ecosystems in ways that maintain *sufficient ecological realism* to trustworthy reflect consequences under field conditions, or alternatively to carry out studies in *field-realistic scales using community-related endpoints*. We will here present the results from our systematic search for literature, providing an overview of empirical studies and relating the conclusions from major reviews and opinion papers specifically targeting the questions above. We will focus on the major methodological challenges and how to overcome them, but not systematically present scattered opinions in a long row of review papers that has neither suggested new approaches to assess indirect effects nor explicitly discussed shortcomings in current methodologies to evaluate risks on biodiversity in ERA of PPPs.

5.1 Approaches to method development to assess indirect effects of PPPs on individuals and populations

Are there any approaches to method development to assess the indirect impact of individual plant protection products on individuals or populations? If so, where are the difficulties or the development potential?

5.1.1 Inventory of approaches from authorities

The environmental protection authorities in Austria, Australia, Belgium, Brazil, Canada, Denmark, and the Netherlands reported that they are not aware of any initiatives that aims to develop methods to assess indirect effects on individuals or populations from PPPs. In Brazil, the focus is on developing risk assessment schemes with only one existing such established scheme, meaning that indirect effects are not covered. The Environmental Protection Agency in the USA mainly focus ERAs on direct effects, but evaluations of indirect effects are sometimes included for certain chemicals and in the assessment on threatened and endangered species. However, we have not found any evaluation of the methods used in US ERA to assess indirect effects of PPPs, and because of time constraints in this project, we have not compiled or mapped these methods, which occur in the current 170 guidance documents.

The German Federal Environment Agency (UBA) developed and introduced an assessment of indirect effects in their ERAs of PPPs in 2018, but this is no longer used due to a court judgement in 2019, stating that the assessment was not harmonized and approved by EFSA and therefore lacked legal basis (Koof, 2020). The concept used by UBA to regulate indirect effects of PPPs encompassed indirect effects on birds, non-target terrestrial plants, and non-target arthropods via trophic interactions in three steps. In the first step, the potential for indirect effects (based on e.g. effect rate (ER_{50}) on the primary producer level (in-field non-target terrestrial plants) and the first consumer level (in field non-target arthropods) of a PPP in a crop were assessed. If the first step showed a high potential for indirect effects, an assessment of the specific potential for indirect effects was required in a second step. In the third step, an assessment was done of whether the indirect effects via trophic interaction could lead to unacceptable effects on biodiversity. The methods have been practiced in a number of cases (Anonymous, 2020, 2019a, 2019b, 2019c) and is therefore an interesting approach. The method has been criticized for being vague and lack sufficient detail (Koof, 2020), but we found no scientific papers evaluating the method, and it was therefore beyond the scope of this report.

In the response of OECD, no additional initiatives were brought up, and EFSA mentioned the German approach. The main message is that the interest in the issue is considerable, but that there are very few approaches to actually assess indirect effects of PPPs on individuals and populations.

5.1.2 Literature review

We identified 124 papers that discussed indirect effects of single PPPs on individuals or populations *in a risk assessment context*. 56 papers dealt with development of risk assessment methods, and 37 papers discussed difficulties or potentials of methods for assessing indirect effects of PPPs on individuals or populations. Of the 56 papers that discussed method development, 14 papers concerned cosms of different sizes, three where field studies, nine were modelling studies, and six papers used combinations of methods.

A large share of the papers (21) were reviews, indicating a large scientific interest in the issues, but also suggesting that the empirical foundation is scant. Among the papers that did not fulfil our inclusion criteria, several treated indirect effects, or effects on biodiversity, but did not do so in the context of risk assessment methods. There may be studies that develop methods that could be suitable to use in risk assessment methods among these.

Studies of indirect effects can be performed in differently realistic settings, ranging from simple species interactions in laboratory-confined studies, over more or less complex model ecosystems (micro or mesocosms) to landscape-scale studies (see Rohr et al., 2016). We found only a few laboratory-scale studies investigating ERA methods for detecting indirect effects (e.g. Del Arco et al., 2015; Zubrod et al., 2011).

5.1.2.1 Semi-field studies

Semi-field studies, often performed as different forms of multispecies model ecosystems (cosms), are used in higher-tier ERA to refine sensitivity assessments. Such studies represent an intermediate scale, where additional complexity can be added compared to simple laboratory studies at the same time as environmental conditions can be better controlled than in full-scale field experiments, which increases precision and reproducibility (Macfadyen et al., 2014; Schäffer et al., 2008). Cosms range from miniature and highly controlled microcosms to large and more complex mesocosms, performed in laboratory or outside (Van den Brink et al., 2005). Among the papers in the literature review using cosms, most were studying aquatic systems (Hayasaka et al., 2019; Mikó et al., 2015; Müller et al., 2019; Riedl et al., 2018; van der Linden et al., 2019) or soil organisms (Macfadyen et al., 2014; Schäffer et al., 2008). If the scale of the cosm is large enough, they can also be used for studies of PPP effects on mobile aboveground invertebrates (Macfadyen et al., 2014), but for larger organisms they are not possible to design in ways that capture indirect effects of PPPs (Schmitt-Jansen et al., 2008).

Cosms most often include artificially constructed community compositions and rarely consider heterogeneous ecological contexts (Ittner et al., 2018). The studied communities can range from species poor to those that include complex food webs across several trophic levels (Brogan and Relyea, 2015; Leeuwangh et al., 1994; Riedl et al., 2018). The included species are sometimes reared but may also be collected from the field (Macfadyen et al., 2014).

In the cosm studies of this review, indirect effects were captured by measures of performance (e.g. biomass, biovolume) or proxies for fitness (e.g. abundance) as endpoints. Consumer-resource interactions was the most commonly studied type of species interaction but also competition was studied. Recovery effects that may interact with indirect effects can sometimes be captured in cosms, but not recovery of populations through dispersal from other habitats in a landscape (Beketov et al., 2008; Halstead et al., 2014; see also Rohr et al., 2016). The ability of mesocosm experiments to detect indirect effects depend on their design, with important issues being sufficient replication to generate enough statistical power and allowing sufficient time since indirect effects may take time to be realized (Müller et al., 2019). However, replication in mesocosm studies may generally be too poor to detect more than major effects (Macfadyen et al., 2014), and most studies were of relatively short duration, ranging from two months to a year.

Designing cosms for studies aiming at targeting indirect effects demands prior knowledge of community compositions and biotic interactions (Macfadyen et al., 2014). Cosm studies allow for experimental manipulation of communities that can help assessing the strength and mechanisms of indirect effects of PPPs (see Fleeger, 2020). It is also possible to study complex communities over several trophic levels in cosms. However, complex communities can be difficult to control and must sometimes be simplified in order to make coms studies feasible (Brogan and Relyea, 2015; Riedl et al., 2018). Furthermore, there is a need for at least some standardization of cosm studies to detect indirect effects, since results from such studies varies largely depending on abiotic, biotic and test conditions (Liebig et al., 2008). Cosms has been successfully used to detect indirect effects of PPPs on dynamics of protists and bacteria in the soil rhizosphere (Imparato et al., 2016), amphibians (Bulen and Distel, 2011), and non-target terrestrial plants (Damgaard et al., 2014), and are recommended by EFSAs scientific opinion to detect long-term and indirect effects on in-soil organisms (EFSA, 2017).

An important use of cosm studies is to confirm and refine the outcomes of laboratory studies (Haegerbaeumer et al., 2019; Leeuwangh et al., 1994), but they can also be used as a tool to inform how complex field studies should be designed (Liebig et al., 2008), or to generate data to inform mechanistic effect models (Riedl et al., 2018). Cosms can be used to extrapolate laboratory results to realistic field scenarios (Rose et al., 2016; Scholz-Starke et al., 2013; Stenrød et al., 2013) and assessments of community effects (Müller et al., 2019), but this is associated with a range of problems described in the following sections.

5.1.2.2 Field studies

Field studies are the only way to assess effects of PPPs on NTOs under fully realistic conditions, both in terms of ecological context and exposure conditions (EFSA, 2015). Targeting indirect effects of PPPs on NTO individuals or populations require knowledge of species interactions, which can be identified as emergent properties in field studies (Saaristo et al., 2018). Field studies including natural communities may reveal surprising effects due to species interactions, which sometimes are possible to subsequently study in more detail in cosm experiments (see Fleeger, 2020; and Rohr et al., 2016). Ideally, field studies are experimental such that exposure and potentially species communities are controlled or manipulated, such that hypotheses on indirect effects can be explicitly tested. However, also observational studies, for example based on monitoring are valuable, not the least because they can generate hypotheses about indirect effects and inform and validate other methodologies such as models or cosm-studies (Fischer et al., 2013).

Among the papers in the literature review, 20 papers that dealt with indirect effects in a risk assessment context used field studies alone or in combination with other methods. Eight of the 20 field studies on indirect effects of PPPs were focussing on birds, four non-target arthropods and aquatic communities, respectively, and three papers on non-target plants. Most of the papers studied consumer-resource interactions, while a few considered competition effects. Effects on abundance and reproduction were the most common outcomes. Of the 20 papers using field studies as study method, only four discussed development of ERA methods to assess indirect effects of PPPs on individuals or populations.

The need and feasibility of performing field studies to assess indirect effects depends on the type of NTO. For example, field studies even performed on small plots, could be used to show indirect effects of PPPs on habitat quality for mobile non-target arthropods, for example lack of food (EFSA, 2015) or non-target terrestrial plants (Isemer et al., 2020). For larger organisms such as birds and mammals, realistic studies in cosms are not practically possible and indirect effects can thus only be captured in field studies (Blus and Henny, 1997).

In field studies, a lot of variation in outcomes may be generated by shifting interaction strengths and varying environmental conditions, making it more difficult to detect casual relationships compared to highly controlled coms studies. However, given the large benefits of field studies, and that they sometimes may be the only way to collect relevant data (e.g. for mobile animals such as birds and mammals), there is a need to minimize their disadvantages, for example by using careful and elaborate designs (Blus and Henny, 1997), which could be inspired by the extensive use of mesocosm in other ecological fields. Field studies could be combined with other methodologies for better process understanding (Damgaard et al., 2014; see also Rohr et al., 2016).

5.1.2.3 Modelling studies

We[1] found 22 papers discussing indirect effects in the light of method development of ERA that used modelling as study method, alone or in combination with other methods. Of these, eleven were about aquatic communities and six about plants.

Risk analyses are fundamentally based on threshold or dose-response data on the effect of PPPs on organisms of different species, which in turn can be used to describe how sensitivity varies between species. Species Sensitivity Distributions (SSD) are widely used in ERA to derive predicted concentrations of chemical substances that are hazardous for 5% of the species (HC_5), which can be combined with assessment factors to account for higher sensitivity in species not directly studied (EFSA, 2016b). Importantly, indirect effects are currently not accounted for in SSDs, but assessment factors could in principle be used to in a generalized way compensate for ecological interactions not considered (Hommen et al., 2010). In general, the occurrence of biotic interactions results in stronger responses in relation to dose compared to estimates from laboratory single-species tests, but the effect size is suggested to be related to ecosystem complexity (Brock, 2013). However, there are approaches to develop SSD-models that account for biotic interactions in assessments of the responses of PPPs within a community, by focussing on how toxicity is modified by species interactions. For example, Baillard et al. (2020) assessed effects of interspecific competition among plants and how it impacted the species sensitivities to the herbicide isoproturon. However, this does not account for how direct effects modify competition and predation.

Community and food-web models can be used to account for indirect effects arising through competitive and trophic interactions between species. A large array of ecological models has been used in ecology to explore indirect effects (see Wootton, 1994 for general theories; and Rohr et al., 2006b for ecotoxicological applications). Rohr et al. (2006a) provides a review of the use of community ecology as a framework for understanding the impact of contaminants when species interacts and cases when this has been done using mathematical modelling. Models include ordinary differential equations such as Lotka-Volterra predator-prey models and models based on network theory (see Rohr et al., 2016). Such modelling requires knowledge on species richness, strength of species interactions, links among trophic levels and distributions. Rohr et al. (2006a) in particular point out Community Viability Analysis

[1] We focus on attempts to include indirect effects in risk modelling, but do not attempt to review approaches on how to in general translate toxicological studies to population effects.

(CVA) as a particularly promising modelling approach, but in our literature search we did not find examples of the use of this in an ERA context.

Mechanistic effect models (MEM) are processed based and focus on how patterns at higher level of organization emerge from processes at lower levels of organization (see Grimm and Martin, 2013). They are suggested to improve the understanding of complex biotic interactions and thus able to assess indirect effects (Forbes et al., 2017; Hommen et al., 2016a). They may also be used to identify to which parameters effects are most sensitive indicating the need of additional studies (Macneale et al., 2014; Reeg et al., 2018b).

MEMs such as toxicokinetic-toxicodynamic (TK-TD)-models, includes one part that describes the fate of PPPs within individuals (TK) and one that describes the response of organs and individuals (TD) (including Adverse Outcome Pathways (AOP) and bioenergetic models) (see Rohr et al., 2016; and EFSA, 2018). These models mechanistically link exposure and population effect and extrapolate findings to a range of conditions such as when exposure varies over time (EFSA, 2018), but also be used to generalize toxicological responses to unstudied species (Van den Brink, 2013). Models that predict community level effects by modelling the processes affecting individual species require a TK sub-model explicitly or implicitly (see Rohr et al., 2016).

MEMs can also be used to account for indirect effect, by explicitly including species interactions and their consequences for fitness of multiple species (Schmolke et al., 2017). MEMs can be on either individual-level, population-level, or for food-webs or ecosystems. A special class of MEMs is individual-based models (IBM), where processes are represented bottom-up by representing individual-level effects assessed in standard studies that are used to simulate emerging population and community level effects (see Rohr et al., 2016). For example, (Reeg et al., 2018a) used IBMs to evaluate consequences of herbicides for grass communities and found accordance with empirical data. IBMs can be combined with statistical or mechanistic toxicity modelling to add realism to the assessments (Hommen et al., 2016a). By being spatially explicit, IBMs can also account for processes such as herbicide drift (Reeg et al., 2017). However, they have been criticised for lack of predictive value because of the high number of parameters that needs to be assessed (see Rohr et al., 2016).

Assessing indirect effects on individuals or populations with models requires insights in species interactions, to make well-informed decisions on parameterization and assumptions (see Fleeger, 2020).

5.2 Studies assessing if current methodology is sufficient to address direct effects on biodiversity

Are there reliable studies on the extent to which current methodology is sufficient to assess the direct impact of individual plant protection products on biodiversity? If such studies exist, have shortcomings in the methodology been identified, are there suggestions for improvements?

The current guidance documents are not explicitly assessing effects of PPPs on biodiversity (EFSA, 2017). We found 69 papers that discussed method evaluation of current methods to assess direct effects of PPPs on biodiversity, but only six of them compared different methods and included some community aspects. We found no papers that compared how well different methods captured effects on biodiversity. Among the 69 papers that discussed evaluation of current methods used to assess direct effects of PPPs on biodiversity, 26 were about aquatic organisms. Nine of the papers focussed on soil organisms, seven papers on non-target terrestrial plants, and five papers on non-target arthropods. The remainder of the 69 papers were more general. Most of the papers that discussed method evaluation, included studies that measured taxa or species richness and abundance, sometimes combined in diversity indices such as Shannon's H'. Studies including phylogenetic richness or diversity were scarce. Few papers studied or discussed diversity partitioning, i.e., diversity within sites (α), between sites (β), and overall biodiversity (γ). We assessed that 98 papers identified shortcomings of current methodology of risk assessments in relation to impacts of PPPs on biodiversity. Furthermore, 119 papers suggested improvement of the current methodology.

Several papers suggest that the current ERA fails to protect biodiversity in agricultural landscapes due to inaccurate predictions of both exposure and effects, followed by inaccurate representation of ecosystems (Boutin et al., 2012; Brühl and Zaller, 2019; Castaño-Sánchez et al., 2020; Hendlin et al., 2020; Iorns, 2018; Ittner et al., 2018; Liess et al., 2019; Maloney, 2019; Mann et al., 2009; Sánchez-Bayo and Tennekes, 2020; Schäfer et al., 2019, 2019; Streissl et al., 2018; Thiour-Mauprivez et al., 2019; Uhl and Brühl, 2019; Vijver et al., 2017). A major criticism detailed in the former section, is that indirect effects are poorly or not at all covered by current methodology, with the consequence that it is not possible to evaluate the full effects of PPPs on NTOs (Prosser et al., 2016).

A common argument is that the ongoing reductions in biodiversity in agricultural landscapes is a showcase for the failure of ERA to safeguard biodiversity (Brühl and Zaller, 2019). While many papers suggest this, we have found few papers providing empirical evidence linking shortcomings of the current ERA methods to biodiversity decline. Some papers link the use of plant protection products to loss of biodiversity. For example, Beketov et al. (2013) found reductions of species and family richness among stream invertebrates on a regional scale and conclude that the current ERA is insufficient to protect regional biodiversity of stream vertebrates. Stanton et al. (2018) performed a systematic review and analysis of farmland bird populations in North America and identified PPPs as a main driver for 57 farmland bird species exhibiting population declines between year 1966 and 2013. Another bird study focussing on grassland species in North America, found that the insecticide acute toxicity was the best predictor of species declines between year 1980 and 2003 (Mineau and Whiteside, 2013). A pan-European study found negative effects on species diversity of birds, wild plants, and carabids in farmland by the use of insecticides and fungicides (however, this study was not captured by our literature search, Geiger et al., 2010). Scientific papers studying PPP effects on biodiversity of other organism groups are scarce. For example it is uncertain if the existing ERA is sufficient to assess effects of PPPs on soil biodiversity in the field (see Gestel et al., 2020). Other under-represented taxa are discussed in section 5.3.8.

More papers have evaluated the level of protection standard test methods offer compared to more complex methods (Brock, 2013; Brock et al., 2004; de Santo et al., 2019; Ernst et al., 2016; Haegerbaeumer et al., 2019; Naito et al., 2003; Reeg et al., 2018a). For example, one paper compared standard first-tier toxicity tests with SSDs for a wider array of species and the model ecosystem approach and found that the first-tier toxicity test was conservative for the studied species (Brock et al., 2004). However, these papers compare mainly individual species-based endpoints such as feeding activity or abundance, and do not assess biodiversity indices that takes relative abundance of species into account. Furthermore, an important finding is that predictions of PPP stress based on single species laboratory derived toxicity data may be conservative for community effects on low concentrations when feedback mechanisms are sufficient to dampen the negative effects. At higher concentrations, negative indirect effects on communities not covered by single-species laboratory tests may lead to underestimations. These relationships may be altered by the level of complexity of a study, and highlights the importance of complex study methods that includes both indirect effects and dampening ecological compensation mechanisms (Brock, 2013).

Effects of PPPs on *genetic diversity* between and within species are yet to be assessed in the current ERA (Brown et al., 2009; EFSA, 2016c).

5.3 Identified shortcomings and development potential of current methods

There is a lack of scientific evidence to show that current ERA methods are sufficient to assess the effects of PPPs on biodiversity, but a widespread view that the ongoing loss of biodiversity is partly linked to the inability of current risk assessment methods to correctly assess the risks PPPs expose to biodiversity. There is a mismatch between the highly controlled and relatively few methods used to assess risks of PPPs and the overwhelmingly variable and complex biodiversity of NTOs that constitutes the goal to be protected from unacceptable negative effects. Several of the shortcomings identified in the literature review originates from this problem. For example, common critiques against the current methods to assess effects of PPPs in the light of potential effects on biodiversity concerns lack of ecological realism, a too limited selection of test species included in the assessments, and lack of clear connections between endpoints measured in ERA and ecological endpoints wished to protect. Since many of the shortcomings and suggestions of improvement of current methodology are common for the assessments of indirect effects on individuals and populations and direct effects on biodiversity, we discuss them jointly below.

5.3.1 Increased mechanistic understanding

The net effects of PPPs may be captured by studying biodiversity endpoints in actual landscape settings, but such studies are difficult to perform. Furthermore, although direct effects of PPPs on biodiversity can be assessed without identification of the underlying processes, results from community level studies can be challenging to interpret without a mechanistic understanding of community processes (Relyea and Hoverman, 2006). As a result, studies on smaller scales are often used to extrapolated to larger scales. However, such upscaling requires mechanistic understanding of interactions between species in a community, as direct effects of PPPs on the behaviours or abundances of target organisms or NTOs can cascade into all trophic levels of a community and cause dramatic indirect effects on NTOs (Boutin et al., 2012; Saaristo et al., 2018). Challenges in upscaling means that the generality of results from cosm studies and their use as a predictor of biodiversity effects of a PPP can be questioned if information about interactions among species in their natural environment are lacking or not accounted for (Saaristo et al., 2018; Streissl et al., 2018). It may even be so that overlooked indirect effects in ERA is an important reason to the continued impacts of PPPs on biodiversity despite considerable efforts to assess and manage risks (Rohr et al., 2006a). The relative importance of indirect effects may even increase as direct effects are reduced by developments of PPPs and assessments (Rohr et al., 2006a). It is not until the underlying mechanisms are understood, that we can develop a general understanding of how PPPs impact biodiversity (Gibbons et al., 2015; Relyea and Hoverman, 2006) and thus, increase the ability to make reliable predictions in ERA of PPPs with sufficient accuracy and power (Bünemann et al., 2006; Forbes et al., 2017; Saaristo et al., 2018; Salminen and Sulkava, 1997).

Smaller scale studies in e.g. mesocosms may provide necessary process understanding that is needed for upscaling efforts, e.g. by using MEMs (see section 5.1.2.3 and 5.3.6). However, both direct effects and the indirect effects PPPs generate are species-specific, hampering the ability to generalize from simplified cosm tests (Saaristo et al., 2018). In studies of indirect effects, it is therefore necessary to include multiple species in environmental realistic settings, for example in mesocosms or field studies (Müller et al., 2019; Relyea and Hoverman, 2006; Saaristo et al., 2018). However, indirect effects in simplified environments may not reflect those in more complex environments (Brock, 2013), and given the large complexity of ecological interactions, the wide range of species interactions and stressors and use of PPPs, it is challenging to demonstrate casual relationships between single PPP-use and indirect effects on individuals and populations (Freemark, 1995; Relyea and Hoverman, 2006). Indirect effects may also be highly context dependent, because community composition and thus potential indirect effects and their interactions strengths differs between environments both in space and time (Saaristo et al., 2018; see also Fleeger, 2020).

It has been suggested that a way forward to understand and model indirect effects related to PPPs is to capitalize on the understanding developed in other ecological fields, that interaction strengths depend on the traits and densities of organisms (Relyea and Hoverman, 2006). Such a framework would allow the information from toxicity tests, cosm studies and observational field studies to be integrated in a framework that enables predictions of different PPPs across shifting ecological contexts.

5.3.2 Adding ecological realism to ERA

For several organism groups, risk assessments are based on investigations of basic toxicity assessed in single species tests under controlled conditions. However, the outcomes from such laboratory studies are difficult or even impossible to extrapolate to realistic community effects in field scenarios (Boutin et al., 2012; Bünemann et al., 2006; Filser et al., 2008; Schmitz et al., 2015), and has therefore been suggested to only be used with some reservation in ERA (Damgaard et al., 2008). Some scientists argue that to protect biodiversity, the assessment factors used to cover for uncertainties linked to the shortcomings of standard single species tests should be increased by a factor of at least an order of magnitude (Schäfer et al., 2019). The general opinion is however that assessment factors do not add realism to ERA and that uninformed use of them may result in conservatism and therefore unjustified rejections of PPPs (Van den Berg et al., 2020). Instead, ERA would become more realistic if ecological context and processes as well as agricultural management context were considered (Beketov et al., 2008; Didden and Römbke, 2001; Schäfer et al., 2019; Schmitz et al., 2014; Streissl et al., 2018). Thus, it seems that the use of assessment factors is poorly founded in explicit scientific understanding of consequences of upscaling.

Higher-tier studies aim to reduce uncertainties stemming from extrapolations by setting up more complex testing methods that adds ecological realism (Schmitz et al., 2015). However, the use of more complex ERAs has been criticised because it may result in lower reliability (Schäfer et al., 2019). Furthermore, while field studies are the only way to fully capture ecological complexity and variable environmental conditions, it may be difficult to identify the causal links between PPPs and ecosystem structure in such studies (Vijver et al., 2017).

Cosm studies are frequently used to assess the effect of PPPs on aquatic organisms. The methodology used in mesocosm studies is continuously refined in attempts to increase their ecological realism, for example concerning pulse exposure (Bayona et al., 2015), abiotic conditions (Wieczorek et al., 2016), and community representation (Bayona et al., 2015). Current ERA for non-target terrestrial plants include greenhouse studies of monocultures that fail to reproduce the natural conditions that non-target plants experience in agroecosystems where they are affected by intra- and interspecific competition, subject to herbivory and pathogens, and experience resource fluctuations and adverse environmental events (Boutin et al., 2019, 2012). However, there does exist relevant terrestrial mesocosm studies and even field experiments that have included species interactions (Baillard et al., 2020; Boutin et al., 2019; Damgaard et al., 2014, 2008; Isemer et al., 2020). Furthermore, in the scientific opinion for soil organisms EFSA (EFSA, 2017) proposes a range of new test strategies with the aim to increase ecological relevance, for example using field samples in micro- or mesocosm studies with entire soil microbial communities. For soil living enchytraeids, Römbke et al. (2017, 2009) proposes that semi-field and field studies are important to increase realism and validate the results of lower tier studies as well as informing the development of models but points out that there is a lack of formal standardization of existing approaches.

Species used in lower tier ERAs may differ systematically in their trait variability variance, e.g. because these species have a reduced genetic and consequently phenotypic variation (Brown et al., 2009). This may result in biased and sometimes underestimated risk assessments when extrapolating to consequences in the field (Saaristo et al., 2018). To increase realism in laboratory and cosm studies, it is therefore essential to ensure sufficient inter-individual variation to acknowledge plasticity in responses to PPPs (Saaristo et al., 2018). In addition, the use of a larger trait variation could contribute to increasing the mechanistic understanding of both direct and indirect effects (Saaristo et al., 2018). This is discussed also in section 5.3.7.

5.3.3 Interactions with other stressors

To accurately assess the risks PPPs exert on biodiversity in ERAs, they must account for how effects are modified by environmental conditions (Fischer et al., 2013). Current ERAs generally extrapolate effects of PPPs to explain wider community impacts on ecological endpoints without accounting for their interaction with other stressors (Bracewell et al., 2019; Filser et al., 2008; Fischer et al., 2013; Pesce et al., 2016). For example, ecotoxicity may depend on ambient temperature, resulting in larger effects on species richness, a relationship that may be particularly important in the light of climate change (Chakandinakira et al., 2019; Mann et al., 2009; van der Linden et al., 2019). The effect of stressors may be considerably modified by environmental conditions such modifications may vary between NTOs (e.g. Mann et al., 2009). Importantly, interactions among PPPs and other stressors may affect indirect effects (Rohr et al., 2006a).

5.3.4 Spatial and temporal scales

The ability of ERA to capture negative effects of PPPs on biodiversity would be improved by considering the multiple spatial and temporal scales that ecosystem processes occur at (Streissl et al., 2018; Trekels et al., 2011). The appropriate spatial scale at which direct effects of a PPP on biodiversity should be evaluated at depends on several factors. First, it depends on the spatial scale of the PPP exposure, including its spread in the landscape because of e.g. drift and runoff (Brittain et al., 2010). Secondly, it will depend on the mobility of the ecological entity of interest (e.g. population or community) with consideration of relevant spatial processes such as cross-habitat movements and dispersal, processes, which in turn depend on landscape characteristics such as habitat composition and configuration (Uhl and Brühl, 2019). This is because it is the relationship between the spatial scale of the exposure and the spatial extent of ecological processes that are affected that will determine the consequences of PPPs on biodiversity. What the appropriate scale to assess risks is will also depend on the magnitude, the duration and the mechanism by which a PPP exerts its effect, because this will affect both the area of biologically meaningful exposure and because it will affect the likelihood that population processes transcend habitat boundaries (EFSA, 2016c).

For relatively sedentary organisms, there may be limited need for evaluating effects of PPPs on large spatial scales. For example, in the scientific opinion for soil organisms, EFSA states that there is no need for studies at landscape scales for soil organisms because of their limited movement ranges (EFSA, 2017). However, for mobile organisms, that for example move between fields where the PPPs are applied and uncultivated edges, larger scale may need to be accounted for in risk assessments (EFSA, 2017). The reason is that without considering such larger scales, the effects of both short-term (e.g. avoidance behaviour) and long-term (e.g. dispersal) processes linked to movement may result in erroneous representation of exposures to PPPs and recovery, in particular for highly mobile or migrating taxa (Uhl and Brühl, 2019). Furthermore, the magnitude, duration, and the mechanism of the effect also influences the appropriate scale to assess risks of PPP impacts (EFSA, 2016c).

Current ERA primarily focusses on short term direct effects on individuals of single species studied in standardized laboratory tests (Jensen, 2019). However, effects of PPPs may only be apparent after a time-lag, such that the current focus may overlook long-term negative effects (Uhl and Brühl, 2019). Long-term chronic toxicity may not be well reflected by short-term effects (Bünemann et al., 2006), which may explain that long-term studies of ecosystems sometimes show unexpected effects from those expected from short-term studies (Jensen, 2019). It has been suggested that toxicological bioassays designed for detecting time-cumulative toxicity should be included in ERA to identify substances that rather than being dose-dependently toxic have effects dependent on time (Sánchez-Bayo and Tennekes, 2020). Consequences of changes in organisms' performance may not realize as population effects until after a time-delay (see Rohr et al., 2016), particularly in habitats other than the field where PPPs are applied. What the suitable temporal scales are depend on the ecology of the ecological entity and of the ecological context, such as life-history traits of an NTO or the ability of a landscape to promote recovery by dispersal from habitats not targeted by PPPs (EFSA, 2016c).

The spatial and temporal scales are particularly important when considering indirect effects. Indirect effects of PPPs caused by species interactions can occur spatially separated from where the PPPs were originally applied and only after a time-lag (EFSA, 2016c). This is obviously especially true for NTOs that largely reside outside fields where PPPs are applied but are indirectly affected. Thus, except from direct short-term effects on several species, effects of PPPs on biodiversity may not be captured in short-term studies (Bünemann et al., 2006; Müller et al., 2019; Rose et al., 2016). This poses a challenge to ERA, since few studies are sufficiently long-term to identify indirect effects (Bracewell et al., 2019). The time necessary for assessing risks of PPPs may sometimes be possible to realize in semi-field and field studies (e.g. soil dwelling Collembola (Ernst et al., 2016)). However, to capture relevant indirect effects, and thereby effects on biodiversity (Reeg et al., 2018b; Zhao et al., 2013), studies may need to continue for months to years (Saaristo et al., 2018). For example, mesocosm experiments on filamentous macroalgae and rooted macrophytes did not show misbalance in community structure until nine months after a single application of a fungicide (Müller et al., 2019). However, it may not be possible to perform studies on relevant timescales for longer-living life forms (EFSA, 2016c). Regarding spatial scale, relevant tests may not be possible to perform in cosms, which means that full-scale field-studies are required (Kattwinkel et al., 2015). It has been suggested that post-approval studies could be used to capture effects not possible to capture in pre-approval ERAs (Streissl et al., 2018).

Risks of PPPs are only assessed for specific levels of organisation and habitats corresponding to the PPPs intended use. For example, it is assumed that biodiversity in agricultural contexts in general is sufficiently safeguarded through the protection of populations of plants and invertebrates in edge-of field habitats, and populations of vertebrates in in-field and off-field habitats (EFSA, 2016c), and in the current ERA, only effects of PPPs on soil organisms in-field are assessed. However, effects can occur in fields where the PPP has been applied, but also in neighbouring fields, field borders, and potential off field which may be important for recovery via dispersal (Streissl et al., 2018). Another drawback of the current ERA is that it does not take into consideration that application of PPPs does not occur at a single event in one field, but on several occasions on larger spatial scales over landscapes and is repeated over the season and over years (Streissl et al., 2018; see also Topping et al., 2020).

5.3.5 Endpoints for indirect effects and effects on biodiversity

To assess the effects of PPPs on NTOs, assessment endpoints (what is to be protected) and measurement endpoints (measurable responses of a PPP by the protected unit) related to the specific protection goals are used, with specified attributes, units, and spatial and temporal scales of the protection (see Garcia-Alonso and Raybould, 2014; Rohr et al., 2016). Current ERA focusses mainly on single individual level endpoints, often related to mortality and sometimes abundance, growth, and reproduction. These endpoints can in themselves be important but are unlikely to capture all potential hazards that have implications for biodiversity, including indirect effects on NTOs (Pandey et al., 2017; Rose et al., 2016; Vijver et al., 2017). Population consequences may not be directly proportional to impacts on individual level endpoints (EFSA, 2016c; Forbes et al., 2017; see also Rohr et al., 2016). For example, toxicity tests generally do not consider variable ecological conditions or recovery, hampering the ability to make predictions from toxicity tests to population and community effects (Boutin et al., 2012; Reeg et al., 2018b). In other words, a fundamental challenge in the current ERA is to overcome the mismatch between the protection goals (e.g. biodiversity) and the measurement endpoints (e.g. individual mortality) (see Rohr et al., 2016).

It may be difficult to determine the causal relationships behind indirect effects of PPPs and NTOs, and negative effects may have to be identified in several steps, e.g. effects on food quality or quantity, condition or reproduction of the NTO, and then be linked to population declines (Boatman et al., 2004; Gibbons et al., 2015). To detect indirect effects, it is necessary to include functional and behavioural endpoints, but given the large variation of behaviours across species and related to species interactions, the understanding of mechanistic responses needs to be improved to define the ecologically relevant endpoints (Pandey et al., 2017; Pesce et al., 2016; Saaristo et al., 2018).

Indirect effect may result in compensatory changes in the abundance of organisms in response to population declines of organisms affected by PPPs, which may result in changed community composition without effects on composite abundance. For example, one field study showed that fungicide effects on earthworm diversity in agricultural soils were not reflected by effects on abundance and biomass because of compensation among species (Amossé et al., 2020). Such compensatory effects may be time delayed. For example, in a long-term mesocosm study, severe effects of neonicotinoids on the composition of functional groups apparent the first year decreased the second year, possibly because functional redundancy resulted in less sensitive species filling vacated niches (Hayasaka et al., 2019).

It is challenging to assess effects of PPPs on the general protection goal biodiversity. This is because there is no single measurable endpoint of biodiversity that is appropriate for all ecosystems, and the “normal operating range” differs between ecosystems (EFSA, 2017). It may also be challenging to interpret diversity related endpoints. They often show high variability (Knauer and Hommen, 2012), and nonlinear responses such that a slight toxic stress may increase species diversity if competitive pressures are released, while further increased chemical stress decrease diversity (“Intermediate Disturbance Hypothesis”) (Bünemann et al., 2006; Pandey et al., 2017).

Biodiversity indices capture different types of information and aspects of biodiversity. Hence, using single diversity indices does not capture all dimensions of biodiversity (Pandey et al., 2017). Some authors propose that to make comprehensive assessments of biodiversity, several indicators of biodiversity could be combined through multivariate analysis (Pandey et al., 2017). Further, combinations of structural and functional aspects of biodiversity as endpoints may improve the sensitivity of predictions in models (Pandey et al., 2017), and allow for separating direct and indirect effects causing altered community composition over time (Bayona et al., 2014).

There are plenty of suggestions in the scientific literature on how to improve endpoints for different organism groups. For plants, for example, abundance and timing of flowering and seed set are suggested to be measured to cover potential effects on biodiversity through indirect effects on pollen and nectar feeding organisms through the provision of pollen- and nectar when these organisms are active (Dupont et al., 2018) and on the soil seed bank which in many cases is the foundation for future biodiversity of wild plants (Boutin et al., 2019; Reeg et al., 2018b). We will not delve deeper into the specific suggestions for each organism group here.

Contemporary molecular approaches may become a tool in the future ERA (Pandey et al., 2017; Römbke et al., 2017). For example, to track effects of PPPs on soil microorganism community diversity and abundance, it has been suggested that specific exposure biomarker-based extraction of DNA or RNA and high-throughput sequencing may become valuable (Thiour-Mauprivez et al., 2019). For soil organisms, abundance is often the only measured endpoint at the community level due to difficulties to determine the taxonomy (Römbke et al., 2017). DNA barcoding and next generation sequencing techniques may simplify the otherwise difficult identification also of fungal species (Bünemann et al., 2006; Ittner et al., 2018), and for aquatic microorganisms these new techniques have the potential to make real-time assessments, identify species, community diversity, and reduce sampling efforts (Pandey et al., 2017). However, there are a few obstacles to overcome before these new techniques can be adopted in ERA, such as high costs linked to reagents and that the genetic reference libraries must be evaluated (Pandey et al., 2017).

5.3.6 Modelling biodiversity effects

ERA relies on empirical investigations of a limited number of species mostly in laboratory and mesocosm settings. Ecological modelling can be used in risk assessment to generalize these empirical findings across species and ecological contexts (Hommen et al., 2016a; Reeg et al., 2020; Whitfield-Aslund et al., 2017; see also Grimm and Martin, 2013). However, generalizations from empirical findings that allow predictions of consequences on whole communities, i.e., on biodiversity, is challenging for several reasons. First, there is a need for approaches able to translate the effect of tests in the laboratory or in mesocosms to consequences for populations in various ecological settings in the field. There is a large literature on this (see Galic et al., 2010; Grimm and Martin, 2013; Pastorok et al., 2003) which we do not cover here. Second, since it is impossible to include the majority of species in laboratory and mesocosm studies, there is a need for principles on how to generalize toxicity results from a few tested to a large number of untested species (EFSA, 2014b). Finally, there is a need to include the interactions between organisms that are differentially affected by PPPs. Such interactions may occur locally but may also be related to organism's movement in the landscape.

SSDs can be used to predict the Potential Affected Fraction (PAF) of a community (e.g. Giddings et al., 2019; He et al., 2019; Maloney, 2019). However, SSDs mainly rely on laboratory derived specific dose-related effects data from a limited number of common test species (EFSA, 2010; Larras et al., 2016), its generalization to natural communities with shifting ecological contexts is fraught with uncertainty (Maloney, 2019). Furthermore, when models subsequently are based on such restricted data, they will have an inherent uncertainty (He et al., 2019). To account for this uncertainty and for potentially higher sensitivity in unstudied NTOs, assessment factors are used in ERA. The assessment factor is higher for lower tiers and lower for higher tiers. This has been criticized because higher tiers often are more specific than lower tiers, and that there is no 'safety loop' that ensures that potential effects not covered by the more specific higher tiers are not missed (see Topping et al., 2020).

Although testing even a large fraction of NTOs for their sensitivity to multiple PPPs in different ecological contexts may seem like an unsurmountable task, it has been demonstrated that consistency of sensitivities across species make it possible to overcome some of the limitations in data availability by combining data across different active ingredients and species (Sala et al., 2012). Risk indices can be constructed by also weighing in the recovery potential of different species (e.g. Sala et al., 2012). Mechanistic approaches to generalize toxicological responses have been suggested. Ecological traits can be used to extrapolate toxicity data between species, including the use of phylogenetic approaches under the assumption that tolerances are governed by evolutionary processes and more similar among closely related species (Guénard et al., 2014). TK-TD-models which can mechanistically link exposure and population effect and extrapolate findings to a range of conditions such as when exposure varies over time, may also provide understanding of interspecific differences in tolerance (Van den Brink, 2013). However, both the statistical and mechanist approaches suffer from lack of ecological realism, since they do not account for context dependencies of toxicities including modifying consequences of indirect effect (He et al., 2019). Although there are attempts to understand how tolerances vary with both ecological context (Stampfli et al., 2014) and species interactions (e.g. Baillard et al., 2020), we have not seen them used in explicit risk analyses.

Apart from full-scale ecological field-studies, ecological models are most likely the only feasible way to estimate consequences of PPPs on biodiversity, since field-relevant variation in ecological context and a true representation of indirect effects is not feasible in laboratory and cosm studies (Reeg et al., 2020; Uhl and Brühl, 2019; Saaristo et al., 2018; see also Fleeger, 2020). A particular promise to link level of biological organization is held by MEMs, since they are process based and therefore can be used to predict effects under novel conditions, given that mechanisms are specified in a robust manner. Mechanistic models therefore considered to be a key tool for future ERA that has the potential to reduce the need for assessment factors - which have been criticised to their lack of realism - that currently are used to extrapolate single species effects to communities and ecosystems (Van den Berg et al., 2020). Multispecies MEMs can account for species interactions and thus indirect effects (Forbes et al., 2017). MEMs used in risk assessments of PPPs are usually IBMs, which by modelling individuals and their interaction with the abiotic and biotic environment, are able to predict emerging properties at the population and community level (see EFSA, 2014b; Rohr et al., 2016). They can use TK-TD models explicitly or implicitly as sub-models, allowing generalization of tolerance to untested species (see Rohr et al., 2016). Mechanistic models can be tailored to account for spatial processes, e.g. by including linked habitat models with multiple population models (Forbes et al., 2017). Given all these advantages, mechanistic models are a promising tool to use in future PPP risk assessments (Forbes et al., 2017; Hommen et al., 2016a).

However, mechanistic models are currently seldom used by risk assessors, and not implemented in the ERA protocols, due to uncertainties considering temporal scale of effects, extrapolations to untested species, populations, and communities, as well as other factors of uncertainty (Hommen et al., 2016a; Streissl et al., 2018). They have also been criticised for being too parameter rich to be of high predictive value (Rohr et al., 2016). Moreover, standardized operating protocols for input data to models dealing with indirect and overall biodiversity effects are lacking (Maloney, 2019), and there is a lack of standardised endpoints and statistics (He et al., 2019; Maloney, 2019). To implement mechanistic models in ERAs a number of challenges need to be solved.

Mechanistic models are only as good as are the process descriptions and data used. To correctly describe mechanisms underlying indirect effects and context dependence of effects, basic ecological knowledge is required, which however often is in scarce supply (Bracewell et al., 2019; Macneale et al., 2014; Uhl and Brühl, 2019). A main challenge for mechanistic effect modelling is to use representations of processes that are complex enough to represent relevant ecological complexity while still having manageable data requirements (EFSA, 2016a). Both model descriptions and parameter estimates contribute to uncertainty in mechanistic modelling, why there is a demand for frequent refining and evaluation (Bartell et al., 2018). An important challenge for ERA is to validate models, e.g. with empirical studies on community level, monitoring information at the ecosystem level or sensitivity analyses (Damgaard et al., 2014; Reeg et al., 2018b). Data from post-approval monitoring programs and field studies can be used to develop, adapt, calibrate and validate models (Schäfer, 2019). They also need to be well documented to be useful for risk managers and risk assessors and avoid exceedance of model capabilities (Reeg et al., 2020; Schmolke et al., 2017). One way to facilitate the use of modelling in ERA to assess effects of PPPs on biodiversity, would be to design a list of standard models or combinations of models along with generalized ecological scenarios that could be used (Brock, 2013; Hommen et al., 2016a, 2016b).

Spatially explicit multispecies models, i.e. landscape models, could be used to predict exposure and responses, and account for species interactions that shapes communities (see Pastorok et al., 2003). Such models that account for current use of PPPs, integrates farm practice data and other land-use data (e.g. satellite data) allows for simulations of landscape effects of PPPs (see EFSA, 2018; Topping et al., 2020).

5.3.7 Poor taxonomic coverage and simplified communities

Native species are seldom included in the current ERA and may need to be considered (Boutin et al., 2012; Iorns, 2018). The current focus in ERA on a limited number of species, results in that they do not sufficiently account for interspecific variation in responses among NTOs (Streissl et al., 2018) and fail to cover variation in community compositions among ecosystems (Filser et al., 2008; Höss et al., 2020). The first tier of ERA focusses on single surrogate species to extrapolate the toxicity of these, established under lab or field conditions, to general biodiversity effects (Saaristo et al., 2018). To this end, a restricted number of species are selected because they are easy to culture and study under laboratory conditions, and not because they are particularly representative of NTOs (see Rohr et al., 2016). This restrictive use of species in current ERA has been criticised (Gibbons et al., 2015), for not accurately representing the variability in sensibility to toxins among and within wild species (Beketov et al., 2008; Bünemann et al., 2006). Furthermore, it has been questioned how useful laboratory reared inbred organisms are in ERA of PPPs. While they have been shown to be conservative, there may be cases where they are insufficient to represent the full spectrum of genetic variation in wild populations (Brown et al., 2009).

Setting up experimental communities is a way to increase ecological realism while at the same time control community compositions such that too much variability is avoided to maintain higher reproducibility compared to a field study. To be able to perform multi-species cosm studies, experimental communities that can be reproduced must be constructed, which for example requires that different species are synchronous. As a result, it is a challenge to encompass the large variation of traits, including sensitivity to toxins, that exists in wild species (Mohr et al., 2012). For example, for non-target terrestrial plants, selection of species in a seed mixture can be logistically challenging, since they must match in sowing dates, be adapted to the climate conditions and be relevant for the tested PPP (Isemer et al., 2020). It can also be difficult to establish the intended composition in species; for example, when sowing seed mixtures with wild plant material in risk assessment the soil seedbank may outgrow the intended species (Isemer et al., 2020).

To overcome the difficulties to identify representative surrogate species and assessments of whole communities, attempts have been made to link susceptibility of PPPs and functional traits (e.g. dispersal capacity and generation time). Determining the role of species traits and taxonomic relatedness may allow for assessments of traits linked to sensitivity instead of species in a community. The Species at Risk (SPEAR) index has been developed for assessing effects of PPPs on NTOs based on ecological relevant traits (Liess et al., 2008; Liess and Beketov, 2011). Inclusion of ecological traits that determine the sensitivity of populations to PPP in combination with toxicity data in risk assessment models have been suggested for several organism groups (Van den Brink, 2008; Rubach et al., 2011; Uhl and Brühl, 2019; Boutin et al., 2012). Furthermore, accounting for traits and taxonomic relatedness may also allow for detecting regional differences in sensitivity of communities (Fischer et al., 2013; Liess and Beketov, 2011).

Functional traits may also allow for prediction of strength and direction of indirect effects arising from species interactions and may therefore be a way forward to mechanistically account for the effect of PPP on communities and biodiversity (Baert et al., 2017; Hashimoto et al., 2019; Liess et al., 2008; Liess and Beketov, 2011; Pandey et al., 2017; Van den Brink, 2008). For example, a species position in the food web, or how connected it is to ecological networks may affect the risk for indirect effects of PPPs (EFSA, 2016b).

An issue with current first tier assessments, is that they do not assess effects on the biodiversity of groups of organisms that are a priori not expected to be directly impacted. As a case in point, herbicides are assumed (but not shown) to not having any direct effect on birds or bees (Hendlin et al., 2020), and indirect effects of herbicides on birds or bees are likely since plants constitute the main forage for bees and several bird species (Brühl and Zaller, 2019); hence not evaluating the effect of herbicides on bees and birds may risk that important effects on biodiversity are not accounted for.

5.3.8 Under-represented groups

Aquatic organisms are among the most well covered organisms in ERA of PPPs. Still, several papers argue that the current ERA is not sufficient taxonomically broad to protect the biodiversity of aquatic organisms (Relyea and Hoverman, 2006). For example, aquatic fungi (Ittner et al., 2018; Zubrod et al., 2015) and marine organisms are largely overlooked even in the scientific literature of ecotoxicology (Relyea and Hoverman, 2006). Aquatic organisms were the most studied group within our review (48% of the papers concerned aquatic organisms), which may be because of a higher feasibility of studying aquatic systems in cosm studies.

For organisms such as mammals and birds, which often operate over large spatial scales, it is difficult to perform field studies that link biodiversity to the use of specific PPPs (Dittrich et al., 2019). In fact, we found only two studies assessing effects of PPPs on bird biodiversity in the light of risk assessments (Dittrich et al., 2019; George et al., 1995). Indirect effects on *birds and mammals* by effects on non-target arthropods are summarised in the EFSA scientific opinion for non-target arthropods (EFSA, 2015). We found no approaches to method development for assessing effects on mammal biodiversity (Freemark, 1995).

Amphibians are generally overlooked in ERA (Mann et al., 2009); only recently direct effects of PPPs on individuals and populations of amphibians and reptiles have been considered in ERA (EFSA, 2018). Scientific papers studying effects on biodiversity of amphibians and reptiles are lacking; no papers in this review assessed if current methodology is sufficient to protect amphibian diversity and only three discussed risk assessment methods in relation to indirect effects of PPPs on amphibians (Bulen and Distel, 2011; Mann et al., 2009).

For *non-target arthropods*, an ESCORT 3 workshop reviewed the current risk assessment as of 2010 and found that indirect effects on non-target arthropods by effects on non-target terrestrial plants should be assessed in the non-target terrestrial plants evaluation (EFSA, 2015). Despite improvements of ERA of bees through the new guidance document (EFSA, 2013b), neither effects on biodiversity nor indirect effects on individuals or populations are included in ERA for flower-visiting insects due to lack of scientific studies (Uhl and Brühl, 2019).

In an EFSA scientific opinion for *non-target terrestrial plants*, research needs related to indirect effects and biodiversity effects are specified, including e.g. accounting for long-term effects of repeated exposure on seed bank diversity (EFSA, 2014a). There are currently few approaches on higher-tier levels for non-target terrestrial plants (Schmitz et al., 2015). In a review of effects of spray-drift of glyphosate on non-target terrestrial plants, no assessment on nonvascular terrestrial plants (liverworts, hornworts, mosses) was found (Cederlund, 2017). Currently, risk assessments of PPPs seldom include perennial species, woody and fern species and rare species, and crop species are often used as proxies for wild species (Boutin et al., 2012).

The diversity of *soil organisms* is greater than that of any other organism group in environments directly affected by PPPs (EFSA, 2017; Imfeld and Vuilleumier, 2012). In current ERA, neither diversity aspects of soil communities nor indirect effects on individuals or populations are included (Römbke et al., 2017; Thiour-Mauprivez et al., 2019). However, a broad range of tests are developed to assess effects of PPPs on the abundance and diversity of soil organisms (Bünemann et al., 2006), and in the scientific opinion compiling the state of the art for in-soil organisms in preparation for updated guidance documents, methods for assessing diversity in higher tiers are proposed, and a systems approach including cosm , field and modelling studies including indirect effects (EFSA, 2017). See also van Gestel et al. (2020) for a detailed overview of soil organism risk assessments.

Non-target microorganisms, living in aquatic or terrestrial environments are in general largely overlooked in the current ERA of PPPs (Dimitrov et al., 2014; Schaeffer et al., 2017; Thiour-Mauprivez et al., 2019; see also Puglisi, 2012). Microorganisms constitutes the base of many food chains and are crucial for ecosystem functioning in soils, water, and even within other organisms, and are sensitive to PPPs. Microorganisms living in animal guts have been shown to be affected by PPPs. For example, PPPs has been shown to modify the microbiota in marine mussels and in honeybee guts, contributing to immune responses and pathological conditions (Iori et al., 2020; see also Alberoni et al., 2021).

Subterranean ecosystems may be affected by runoff of PPPs, but effects on their biodiversity are neglected in current ERA, despite that they can suffer from detrimental effects of PPPs (Castaño-Sánchez et al., 2020). Due to this, standardized testing protocols must be developed urgently. Bioassays for subterranean organisms are limited and ecotoxicological data to underpin assays are scarce and scattered, e.g. there is a major knowledge gap on their sensitivity to pollutants. Community-level effects of subterranean ecosystems likely differs from surface ecosystems, due to truncated trophic chains, small population sizes, slow life-history strategies, reduced possibilities for recolonization and recovery of their often endemic species. Thus, risk assessment standard procedures for surface ecosystems need to be modified to account for the specific traits of subterranean communities (Castaño-Sánchez et al., 2020).

5.4 Design of future methods to assess PPP effects on biodiversity

How would a method be designed to assess the impact of the use of individual plant protection products on biodiversity?

There is a lack of evidence to assess if current ERA methodology is sufficient to protect biodiversity, but there is plenty of circumstantial evidence suggesting that it is not sufficient (Beketov et al., 2013; Geiger et al., 2010). To approach the complex and daunting task of assessing the risks of the large number of PPPs on biodiversity, including indirect effects on individuals and populations, the above-mentioned shortcomings and challenges needs to be addressed by considering a number of the suggested developmental approaches we have found. In summary, the major challenges for future risk assessments are to consider the representativeness of evaluated species (Brown et al., 2009; Streissl et al., 2018), the context dependency of PPPs' impact on NTOs (Schäfer et al., 2019), the spatial and temporal scales that relevant ecological processes occur at (Streissl et al., 2018), and trustworthiness of links between measurement endpoints and the biodiversity aspects that are to be protected (see Rohr et al., 2016). Since pre-approval full-scale experiments covering a sufficient range of taxa may not be feasible, the most realistic way to meet this is by developing modelling approaches (see Topping et al., 2020). However, since models are generalizations of ecological findings, a combination of methods will be needed to assess effects of PPPs on biodiversity (Brock, 2013). Several papers also suggest that the current ERA should be complemented with new approaches, such as large-scale post-registration monitoring, pesticide vigilance, and supervised provisional authorization of PPPs (Brock, 2013; Schäfer et al., 2019; Streissl et al., 2018), to complete the current bottom-up oriented structure of ERA that extrapolates effects on very small scales and few organisms to general biodiversity effects across landscapes (e.g. Beketov and Liess, 2012).

The German approach to assess indirect effects of PPPs on birds is interesting, since it has been applied in practical risk assessment reports. However, the method has not been evaluated scientifically and we have not found the documents that has been used to underpin the method.

5.4.1 Combination of methods

To make accurate assessments that are sufficiently protective without being overly conservative, multiple lines of evidence need to be combined and weighed together (Brock, 2013; Liess et al., 2019). The inherent bias and advantages of the different methods currently used in ERA to assess effects on biodiversity implies the application of combination of techniques to give comprehensive pictures of PPP effects (Filser et al., 2008; Rose et al., 2016). Combinations of methods may also allow for assessments that balance sufficient realism and reduction of complexity (Relyea and Hoverman, 2006).

Laboratory methods can render basic toxicity data useful to develop predictions of how PPPs affect organisms (Relyea and Hoverman, 2006), but are difficult to extrapolate in space, time and across biological organisation levels. Laboratory studies combined with semi-field or field experiments as logistically possible, are valuable to reduce uncertainties, because they consider both direct toxicologically laboratory derived effects and contextualize them ecologically (e.g. de Santo et al., 2019). Combination of laboratory or modelling studies with field studies is also a promising alternative to identify mechanisms underlying indirect effects on populations and effects on biodiversity (Relyea and Hoverman, 2006; Vijver et al., 2017). However, field studies require extensive resources and are rare in ERA, especially those that account for processes occurring across habitats, on the landscape level. Modelling approaches can be used to predict general and long-term effects on communities on larger temporal and spatial scales under different scenarios (EFSA, 2017; Reeg et al., 2018a, 2017), but rely on outcomes from laboratory, semi-field, field, landscape-scale and monitoring data.

Modelling is a way to handle upscaling and generalize results among contexts, where process-based modelling and in particular individual-based models hold particular promise because of their ability to utilize process-understanding from smaller scale studies and predict consequences as emerging properties at higher levels of biological organization and novel conditions (see Rohr et al., 2016). Multi-species spatially explicit models are, in principle, able to account for indirect effects (see Pastorok et al., 2003) and thus predict consequences on biodiversity. However, it remains a large challenge to construct models which account for relevant ecological processes (which may be partly unknown at present) yet are feasible to use in a risk assessment context (see EFSA, 2014b). Expert knowledge elicitation techniques may be a way to handle some of these challenges (Schäfer, 2019; Streissl et al., 2018).

Parameterizing and validating models are remaining problems that may require extensive collection of data to solve. Higher tier studies should be used to validate and calibrate lower tiers, but until now, this has mostly been done for acute toxicity tests (mesocosms and laboratory studies) (Brock, 2013). There is a need for validation studies on long-term effects and effects on higher levels of biological organisation (see Rohr et al., 2016). Monitoring data on landscape scales (see 5.4.2) should be used to improve and validate models (Brock, 2013; EFSA, 2016a; Schäfer et al., 2019; Wendt-Rasch et al., 2014).

5.4.2 Post-approval assessments and monitoring

Given the large complexity of ecological systems, including that interactions among species and the environment are dynamic and varies in space and time, the full range of direct and indirect effects of PPPs is difficult to predict (Didden and Römbke, 2001; EFSA, 2016a; Forbes et al., 2017; Vijver et al., 2017). Large-scale post-registration monitoring that includes both chemical and ecological aspects could complement the ERA by providing a safety lock, and thereby protecting biodiversity from negative effects of PPPs (Schäfer et al., 2019; Vijver et al., 2017). Monitoring can be used to estimate the combined consequences of exposure and effects on population (Streissl et al., 2018), and thereby reveal underestimations (and overestimations) of actual risks for biodiversity (Vijver et al., 2017).

Large-scale post marketing frameworks are proposed to be developed based on existing (e.g. within the Water Framework Directive) and new monitoring systems for gathering of chemical and ecological data, to track long-term effects of PPPs under actual field conditions

(Brock, 2013; Bünemann et al., 2006; Schäfer et al., 2019; Streissl et al., 2018; Vijver et al., 2017; see also Gestel et al., 2020). However, post-registration monitoring require that landscapes and catchments are assigned for risk assessment, selected based on representativeness for a range of factors, and that farmers within these areas are sharing data on when, where, what and how much PPPs are used (Schäfer et al., 2019). Patterns of altered biodiversity captured by post-registration monitoring can be difficult to link to the use of a specific PPP, and it may be difficult to separate from effects of other stressors (Schäfer, 2019; Streissl et al., 2018). Post-registration monitoring is also being criticized because it only discovers negative effects when the damage has already occurred (see Topping et al., 2020). In other applied fields of ecology, the power of different evaluation techniques has been discussed, suggesting that designs that includes before-and-after measurements as well as randomized controls (BACI-designs) are much more powerful than less complex designs that either lacks pre-treatment data or use space-for-time substitution to generate quasi-experiments (see Christie et al., 2019; Mancini et al., 2020; Rundlöf et al., 2016). What is feasible in post marketing monitoring programs, will be a matter of what costs are regarded as legitimate. However, space-for-time substitution may offer a decent alternative if carefully designed (see Pickett, 1989). True BACI designs would be possible if higher tiers were complemented or replaced with supervised provisional authorisation of PPPs linked to risk assessment studies under real-world conditions prior to final approval (inspired by the regulations for pharmaceuticals) (Schäfer et al., 2019; see also Milner and Boyd, 2017). However, with good designs of monitoring programs in combination with landscape modelling, complex causality can be trustworthy examined for large scales (Streissl et al., 2018; Vijver et al., 2017).

6 Final remarks

The overall protection goal to preserve biodiversity and the ecosystem is often on population, community, or ecosystem level, while the current ERA is mainly based on data on toxicity of individuals of a few test species (Schmitz et al., 2015). Extrapolation of effects on the individual or even at the population level to effects on communities and biodiversity in general is daunting challenge (Forbes et al., 2017). In lieu of realistic settings under which biodiversity can be estimated as endpoints, interactions among species need to be addressed to be able to make accurate assessments of the effect PPPs has on biodiversity (Relyea and Hoverman, 2006; Streissl et al., 2018). Short-term laboratory studies may provide information on acute toxicity, while chronic toxicity, indirect effects, interaction with environmental conditions and in the end community effects requires other study methods that extend to more realistic scenarios that account for relevant spatial and temporal scales when upscaling both lethal and sublethal effects of PPPs. In particular, assessments of indirect effects of PPPs on communities require mechanistic understanding of species interactions. To assess such fully, a combination of methods is required. Field studies are valuable to pinpoint important species interactions and to account for ecological compensatory mechanism and indirect effects. These can inform and validate semi-field studies in e.g. cosms. Both semi-field and field studies are important to inform and validate models, which that are the most promising way to extrapolate individual level effects to realistic effects on higher orders of biological organisation based on mechanistic understanding, and thereby predict and assess effects of PPPs on biodiversity. Ecological monitoring linked to ERA is a promising proposal on how to verify that the future ERA methods are sufficient to safeguard biodiversity.

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8 Annex I. Inventory data

Letters were sent between 2020-09-10 and 2020-11-11 to:

Australia: Australian Pesticides and Veterinary Medicines Authority

Austria: Austrian Agency for Health and Food Safety

Belgium: Federal public service, Health, Food chain safety and Environment

Brazil: Brazilian Institute of Environment and Renewable Natural Resources

Canada: Pest Management Regulatory Agency, Health Canada.

Denmark: Ministry of Environment and Food of Denmark

France: the French Agency for Food, Environmental and Occupational Health & Safety

Germany: German Environment Agency

Japan: Ministry of the Environment

Netherlands: Board for the Authorisation of Plant Protection Products and Biocides

Norway: the Norwegian Scientific Committee for Food and Environment

United Kingdom: Health and Safety Executive

United States of America: US Environmental Protection Agency

EFSA

OECD

8.1 Letter to EFSA

Dear NN,

We are working on a report for the Swedish government to use for improving the ecological risk assessment for plant protection products in agriculture, with the long-term goal to reduce the negative effects of such products on biodiversity and the ecosystem.

One part of the report aims to inventory and describe existing initiatives to develop methods that assess the indirect impact of individual plant protection products on individuals or populations. We are writing to you to ask if you have information on the following questions:

- Can you suggest contact persons for us to approach among the EFSA-member states?

- Do you have suggestions of non-EFSA member organisations for us to contact?

- Are the current risk assessment programmes in the EFSA-member countries assessing indirect effects of plant protection products on individuals or populations?

- Are there any initiatives among the EFSA-members to develop methods to assess indirect effects on individuals or populations from plant protection products? If so, what are the difficulties and/or the development potential?

We would be very grateful if you have the opportunity to indicate if your organization have addressed this issue, and if there are any written reports that we may take part of.

We are happy to provide further information and look forward to your reply.

Yours sincerely,

Agr. Dr. Sandra Lindström with colleagues

Centre for Environmental and Climate Science

Lund University, Sweden.

https://www.cec.lu.se/

8.2 Letter to government officials

Dear NN,

We are working on a report for the Swedish government to use for improving the ecological risk assessment for plant protection products in agriculture, with the long-term goal to reduce the negative effects of such products on biodiversity and the ecosystem.

One part of the report aims to inventory and describe existing initiatives to develop methods that assess the indirect impact of individual plant protection products on individuals or populations. We are writing to you to ask if you have information on the following questions:

1. Who is the right contact person for us to approach?

2. Is the current risk assessment programme in your country assessing indirect effects of plant protection products on individuals or populations?

3. Are there any initiatives in your country to develop methods to assess indirect effects on individuals or populations from plant protection products?

4. If so, what are the difficulties and/or the development potential?

We would be very grateful if you have the opportunity to indicate if your organization have addressed this issue, and if there are any written reports that we may take part of.

We are happy to provide further information and look forward to your reply.

Yours sincerely,

Agr. Dr. Sandra Lindström with colleagues

Centre for Environmental and Climate Science

Lund University, Sweden.

https://www.cec.lu.se/

9 Annex II. Decision tree for the systematic map

Category	Sub-category	Value	Explanation
NTO		1 = yes, 0 = no	The study deals with non-human non-target organisms.
Single PPPs		1 = yes, 0 = no	The study is about single PPPs, not only mixtures.
Indirect effects on non-target individuals or populations OR direct effects on non-target biodiversity	Indirect effects on individuals or populations	1 = yes, 0 = no	The study deals with at least one method to assess indirect effects on non-target individuals or populations.
	Direct effects on biodiversity	1 = yes, 0 = no	The study deals with at least one method to assess direct effects on non-target biodiversity, but not only effects on individuals or populations.
	Sum of sub-categories	0,1,2	The study deals with either indirect effects on individuals or populations and/or direct effects on biodiversity.
Include full-text?		1 = yes, 0 = no	Articles need to comply with the two first and one of the sub-categories of the last criteria to be included.

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