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E-CHAPTER FROM THIS BOOK



Assessing the impact of pesticides on pollinators

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1 Introduction

In agriculture, pesticides are widely used to control hazardous pests, diseases, and weeds which would otherwise affect the health of crops and agricultural productivity (Popp et al., 2013). The need to control these factors varies across crops and regions, depending on pest pressure, crop variety and its tolerance to stress, and local agronomic and climatic conditions. However, where no or insufficient control is provided, yield losses of up to more than 80% may be the consequence (Oerke, 2006). On the other hand, pesticides are designed to control target populations of organisms which are damaging crops (e.g. insect pests, fungi, weeds) by killing individuals of these organisms or acting on their development or presence in a field. Due to these inherent properties, pesticides may have undesired side effects to the environment if they are not applied with the necessary caution.

The selectivity of pesticides to a group of target organisms is variable and depends on a number of factors such as mode of action, mechanisms of

uptake, metabolization, and administration. The inclusion of a large number of screening tests in the product development has overall improved the selectivity of pesticides over time. However, only very few pesticides are so selective that they inherently would only affect pest species and would not have the potential to harm non-target species taxonomically related to pests. Therefore, environmental safety requirements, along with regulatory systems to enforce them, have been established for the registration of pesticides to ensure that the use of all pesticide products in the field will not cause unacceptable effects to the environment. For this purpose, the use and commercialization of pesticides is subject to the most stringent regulatory systems which are globally in place for chemicals, which are defining principles and requirements of environmental risk assessment and management, and directions for the implementation of environmental risk assessment and management. One of the most debated and most intensively researched examples of non-target organisms and their protection from unwanted pesticide side effects in this regulatory context are bees and other pollinators.

The reasons for this are diverse: first, bees are important pollinators and, as an organism group exhibiting mutually beneficial trophic interactions with crops, an essential production factor in agriculture (e.g. IPBES, 2016). Therefore, growers have a genuine interest in having them protected from possible pesticide side effects. Second, honey bees are managed as livestock, especially in regions like Europe and North America (e.g. Pirk et al., 2017), and for managed colonies, there is a beekeeper attending them who is concerned about their health and safety, which fostered the specific attention of pesticide safety to bees earlier than for many other organisms of the environment. Finally, honey bees can be readily bred and managed, and there is long-standing experience with handling their colonies (van Engelsdorp and Meixner, 2010). As such, they could be used for tests under semi-natural conditions from the beginning of ecotoxicological research, without having to upfront develop techniques for breeding and handling.

These specific features ensure a special position of the honey bee in risk assessment systems. Whereas most other standard testing organisms in risk assessment have from the beginning been established as surrogate species, representing a more or less broad spectrum of other species that could not be directly tested, the honey bee was, at least initially, considered as a standalone species, representing in the first place itself rather than other taxa. Although in the meantime honey bees are debated as surrogate organisms for other pollinators, especially solitary bees and bumblebees (e.g. European Food Safety Authority, 2013a, United States Environmental Protection Agency et al., 2014), this unique feature still involves specificities in the risk assessment (e.g. the Hazard Quotient), which will be discussed in more detail in subsequent sections.

2 History of bee testing and risk assessment

The first steps to investigate the side effects of pesticides on bees started early in the history of ecotoxicology. The historic development of bee testing and risk assessment in Germany and in the European Union, which is well-documented, serves as a case study, yet the prerequisites and processes which governed these developments were basically comparable in other countries.

When the use of pesticides became prevalent in agriculture in the early twentieth century, many of the products used to control insect pests contained highly toxic substances like arsenic or lead. Their application in flowering crops led in some cases to bee kills, and subsequently to complaints from beekeepers, which prompted authorities to develop solutions for this issue. As an example, in Germany, a Testing Center for Pesticides ('Prüfstelle für Pflanzenschutzmittel') at the Imperial Institution for Biology (Biologische Reichsanstalt) was established in 1920, which dealt with the assessment of pesticide side effects to bees, initially on the basis of simple oral, and later also contact acute tests. At this time, a specific registration process for pesticides with integral environmental safety assessment was not in place and thus bee testing was not mandatory. The Testing Center issued, based on their findings, recommendations for risk mitigation measures to protect bees (Brasse, 2007).

Later, testing approaches became more sophisticated, and tent tests with bee colonies were used in safety assessments (e.g. Finkenbrink, 1940). The 1950s saw an increasing need to further optimize the approaches to protect bees from potential pesticide side effects, and, in this context, to generate accurate figures describing intrinsic bee toxicity. In 1956, the International Commission on Bee Botany organized a meeting in Bern (Switzerland) on the theme of the protection of bees from pesticide effects (Pettinga, 1980), which triggered further work and follow-up consultations and congresses by various organizations to further elaborate approaches for testing solutions. At the same time, approaches to systematically compile methods to analyze pesticide residues in dead bees were initiated (Stute, 1956).

In the 1970s, the progressive development of testing methods triggered the need for method harmonization between European countries and research organizations. The Symposium on the Harmonization of Methods for Testing the Toxicity of Pesticides to Bees took place in 1980 in Wageningen (The Netherlands) (Pettinga, 1980, Stevenson, 1980). Further international consultations on the topic followed, and a few years later, Oomen (1986) published a sequential scheme for evaluating the hazard of pesticides to bees. On the basis of the fundamentals elaborated during these exchanges, the European and Mediterranean Plant Protection Organization issued harmonized testing guidelines (EPPO, 1992) and a risk evaluation scheme (EPPO, 1993) for honey bees in the early 1990s. The EPPO Guidelines and Schemes have been subject to revisions since that time,

reflecting scientific knowledge and experience gained from their use. The latest version was issued in 2010 (EPPO, 2010a,b). The EPPO Risk Assessment Scheme was officially withdrawn in 2018 (European and Mediterranean Plant Protection Organization, 2021) but is effectively still in use in the European Union.

In 2012, the European Food Safety Authority took over the review of risk assessment guidance for bees and proposed a new Guidance Document (EFSA, 2013a). The document expands the risk assessment to bumble bees and solitary bees but so far could not be fully implemented, among other reasons because of the lack of complete testing schemes for these groups and the lack of methods to experimentally measure the protection goals proposed in the document. A revised version has been prepared by EFSA (EFSA, 2019) and is up for public commenting (EFSA, 2019).

3 Testing pesticide effects to pollinators

In order to evaluate the risk of a pesticide to pollinators, one of the essential steps is determining the intrinsic toxicity of the pesticide to the pollinator. In regulatory ecotoxicology, this is done by means of an elaborate system of testing methods and approaches, which are described in the following.

3.1 Test species

Assessing the effects of chemicals on non-target species populations and communities is not a simple task. Evaluating effects on the environment, biodiversity and ecosystems must concede that testing every species in any type of ecosystem is impossible. Thus, risk assessments, and particularly regulatory risk assessments that support regulatory decision making, typically rely on a set of standardized and reliable data aimed at providing a representative indication of a chemical's potential effects on the environment, with a high level of certainty.

In this context, standardized ecotoxicity testing has seen an increased development in the last decades, offering a range of testing methods on standard species that provide a sensitive assessment of the intrinsic toxicity of chemicals, in a reproducible and robust way (OECD, 2021a).

For pollinators, most regulatory studies are performed on the honey bee, *Apis mellifera*. The choice of the honey bee as a standard or surrogate species in the risk assessment of chemicals has been extensively discussed (e.g. Boyle et al., 2018, Hinarejos et al., 2018) and is based on several rationales as follows:

- Historically, honeybees appear to be the first non-target insect species farmers and beekeepers were adamant to protect in a crop, because of its role in pollination and the provision of hive products such as honey.

- Non-intentional effects of pesticides on honeybees have hence been studied since the early twentieth century (e.g. Brasse, 2007, Palmer-Jones, 1958, Everts, 1990) even before testing methods on non-target arthropods used in biocontrol were developed (Barrett et al., 1994).
- Testing methods could be rapidly developed, as honeybees are easy to raise and maintain under human management, and well suitable for the development of accurate, reproducible, and measurable exposure dosing, such as controlled oral administration and topical application of a test item in the laboratory.
- Honey bee behavior enabled testing under confined laboratory conditions, in tunnels in the field, and in the open field, allowing testing of effects in a broad range of scenarios and testing tiers from the laboratory at an individual level to the field scale at the colony level.
- Individual bees can learn to respond to a stimulus and thus testing can involve behavioral effects such as foraging behavior (OECD, 2021c, Decourtye et al., 2005) or learning capacity.
- The biology of the honey bee is well described which allows for an easy interpretation of testing results.
- Ecotoxicity data indicate that the honey bee is usually an inherently sensitive species to pesticides, and thus a good indicator of pesticide intrinsic toxicity. Exceptions exist that usually relate to differences in body weight and/or differences in the metabolic pathways that degrade pesticides (Arena and Sgolastra, 2014, Pamminer, 2021).

Differences in biology and behavior between bee species may greatly influence their factual sensitivities to pesticides under field conditions, which has stimulated the development of new testing methods on bee species other than the honey bee. To date, acute oral and contact toxicity testing guidelines have been developed for bumblebees *Bombus terrestris* (OECD, 2017b,c). Method development is ongoing for a chronic bumblebee laboratory test (Exeler et al., 2020), an acute contact (Roessink et al., 2018) and oral (Hodapp and Kimmel, 2018, Roessink et al., 2018, 2020) laboratory test with the mason bee (*Osmia* spp.), a larval laboratory test with *Osmia cornuta* (Exeler and Quambusch, 2020), and an acute laboratory contact test (Nocelli et al., 2020) as well as a larval laboratory test (Rosa-Fontana et al., 2020) with stingless bees (Meliponini). Moreover, higher-tier testing methods are under development for bumble bees and solitary bees (Cabrera et al., 2015, Knaebe et al., 2018, Franke et al., 2020).

Overall, the robustness of a risk assessment to pollinators relies on the possibility to assess whether the effects that were observed in laboratory studies for a tested species will express under higher-tier testing conditions reproducing the conditions of exposure expected after the intended use,

addressing the uncertainties identified in lower-tier studies, and enabling extrapolation to other (untested) species. While ideally complete testing schemes for each species could reduce uncertainties in the risk assessment and adapt the testing requirements to the most representative pollinator of a cultivated crop, alternative ways to perform the risk assessment are necessary in real life, since testing each possibly exposed species is impossible in practice. A possible way forward is to design risk assessment scenarios according to the concept of 'focal species', based on the model developed for example in the risk assessment performed for birds and mammals (European Food Safety Authority, 2009). In such systems, effect testing is performed through methods developed on two standard species of different bird groups. The risks to the bird species expected to use a specific crop as a habitat and thus being the most likely to be exposed are evaluated through exposure scenario and biological traits defined for those species, to account for differences in traits, diet, and behavior. The possibilities to develop such a system for pollinators were discussed in a dedicated workshop, and recommendations were published by Boyle et al. (2018).

3.2 Testing methodologies and designs

Ecotoxicological tests with bees expose bees or bee colonies to a pesticide in a standardized and defined way and include regular observations to evaluate whether effects are induced and which effect the tested substance may have on the test organisms. All testing types include an untreated control group, one or several treatment groups which mostly evaluate different test concentrations, and a toxic standard group with a substance that causes known, defined, and reproducible effects to the test organisms. Only in open field tests, a toxic standard is usually not applied to avoid the deliberate release of toxic concentrations of pesticides into the environment (see e.g. Lewis et al., 2001, 2009). Mostly, tests are conducted in a replicated design (i.e. each treatment group is run in a number of replicates) to allow for statistical evaluation.

For all tests, there are defined parameters to be evaluated, which may be affected by the test substance. Typical parameters measured in laboratory tests are mortality, food uptake, and behavior. In more complex tests involving entire honey bee colonies, other parameters such as flight and foraging activity, colony strength, hive weight, nectar and pollen storage, breeding activity and hatching success, or even overwintering success can additionally be tested. The more complex a test design is, the more parameters can generally be assessed. The values of these parameters measured for the test substance are subsequently evaluated by comparison with the data from the control and the toxic standard groups. From those measured parameters and their comparative evaluation, a study endpoint is derived (for a more detailed definition, see e.g. United States

Environmental Protection Agency, 2003). Each test type has a defined endpoint, e.g. an LD50 (concentration that is lethal for 50% of the test organisms), a Non-Observable Effect Concentration (NOEC) (concentration that causes no observable effects), an NOED (individual dose that causes no observable effects), or an NOER (application rate that causes no observable effects).

Most risk evaluation systems are based on studies of different complexity. Studies of lower complexity are characterized by a simplified design, whereas the more complex studies reflect more realistic exposure scenarios. These different levels of complexity are referred to as study 'tiers'. Lower-tier studies are mostly conducted in the laboratory under artificial exposure conditions, and, in the case of honeybees, usually with individual worker bees. By their simplistic design, they exclude as far as possible factors that may influence exposure and therefore generate variability in the results. These studies focus on the test organism and its intrinsic biological traits, and on the test substance, and are typically well standardized. Lower-tier studies have the advantage that they can usually be conducted relatively easily and frequently without great efforts and that their interpretation is accordingly straightforward.

Higher-tier studies, in contrast, are typically exposing bees under more realistic conditions in the semi-field or field, in the case of honeybees with entire colonies. They assess the effects of a substance in a field-relevant scenario, considering additional relevant factors other than the test organism and the substance. The bees are typically exposed to the test substance through matrices that are exposed to the treatment, such as flowers, nectar, or pollen. However, in certain study types, e.g. colony feeding studies, artificial standard exposure designs can be employed in order to achieve controlled exposure conditions (e.g. Oomen et al. 1992).

In honey bee risk assessment, the following study tiers are distinguished (United States Environmental Protection Agency, 2021a):

- 1 Tier 1: laboratory testing (individual bees tested, simplistic design, artificial exposure).
- 2 Tier 2: cage studies, semi-field and colony feeding studies (bee colonies tested in a compartment (tunnel/tent) and exposed to a realistically treated crop, or free-flying colonies exposed to an artificially spiked diet).
- 3 Tier 3: field testing (free-flying bee colonies exposed to realistically treated crops in a controlled design).
- 4 Tier 4: post-registration field monitoring studies, involving observations under practical use conditions without a controlled design.

The progression across the tiers from 1 to 3 in a risk assessment is illustrated in Fig. 1.

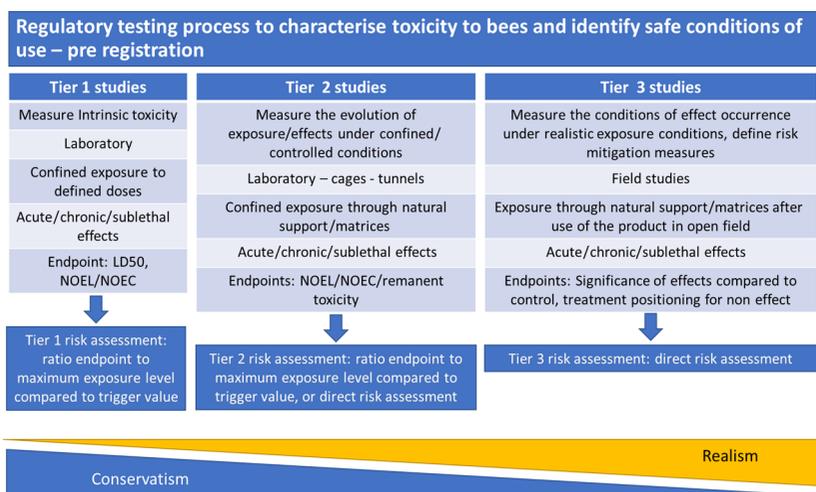


Figure 1 Illustration of the organization of studies in a tiered risk assessment.

A broad spectrum of different study types has been developed, which are testing different endpoints at different testing tiers. Some important study types for honey bee and bumblebee tests are listed in Table 1.

3.3 Testing guidelines and risk assessment guidance documents

In order to generate test results that are comparable to each other and with benchmarks relevant to the safety assessment, ecotoxicological tests have to be conducted in a standardized way, according to defined testing guidelines. A testing guideline is a detailed, officially approved method description for a test procedure. The degree of detail with which a guideline stipulates test design and procedures depends on type and complexity of the test; the simpler the test setup (e.g. laboratory tests), the more detailed and prescriptive the guideline can be, whereas in more complex setups (e.g. field testing), more flexibility is needed to adjust the design for specificities of the crop, the tested product, and prevailing environmental conditions.

The studies to be used for the pesticide safety assessment usually have to follow an officially recognized guideline which sets out the test design and procedure, but also the prerequisites under which a test and its results can be considered valid (so-called Validity Criteria). Guidelines are issued or approved by international organizations, for example, the Organisation for Economic Co-operation and Development (OECD) or the European and Mediterranean Plant Protection Organization (EPPO), or authorities (e.g. United States Environmental Protection Agency). Some important testing guidelines

Table 1 Some important study types and the guidelines according to which they are conducted

Study Type	Guideline	Source
Tier 1		
Acute oral toxicity (honey bee adult) (Fig. 2)	OECD 213	OECD (1998a)
Acute contact toxicity (honey bee adult) (Fig. 2)	OECD 214	OECD (1998b)
	OCSPP 850.3020 (USA)	United States Environmental Protection Agency (2012a)
Acute toxicity/single exposure (honey bee larvae) (Fig. 3)	OECD 237	OECD (2013)
Chronic oral toxicity (honey bee adult)	OECD 245	OECD (2017a)
Chronic toxicity/repeated exposure (honey bee larvae)	OECD GD 239	OECD (2021b)
Acute oral toxicity (bumblebee adult)	OECD 247	OECD (2017c)
Acute contact toxicity (bumblebee adult)	OECD 246	OECD (2017b)
Toxicity of foliar residues (honey bees)	OCSPP 850.30309 (USA)	United States Environmental Protection Agency (2012b)
Tier 2		
Semi-field (tunnel or tent) testing (Figs. 4–6)	EPPO 170	European and Mediterranean Plant Protection Organization (2010a)
	CEB 230 (France)	Végéphyll (2013)
	EFSA Guidance Document (European Union) ^a	European Food Safety Authority (2013a)
Semi-field (tunnel) brood testing	OECD GD 75	OECD (2014)
Brood feeding test	No officially validated guideline	Oomen et al. (1992); Lückmann and Schmitzer (2019)
Colony feeding test	No officially validated guideline	Overmyer et al. (2018), Thompson et al. (2019)
Tier 3		
Field testing (Fig. 7)	EPPO 170	European and Mediterranean Plant Protection Organization (2010a)
	OCSPP 850.3040	United States Environmental Protection Agency (2012c)
	EFSA Guidance Document (European Union) ^a	European Food Safety Authority (2013a)
Tier 4		
Field monitoring	No officially validated guideline	

^a Draft guidelines for semi-field and field testing.



Figure 2 Acute laboratory test with adult honey bees according to OECD 213/214.

are compiled in Table 1. Besides internationally accepted testing guidelines, there are also some specific guidelines on a national level in certain countries (e.g. the Foliar Residues Test according to OCSPP 850.3030 (USA, Brazil, South Korea) or the semi-field test according to CEB 230 (France)).

3.4 Test method development and validation

For a test design to be useful in risk assessment, it must be capable of yielding reproducible and consistent results. This means that wherever and by whomever a study of a certain type with given parameters like test substance and test concentrations is conducted, it must be assured that the results are always the same or at least do not exceed a defined range of variation (e.g. a certain maximum control mortality). This sounds trivial but is not. Experience has shown that it is relatively straightforward for a testing laboratory to implement and successfully apply a new testing design; however, using a new method in a way that ensures results consistent with those of other testing facilities is much more difficult, and in most cases, it has taken years for experienced laboratories to implement new methods according to consistent standards. This is because even small details in the test design (e.g. air humidity, light regimen, food composition, etc.), the relevance of which may not be initially evident, can have a substantial influence on the results of a study. In order to address this, new



Figure 3 Honey bee larval laboratory test according to OECD 237.



Figure 4 Semi-field (tunnel) test with honey bees in oilseed rape according to EPPO 170.



Figure 5 Semi-field (tunnel) test with honey bees in phacelia according to EPPO 170.



Figure 6 Application in a semi-field (tunnel) test with honey bees in phacelia according to EPPO 170.



Figure 7 Field test with honey bees in oilseed rape according to EPPO 170.

testing guidelines normally go through a validation process called ring testing (OECD, 2005) before they can be finalized and officially endorsed. Ring testing is a stepwise process where different testing facilities apply a new test design in a coordinated way in parallel and compare the results. When the results between the different laboratories are not consistent, parameters are identified which may be causing this variability, and eliminated to harmonize the design. This is done iteratively until consistently reproducible results can be produced by all participating testing units. Considering the importance of validation for the generation of significant results, risk assessment should always be based on validated test methods.

3.5 Good Laboratory Practice compliance ensuring quality standards for regulatory studies

To ensure that safety studies are conducted according to globally comparable quality standards and to ascertain that study procedures and study results are clearly traceable and reproducible, the principles of Good Laboratory Practice (GLP) have been developed and established as a framework for conducting and reporting studies. The principles of GLP set out clear regulations for responsibilities and documentation in the course of study conduction and evaluation. Adherence to the GLP regulation prevents unreproducible study

results and precludes data falsification and fraud. The principles of GLP were established in the 1970s and adopted in many countries around the world in the 1980s and 1990s under the auspices of OECD (OECD, 1998c, 2021d). Pesticide studies for regulatory purposes must be conducted under GLP in most countries.

4 Ecotoxicological risk assessment for pollinators

Once the intrinsic toxicity of a pesticide to a pollinator has been determined, the risk posed to the pollinator by the use of the product can be determined by respective risk assessment approaches. One of the basic features of these risk assessment approaches is that it compares effect data and exposure data (see e.g. European Food Safety Authority, 2012). This step is essential, since not only the intrinsic toxicity of a substance but also the exposure to the substance drive the risk. In the risk assessment, both parameters are brought together and integrated.

4.1 Fundamental principles of risk assessment

In the registration process for pesticides, studies that have been conducted to assess the toxicity of a product are followed by procedures that assess the product for its risk to pollinators. Risk assessment is based on some fundamental principles and follows respective rules and procedures which have been stipulated by the legislator. Principles, details, and backgrounds of the most important risk assessment approaches are outlined in the following sections.

4.1.1 Protection goals

Protection goals are the benchmarks used to interpret the outcome of a risk assessment for regulatory decision-making purposes.

In the pesticide regulatory process of the European Union (European Parliament and Council of the European Union 2009, Article 4 (3)(e)), uniform principles for the placing of pesticides on the market are defined. They stipulate that 'a pesticide must not have unacceptable effects on the environment, and no impact on biodiversity and ecosystems'. The compliance of a pesticide to these protection goals is verified by the risk assessment, according to dedicated guidance documents. The data needed to perform a risk assessment are also listed as regulatory data requirements (European Commission, 2013a,b) in the regulation itself. Adherence to this list is part of a completeness check process for the active substance, its metabolites or degradation products and the

formulated product.¹ A risk assessment is performed for each anticipated use of a pesticide and thus for each use, the outcome of the risk assessment is compared to 'Specific Protection Goals'.

Specific Protection Goals for pollinators are defined to ensure a high level of protection for each intended use. More specifically, they describe what to protect, where to protect it, and the timescale over which transient effects might be tolerated without affecting the protection goal, where relevant.

In the European Union, the Specific Protection Goals developed for pollinators are based on ecosystem services that need to be preserved in cultivated areas, such as pollination, the production of hive products (for honeybees only), and biodiversity (specifically addressed under genetic resources and cultural services). This approach was also proposed by experts of the Pellston Workshop on the Risk Assessment to Pollinators in North America (Fischer and Moriarty, 2014), and in most regions globally (e.g. APVMA, 2017, Cham et al., 2017, United States Environmental Protection Agency et al., 2014).

Protection goals valid for pollinators (and other insects) always relate to entities like the colony (in the case of honeybees), or the population (in the case of solitary bees), or to services like pollination, but never to the individual. This means that the individual bee is not the target of the protection goals, and effects on individual bees can be considered acceptable, as long as the population, the colony, or relevant functional endpoints are not affected. This is because organisms like insects are characterized by an intrinsically high loss rate (and colonies by an intrinsically high turnover of individuals), therefore effects on individuals will not necessarily result in biologically relevant effects at the population or colony level.

In the European Union, the European Food Safety Authority (EFSA) defines the Specific Protection Goals attributes, such as survival and development of honey bee colonies and effects on larvae and bee behavior to preserve those ecosystem services (European Food Safety Authority Scientific Committee, 2016).

For those attributes, experts have derived the magnitude of effects that must be met for the ecosystem services to be protected and thus for the risks to be acceptable. Effects can e.g. be defined as a percentage of colony size affected (e.g. 10%, 15%, or 20%). Exposure also enters in the Specific Protection Goals definition, through a given percentage of colonies located at the edge of a field whose exposure is to remain below the levels of exposure calculated in the risk assessment.² Specific Protection Goals can be defined for other groups such as solitary bees and can be similar to that for the honey bee or adapted to their particular conditions.

¹ A dossier typically counts ca 450-500 studies, 150 of which are ecotoxicological studies.

² Specific Protection Goals were, for example, set to 7% colony size, and 90th of colonies at the edge of a field would be exposed to lower levels than the calculated exposure levels for a particular use.

An important criterion in defining Specific Protection Goals and in particular the magnitude of effects to be used is the distinction of effects induced by the exposure to a pesticide from other effects and thus to be able to measure the compliance of a pesticide use to the Specific Protection Goal in situ/in the field. While Specific Protection Goals can be directly assessed in a field study, it may be necessary to define benchmark, or trigger values, to be used when the risk assessment relies on laboratory studies and on a comparison of ecotoxicological reference values derived in laboratory studies to calculated or expected exposure levels. Those benchmark or trigger values are then calibrated based on the magnitude of effect for acceptable risks. These benchmark or trigger values become an indicator that the acceptability criteria of the regulation are met on the basis of the outcome of a screening risk assessment, relying on screening data, and a high level of conservatism in the exposure assessment.

4.1.2 Hazard vs. risk

One of the fundamentals of each ecotoxicological risk assessment is the interrelation between hazard, exposure, and risk. Hazard is the potential of a substance to cause an effect in certain organisms; it is determined by the intrinsic toxicity of the substance. Risk is the product of hazard times exposure. Consequently, the risk may be low when exposure is lower than the thresholds for effects, even in case of inherently toxic substances, whereas, when exposure is higher than these thresholds, there may be a risk even in case of low intrinsic toxicity. When exposure is zero or negligible, the risk is negligible too. As for any other chemistry including pharmaceuticals as well as for foodstuffs, this also means that toxicity does not equal risk. This is commonly and intuitively understood for things we consume (e.g. sodium chloride, fluoride, sugar, caffeine, and ethanol). Paracelsus captured the concept well, 'All things are poison, and nothing is without poison, the dosage alone makes it so a thing is not a poison', but in the public discussion of pesticide risks, the difference between hazard and risk is frequently misconceived.

4.1.3 Effects and exposure

As in all risk assessment approaches, the ultimate goal of a pollinator risk assessment is to compare the exposure of the pollinator species under consideration to a substance under defined use conditions with the respective toxicity thresholds of the substance to the species under evaluation (e.g. honeybees) and to derive from this comparison the risk that the use of products containing the substance poses to the evaluated species.

In the case of laboratory data, measured toxicity data are compared to practically measured or mathematically modeled exposure data. Exposure can

be expressed in different dimensions, e.g. concentration (quantity of substance per unit of a medium (e.g. diet, water, or soil)), dose (quantity of substance taken up per individual of the assessed species (or per body mass unit of the individual)), or rate (quantity of substance applied per area unit). Which dimension is relevant depends on the test design. For instance, in studies where animals can be individually dosed, a specific dose can be tested, whereas where the exact exposure per individual cannot be accurately determined (e.g. in water for aquatic organisms, or in honey bee colony feeding studies), exposure is expressed as a concentration. Where the test substance is applied by overspray, a relevant application rate can be established (Fig. 1). Likewise, effects can be measured by a variety of parameters and endpoints, the most important of which have been described above (Section 3.2). In higher-tier studies, where the exposure of the test organism according to realistic scenarios is already a part of the study design, a quantitative comparison between exposure and effects can be omitted since both elements are already integral parts of the study design (Fig. 1).

4.1.4 Assessment factors and risk quotients

The result of a study is expressed as an endpoint, i.e. an exposure concentration, dose, or rate which is causing a defined effect (or no effect) to the test organism. In risk assessment, there are frequently additional assessment factors (or safety factors) applied against an endpoint. Assessment factors serve to introduce an additional margin of safety, to account for natural variability of the test system, and to compensate for inter-species variabilities where a test organism is a representative surrogate organism, covering other species too. Typically, the lower the tier of the study used to generate an endpoint, the higher the assessment (safety) factor. Lower-tier study endpoints may not be directly defined as a safe concentration in the assessment unless an assessment factor (or safety factor) is applied against them. In toxicology and ecotoxicology in general, assessment factors span from one up to 1000, depending on the study type, the number of studies that have been performed of the respective type, and the test organism.³ LD50 endpoints are almost always used in the connection with a substantial assessment factor, accounting for the fact that the underlying studies are lower-tier, and to account for the relevant protection goals in the risk assessment. Endpoints derived from higher-tier studies can typically be directly used in the risk assessment and mostly do not require an assessment factor since their design is close to realistic field conditions.

³ For example, the derivation of a PNEC or Predicted No Effect Concentration for aquatic organisms uses a factor of 1,000 when a limited number of acute toxicity studies is available, 100 and 10 for acute and chronic studies where both are available and the PNEC will be the lower of the two values (European Chemicals Agency 2008).

Especially in lower-tier risk assessment, the application of an assessment factor against a toxicity endpoint is frequently done in the calculation of a risk quotient. Risk quotients include the Hazard Quotient (HQ), the Exposure-Toxicity Ratio (ETR), and the Toxicity-Exposure Ratio (TER). All these quotients are based on a division of an exposure value by a toxicity value (HQ, ETR) or reversely (TER). These are compared to a trigger value reflecting the protection goal or can directly include the assessment factor.⁴

Whether the outcome of this calculation is above or below a pre-defined trigger value indicates whether the evaluated use can, at the respective tier of the risk assessment, be considered safe, or whether a risk cannot be excluded at this tier. For example, in the assessment of chronic risks to adult bees, with a TER trigger of 10, a dietary concentration of the evaluated substance of 2 mg/kg diet, and an NOEC of 30 mg/kg diet as the relevant toxicological endpoint, the TER would be calculated as 30 divided by 2, i.e. 15, meaning that the exposure is 15 times lower than the threshold for toxicity. In such a case the use could therefore be considered safe.

Where the dimensions of the toxicity endpoint and the exposure endpoint are not the same (e.g. effect data expressed as mg substance ingredient/bee and exposure data expressed as mg active substance/kg diet), conversion factors have to be included in the equation in order to make them directly comparable. A specific case is the HQ as used in the EPPO Risk Assessment Scheme (European and Mediterranean Plant Protection Organization, 2010b). In this approach, exposure (expressed as an application rate of the product or substance under evaluation in g/ha) is divided by an effect endpoint (an LD50 expressed in µg/bee). Here, the different dimensions of numerator and denominator are not adapted by an adjustment factor. In contrast, the approach is based on a systematic evaluation of historic incident data undertaken by Aldridge and Hart (1993) that established a relationship between the HQ and the likelihood of a respective pesticide use to be involved in bee intoxications in the field. Pesticide uses with a HQ greater than the trigger value of 2500 were frequently implicated in incidents. Uses with an HQ smaller than 50, however, can be considered intrinsically safe according to their analysis. Thus far, the HQ in this approach is one of the few evaluation factors in ecotoxicology which have been validated against realistic field data.

4.2 Tiered risk assessment systems

A risk assessment system based on studies of different tiers is called a tiered risk assessment system. Therein, the different study tiers as described in the previous

⁴ Note that additional refinement or correction factors may be included in the expression of exposure in the risk equation.

section are mirrored, and their specific strengths and limitations accordingly inform the respective risk assessment steps or tiers (see e.g. Alix and Lewis, 2010) (Fig. 1). Lower-tier (especially laboratory) studies are most suitable to determine the intrinsic toxicity of a substance to a test organism; on the other hand, they are normally not appropriate to directly deduce information about the existence of a risk that a substance may pose under realistic conditions. Since they exclude all naturally mitigating factors, their results, if directly extrapolated to field conditions, would frequently lead to an overestimation of risks. Higher-tier studies, in contrast, are suitable for directly deducing information about potential risks of a test substance; the more realistic the scenario is, the better. Especially field studies are complex in their setup, their conduction requires significant efforts and resources, and the interpretation of their results needs to be based on expert judgment. Whereas lower-tier studies, since focusing on intrinsic features of the test substance and the test organism, are universally valid, the extrapolatability of higher-tier studies may be more limited, especially when they do not represent worst-case conditions in all parameters, or when specific scenarios (e.g. in terms of crop, environmental conditions, application of the test substance) are tested, which do not reflect standard conditions.

Risk assessment systems in which lower-tier and higher-tier studies are related to each other in a hierarchical configuration are called tiered, hierarchical systems (Fig. 1). They aim at assessing the compliance of pesticide products with defined protection goals, based on a stepwise approach to assessing effects and risks, from highly standardized laboratory-derived parameters to realistic field studies. All existing major risk assessment schemes for bees belong to this category. The rationale of such a system is that different studies and their results have a different weight and a different significance for the understanding of a potential risk. The more realistic a study design and an exposure regime is, the higher weight is assigned to a study tier, and the results of a higher-tier study can override the results of a lower-tier study.

In terms of study conduction in the framework of a risk assessment scheme, most systems are operating as follows: the first studies to be conducted are lower-tier studies. In the case of substances that are intrinsically non-toxic to bees (as many herbicides and fungicides), there are no adverse effects seen even under the extreme conditions of lower-tier studies. In such cases, testing can stop, if the findings of the toxicity test in connection with the expected exposure under field conditions clearly suggest the absence of a risk. If the results of the tested tier do not attest to the absence of a risk, the next testing tier is triggered. This process continues up to the tier where the safety of the product can be demonstrated, including risk mitigation measures where needed. In other words, if the outcome of the risk assessment does not meet the benchmark or trigger value indicating a safe use, this suggests that a refined risk assessment is needed, which assesses whether or not the pesticide meets

the Specific Protection Goals under the proposed conditions of use, based on high-tier studies in tunnels or open field (European Commission, 2011a).

To some degree, studies of a lower-tier may be waived, if studies of the next higher-tier are available (e.g. Alix and Lewis, 2010). For instance, if a field study is provided, a corresponded semi-field study may not be necessary. If there are adverse effects even in the highest tiers, use restrictions for the product to minimize exposure need to be designed, or the product cannot be registered for the respective use.

The advantage of the tiered system is that intrinsically low-toxic substances can be filtered out early and with a limited effort, so that the focus can be directed to substances that have a higher intrinsic toxicity, allowing unnecessary complex higher-tier tests on intrinsically safe substances to be avoided.

An example of the application of the tiered system is provided in Box 1.

Box 1 Case study: Honey bee safety of the neonicotinoids

One of the most controversially discussed topics related to pesticide environmental compatibility is the honey bee safety of the neonicotinoids (e.g. Eisenstein, 2015). This is a class of insecticides which selectively act on insect nicotinic acetylcholine receptors and which are on the market since the 1990s (Jeschke and Nauen, 2010). Due to their efficacy against pest insects, their systemicity in plant tissues, which allows their use as seed and soil treatment, and their high level of operator safety, they are widely used in a broad variety of crops (Jeschke and Nauen, 2010). A sub-class of the neonicotinoids, the so-called nitro-substituted neonicotinoids exhibit a high intrinsic toxicity to bees, yet of a comparable order of magnitude as other insecticide classes; imidacloprid, thiamethoxam, and clothianidin for instance belong to this group (Iwasa et al., 2003).

In the mid-1990s, seed treatment applications of imidacloprid in sunflower in France were suspected as a cause of honey bee colony losses due to systemic residues in nectar and pollen (Maus et al., 2003). In 2008, a severe incident took place in Germany where a significant number of bee colonies were intoxicated by dust particles from clothianidin-coated corn seeds of deficient seed treatment quality (Forster, 2009, Nikolakis et al., 2009). In the early 2010s, some studies about sublethal effects of neonicotinoids to bees attracted the attention of the public and the scientific and regulatory communities (Henry et al., 2012, Whitehorn et al., 2012), which triggered political and regulatory processes which eventually led to far-reaching regulatory restrictions for imidacloprid, thiamethoxam, and clothianidin in the European Union (European Commission, 2013c, 2018a,b,c), whereas they are continued to be used in other regions of the world, yet in

some countries with use adaptations in order to optimize their bee safety.

The regulatory risk assessment for the neonicotinoids under discussion is driven by higher-tier data, which exemplifies the principles of risk assessment as outlined in this chapter. The substances usually do not pass the lower-tier risk assessment due to their intrinsic toxicity to bees. Therefore, higher-tier studies had to be performed, or risk mitigation measures minimizing exposure were stipulated. For seed treatment uses which can lead to trace level residues in nectar and pollen of treated crops (and, to some degree also of succeeding crops), the regulatory risk assessment is largely based on colony feeding study data in combination with residue data from bee-relevant matrices in treated and succeeding crops (second tier), and field data (third tier). These data largely revealed no or little concern about exposed honey bee colonies (e.g. Maus et al., 2003, Schmuck and Keppler, 2003, Schmuck et al., 2005, Pilling et al., 2013, Thompson et al., 2019).⁵ In the case of foliar uses, the risk to bees can in practice be easily mitigated by the avoidance of application during flowering of the crop and the restriction of pre-flowering applications.

Public concerns about the neonicotinoids were predominantly triggered by studies on sublethal effects which were conducted off the regulatory study system and mostly off the regulatory standards for safety studies. Hundreds of studies on neonicotinoid sublethal effects have been published (e.g. by Godfray et al., 2014, 2015, Siviter et al., 2021). The existence of sublethal effects as such is neither new nor unexpected, since any intrinsically toxic substance which is administered at a non-lethal level will inherently cause sublethal effects at certain exposure concentrations. This principle is well known to us from substances of our everyday life (caffeine, sodium chloride, ethanol, glucose). However, it is important to consider that most of the studies which have been conducted on sublethal effects were done in the laboratory (or under otherwise unrealistic exposure conditions) and with individual honeybees rather than with honey bee colonies. Moreover, exposure concentrations were not reflecting realistic field exposure scenarios in the vast majority of cases (see e.g. Carreck and Ratnieks, 2014). Accordingly, most of these studies would in a regulatory system be categorized as first-tier studies (see Fig. 1), which can inform about the intrinsic toxicity of the test substance, but which do not allow conclusions about the safety of a product under realistic conditions. Interestingly, a majority of studies which were conducted off the regulatory system but would correspond to higher-tier studies largely confirmed the conclusions of the regulatory risk assessment: most field studies either showed no or only insignificant, or only inconclusive effects to exposed honey bee colonies (Stadler et al., 2003, Cutler and Scott-Dupree, 2007, Nguyen et al., 2009, Cutler et al. 2014, Rundlöf et al., 2015, Rolke et al., 2016, Woodcock et al., 2017). This conclusion has in the meantime been confirmed by a comprehensive meta-analysis of available data

⁵ The EFSA risk assessment came to a different conclusion since, among other reasons, large part of the available higher-tier studies was not taken into consideration (European Food Safety Authority 2013b, c, d).

on the honey bee safety of the neonicotinoids (Stephenson and Solomon, 2017, Solomon and Stephenson, 2017a,b). This case study illustrates the critical importance of interpreting individual studies in their context and according to their purpose and context, which can be fundamentally different between the regulatory framework and academic research.

4.3 Risk assessment schemes and guidance documents

Risk assessment schemes or guidance documents provide guidance on the types of studies to conduct depending on the uses or product type, which procedures are to be used, how the study results are to be interpreted, and how the different study types and their results relate to each other in the risk assessment.

Like guidelines, risk assessment schemes and guidance documents are issued by international organizations or authorities. Examples are the EPPO Risk Assessment Scheme (European and Mediterranean Plant Protection Organization, 2010b) (Europe), the EFSA Guidance Document on the Risk Assessment of Plant Protection Products on Bees (European Food Safety Authority, 2013a) (European Union), the Guidance for Assessing Pesticide Risks to Bees (United States Environmental Protection Agency et al., 2014) (USA, Canada), the Guidance on Exposure and Effects Testing for Assessing Risks to Bees (United States Environmental Protection Agency, 2016) (USA, Canada), the Manual de Avaliação de Risco Ambiental de Agrotóxicos para Abelhas (Cham et al., 2017) (Brazil), and the Roadmap for Insect Pollinator Risk Assessment in Australia (Australian Pesticide and Veterinary Medicines Authority, 2017) (Australia).

Since the publication of the first risk assessment scheme for pollinators by EPPO in 1992 (European and Mediterranean Plant Protection Organization, 1992), various countries and geographical regions have developed their own guidance documents for pollinator risk assessment. A summary of their main contents including protection goals, testing requirements, and risk assessment principles are outlined in Table 2 for the European Union, North America, Brazil, and Japan, which taken together represent the diversity that may be observed in the existing risk assessment schemes. The risk assessment schemes in other countries such as Australia, New Zealand, South Korea, and Mexico have adapted and updated the North American standards to meet their own specificities.

Risk assessment schemes in all regions are based on comparable protection goals and thus share similar perspectives on the role and importance of pollinators to agriculture, beekeeping, and biodiversity. For

Table 2 Exemplary overview of risk assessment schemes in different countries and regions

Country/geographical region	Protection goals	Fundamental lower-tier testing requirements	Risk assessment principles	High-tier studies
Europe: EPO Risk Assessment Scheme ^a (European and Mediterranean Plant Protection Organization, 2010b)	Colony development and survival	OECD 213, 214	Sub-schemes for foliar and soil-systemic treatments HQ as decision criterion for chronic and higher-tier testing	Residue refinement, semi-field and field studies
European Union: Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (European Commission 2002)	Overall survival and productivity of the hive	OECD 213, 214	Adaptation of the EPO Scheme. HQ as decision criterion for higher-tier testing	Residue refinement, semi-field and field studies
European Union: EFSA Guidance Document (European Food Safety Authority, 2013a)	Colony development and survival; brood development and bee behavior. Provision of pollination services Production of hive products Non- <i>Apis</i> bees' populations (biodiversity)	OECD 213, 214, 239, and 245	Risk assessment for major and minor exposure routes Trigger values developed for each bee stage and assessment scale	Residue refinement, semi-field and field studies
USA and Canada: Guidance for Assessing Pesticide Risks to Bees (United States Environmental Protection Agency et al., 2014)	Colony and individual level assessments Honey bees as proxy for biodiversity Provision of pollination services Production of hive products	OECD 213, 214, 237, 239, and 245	Oral and contact exposure via treated crop Trigger values developed for acute and chronic risks	Residue refinement, semi-field and field studies
Brazil: Manual de Avaliação de Risco Ambiental de Agrotóxicos para Abelhas (Cham et al., 2017)	Colony development and survival; brood development and bee behavior. Provision of pollination services Production of hive products Non- <i>Apis</i> bees' populations (biodiversity)	OECD 213, 214, 237, 239, and 245	Oral and contact exposure via treated crop Trigger values developed for acute and chronic risks	Residue refinement, semi-field and field studies
Japan: Data Requirements for Registration of Agricultural Chemicals Food and Agricultural Materials Inspection Center (2019)	Maintenance of honey bee colony for beekeeping Colonies to be protected Focus on <i>Apis mellifera</i> (plan to introduce guidance for native pollinators e.g. <i>Apis cerena</i>)	OECD 213, 214, 237, 239, and 245	Oral and contact exposure via treated crop Trigger values developed for acute and chronic risks	Residue refinement, semi-field and field studies

^a The EPO Risk Assessment Scheme has officially been withdrawn in 2018 (European and Mediterranean Plant Protection Organization 2021), but is effectively still in use in the European Union.

study requirements, OECD guidelines remain the standard for all regions to address intrinsic toxicity assessments. For risk assessment calculations, most schemes primarily focus on direct exposure to a treated crop through oral and contact exposure routes, though e.g. in the European Union other possible exposure routes have been added to the scheme (see Chapter 15). Trigger values, also called levels of concern in North America, usually distinguish acute and chronic risk assessments. The EFSA Scheme has introduced additional distinctions between bee life stages and species, based on the models and calculations used to derive those trigger values.

Despite the differences between the approaches, the systems are based on the same principles and fundamentals, and theoretically lead to largely similar results in most cases, yet the margin of discretion which is granted in all systems may in practice lead to diverging conclusions in individual cases. All systems consider higher-tier tests under realistic exposure conditions, which are mostly required for intrinsically toxic substances, as overriding other study types. In contrast to the other systems, the EFSA system does not discriminate and filter out intrinsically non-toxic products in the first tiers, while simultaneously complicating higher-tier testing options by extremely rigorous requirements for study validity. The EFSA Scheme has so far not been fully implemented in the European Union, among other reasons because methods to experimentally measure the protection goals proposed in the document are lacking. A revised version has been prepared by EFSA (EFSA, 2019) and is up for public commenting (EFSA 2022).

5 Indirect and sublethal effects

Indirect effects are an important source of uncertainty in the risk assessment. For pollinators, indirect effects may result from effects of pesticides on habitat and food resource or quality. Hence weed management (chemical or mechanical) could result in indirect effects that affect the survival of a colony to a similar extent as direct effects.

In the regulatory risk assessment of pesticides, indirect effects on pollinators are addressed through the risk assessment performed for non-target plants that are present at the edge of a field and may be exposed to spray drift, for example. This risk assessment is performed for all types of products including herbicides and plant-growth regulators, and guidance documents are available that provide detailed recommendations on how to perform laboratory and field studies (European Commission, 2002, Arts et al., 2017).

Besides effects on mortality or reproduction, pesticides may affect other parameters such as behavior, longevity, immune defense, sensory perception, cognitive skills, and many others. Such effects are referred to as sublethal effects. Relatively little is known about how far sublethal effects may affect bee

colonies or populations in a way that would be measurable on the basis of population parameters. In fact, there are very few documented cases where an adverse impact at the honey bee colony level has been seen to be caused by sublethal effects in a field-realistic scenario. Therefore, sublethal effects that are measurable at the level of individual honeybees may not necessarily be biologically relevant effects, or do at least not necessarily affect the protection goal (see e.g. Henry et al., 2015, Siede et al., 2017). In existing risk assessment systems, sublethal effects are mostly accounted for in the same study types that are also assessing mortality and other standard endpoints (e.g. Thompson and Maus, 2007). Some study designs have been developed to test for specific sublethal effects (e.g. OECD GD 332, effects on homing capability (OECD, 2021c)), but they are not mandatory in most risk assessment schemes, as our understanding of the biological significance of sublethal effects is limited. However, sublethal effects can generally be considered to be essentially covered by the existing testing methods, as they would either manifest in effects on other endpoints (e.g. mortality, foraging, reproduction, and colony performance) or alternatively would not be relevant in relation to the protection goal.

6 Risk mitigation

Where the outcome of the risk assessment does not exclude the presence of risks to pollinators under practical use conditions of a product, risk mitigations have to be defined to ensure a safe use by minimizing exposure. The registration process for pesticides in the European Union defines specific risk mitigation measures to accompany the registration of pesticides where they are triggered by the outcome of the risk assessment (European Parliament and Council of the European Union, 2009). Risk mitigation measures for pesticides may be implemented at various levels. The regulations stipulate a range of precautionary or safety phrases describing appropriate conditions of use on the product's labeling (European Commission, 2011b). Labeling recommendations can hence reduce exposure of both managed and wild bees (for examples see **Box 2** Safety phrases on product labels). For managed species, beekeepers can also efficiently manage colonies in space and time thanks to adapted recommendations. Mitigation measures may include a variety of crop or hive management approaches that reduce the exposure of bees to the treatment, and which are defined on a case-by-case basis, considering the intrinsic properties and characteristics of the product, the crop, and pollinator species of concern in the mitigation measure. Examples are avoiding applications during flowering and during pre-flowering safety intervals depending on the residual toxicity of the product, applications in the evening after daily bee flight, avoiding spray drift to bee-attractive crops or

weeds, removal of flowering weeds in the crop's understory before application, or covering or removal of bee hives in or nearby the crop prior to the treatment (see e.g. Australian Government – Rural Industries Research and Development Corporation, 2012, Hooven et al., 2013, CropLife International, 2017, Johansen and Wu-Smart, 2021). Label information is mandatory and is implemented in all countries, adapted to national situations and farming practices, and designed specifically for each product. These mitigation methods have been developed with a focus on honeybees and may not in all cases applicable to wild pollinators.

Box 2 Safety phrases on product labels

Examples of safety phrases on product labels to protect pollinators

European Union (European Commission, 2003):

SPe 8: Dangerous to bees./To protect bees and other pollinating insects do not apply to crop plants when in flower./Do not use where bees are actively foraging./Remove or cover beehives during application and for (state time) after treatment./Do not apply when flowering weeds are present./Remove weeds before flowering./Do not apply before (state time).

Australia (Australian Pesticide and Veterinary Medicines Authority, 2018):

Mandatory label for 'Protection of Livestock' with the precaution to not spray onto bees or beehives and the mention that 'once the spray deposit has dried, foraging bees will not be affected'.

New Zealand (New Zealand Ministry for Primary Industries, 2020):

'POLLINATORS: When applied during non-foraging periods the Product will not interfere with the activity of honeybees once the spray has dried. At least 3 hours drying time should occur before bee foraging is expected. At times when bees aggregate in large numbers outside the hive, ensure they are not directly contacted by the spray.'

United States (United States Environmental Protection Agency, 2021b):

'This product is toxic to bees exposed to treatment during the 3 hours following treatment. Do not apply this pesticide to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on the plants during this time period.'

Besides labelling of pesticides, crop management practices adopted by farmers at the farm scale may greatly reduce exposure of pollinating species visiting crops (Alix and Garrido, 2015, Alix et al., 2017). A range of farm management tools beneficial to pollinators has been identified, ranging from

establishing natural and semi-natural field margins to managed field margins, dedicated pollen and nectar seed mixes, wildflower sown margins, grass strips, or conservation headlands. Each provides advantages to pollinating insects, either as a refuge area or in providing a dedicated source of food or nesting habitat. A ranking of the benefits represented by each type of farm management was provided by the MAgPIE Project.

An inventory of mitigation measures for pollinators has been compiled by the OECD-PEIP working group (OECD, 2021e). The inventory includes regulatory risk mitigation recommendations as communicated through the information on pesticide labels, and education and training programs. Education and training programs for farmers and beekeepers are also key components of risk management as they drive the accuracy with which risk mitigation measures are implemented. These programs may be organized by any stakeholder and are most often voluntary initiatives, thus indicating a real commitment of countries.

7 Pesticide incident monitoring

Monitoring approaches aim to assess the potential effects of pesticides used in crop protection according to label recommendations under practical conditions on specific populations and communities. These studies complement the risk assessment performed under relevant regulations (European Parliament and Council of the European Union, 2009) and describe the conditions of exposure of organisms in their environment, the conditions of occurrence of risks, and provide in situ assessment of the efficacy of risk mitigation measures that were recommended thus enabling further adaptation based on the collected information.

Despite an increasing interest in field observations of pesticide impacts on pollinators, there is to date no harmonized regulatory guidance or monitoring methodology for honeybees or other pollinating species, nor is there regulatory guidance on the use of generated data in support of risk assessment or decision making. An inventory of published monitoring studies was undertaken by the International Commission on Plant-Pollinator Relationship (ICPPR) a few years ago (Alix and Garrido, 2015), which analyzed 24 monitoring studies and was further expanded to 56 studies in 2016, as a basis for a possible guidance on good monitoring practices. The analysis of the data revealed distinct approaches depending on the species monitored and on the purpose of the study. For managed species, the baseline can be defined at the introduction or release of pollinators in the cultivated system whereas wild bees require setting study designs reflecting ecological approaches to first define the expected occurrence and diversity from the collected environmental features of the ecosystem. In both cases, a dedicated description of the environment

is critical to the interpretation of the data. There is a need to gather existing knowledge on the specific landscape management initiatives and their benefits to pollinators in cropping systems in order to build a decision tree to assess the potential of a crop to induce pollinator exposure and to identify the risk mitigation measures and crop management actions with the highest potential to limit exposure. However, limited knowledge is available on species traits and relationship to specific flora to accurately predict the expected community in a particular crop system, which would define which species need to be protected and where.

Projects that are initiated should allow a better implementation of monitoring data collection, as for example in the European Union (European Commission, 2021) and propose protocols. A first financial analysis shows that despite the relatively high financial costs of systematic monitoring data generation, the benefits to pollinated crops and systems from the resulting learning can largely compensate these expenses (Breeze et al., 2020). Besides a harmonized method to collect data that can be compared and used to feedback to regulatory processes, basic ecological data are needed to better understand plant-bee relationships and depict the species and assemblage of species that can be expected in particular systems, to inform protection goals and design risk assessments and monitoring.

In some countries, there are passive monitoring systems in place, where reported incidents of bee intoxications are investigated by official institutions to analyze whether an incidence of mortality was caused by pesticide intoxication and, if so, which products and application practices were involved. Examples for such incident monitoring approaches are the British Wildlife Incident Investigation Scheme, the Incident Monitoring of the Julius Kühn Institute in Germany, and the Pesticide Incident Reporting Program in Canada (Seefeld, 2006, Barnett et al., 2007, Thompson and Thorbahn, 2009, Mineau et al., 2008, Julius-Kühn-Institut, 2021a,b, Government of Canada, 2018, 2021).

8 Conclusion

Overall, the development of the described approaches and efforts, which have been continuously extended and refined over the last decades, underscores the importance of bees and other pollinators for agriculture and crop protection. Incident monitoring systems confirm that in countries where systematic long-term figures are available, numbers of bee intoxications with pesticides have shown a decreasing trend over the last decades and are currently on low absolute numbers (e.g. Thompson and Thorbahn, 2009, Carreck and Ratnieks, 2014). This reflects the improvement in the level of protection provided by regulatory systems developed over the years as well as the increasing awareness of the importance of pollinator protection among the involved

stakeholders. Nonetheless, environmental safety testing and risk assessment schemes in regulatory ecotoxicology are not static constructs, but dynamic systems, which can be constantly optimized to always reflect the latest state of science and address emerging research issues; in so far, we can expect further new developments and optimizations in the years to come.

9 Where to look for further information

Key resources for further information on the topics outlined in this chapter are in the first place the guidelines and guidance documents cited in the main text. Beyond this, a broad overview of methodologies for scientific studies on honeybees (not restricted to pesticide assessment and evaluation) is provided in the COLOSS BEEBOOK (<https://coloss.org/beebook/>). General information can also be found in Whitford et al. (2017).

Further information about the regulation of pesticides with regard to pollinator safety can be found on the websites of a variety of authorities and agencies in charge of environment, agriculture, or pesticide registration, such as the United States Environmental Protection Agency (US EPA) (<https://www.epa.gov/pollinator-protection/epa-actions-protect-pollinators#:~:text=EPA%20has%20taken%20the%20following,contract%20to%20provide%20pollination%20services.>), the United States Department of Agriculture (e.g. <https://www.usda.gov/sites/default/files/documents/pollinator-priorities-2021R4-508-version.pdf>), the European Food Safety Authority (EFSA) (<https://www.efsa.europa.eu/en/topics/topic/bee-health>), the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) (<https://www.anses.fr/en/content/bee-health>), the German Julius Kühn-Institut (<https://www.julius-kuehn.de/en/bs/>), and others.

Standardized testing methods for pollinators are issued by the Organisation for Economic Co-operation and Development (OECD) (<https://www.oecd.org/chemicalsafety/testing/work-related-beepollinators.htm>).

Major scientific programs and networks which are looking at a broad spectrum of aspects related to pollinator health (including, but going far beyond pesticide topics) comprise COLOSS (<https://coloss.org/>), PoshBee (<https://www.poshbee.eu/>), Safeguard (<https://www.safeguard.biozentrum.uni-wuerzburg.de/>), STEP (<http://www.step-project.net/>), Salud Apicola (<https://en.saludapicola.com/>), and others.

Important scientific organizations working on pollinator safety topics in agriculture include the International Commission of Plant-Pollinator Relationships (ICPPR) (<https://www.icppr.com/>), the Society of Environmental Toxicology and Chemistry (SETAC) (<https://www.setac.org/>), Project Apis m. (<https://www.projectapism.org/honey-bee-research.html>), the Pollinator Partnership (<https://www.pollinator.org/>), the Pollinator Research Task Force

(<https://pollinatorresearchtaskforce.com/>), the Honey Bee Health Coalition (<https://honeybeehealthcoalition.org/>), the Bee Informed Partnership (<https://beeinformed.org/>), and ABELHA (<https://abelha.org.br/en/>).

Information about the engagement of the crop protection industry in pollinator protection can be found on the websites of CropLife International (e.g. <https://croplife.org/case-study/pollinator-protection-vital-to-crop-production/>, <https://croplife.org/crop-protection/pollinators-2/>), CropLife Europe (e.g. <https://croplifeeurope.eu/report/pollinators-and-agriculture/>), CropLife America (e.g. <https://www.croplifeamerica.org/resources>), CropLife Latin America (<https://www.croplifela.org/en/whats-new/pollinators>), and CropLife Asia (https://www.croplifeasia.org/video_tag/pollinators/#/). CropLife International also provides an overview of stewardship approaches to protect pollinators (<https://croplife.org/wp-content/uploads/2017/04/Protecting-Pollinators-Through-Good-Stewardship-Practices-v7.pdf>).

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