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Non-*Apis* bees as model organisms in laboratory, semi-field and field experiments

Non-*Apis* Bienen als Modelorganismen in Labor-, Halbfreiland- und Freilandversuchen

Abstract

As part of the registration process of plant protection products (PPPs) and their active substances in the EU, the risk of PPPs for bees has been assessed so far by using the European honey bee (*Apis mellifera* L.) as a surrogate species. In the past few years other bee species have been discussed to augment data on honey bees. The addition of bee species in the registration process goes along with adapting test methodologies to new bee species and understanding how to use these species at different tiers (laboratory, semi-field and field levels). Here we first discuss the importance of bees as test organisms, outline the current state of research relevant to the methodology and design of experiments with bees and highlight recent activities in the standardization of test procedures.

Key words: honey bee, bumble bees, solitary bees, ecotoxicology, risk assessment, sensitivity, method development

Zusammenfassung

Im Rahmen der Zulassung von Pflanzenschutzmitteln und ihren Wirkstoffen in der EU wurde das Risiko für Bienen bisher anhand der Westlichen Honigbiene (*Apis mellifera* L.) als Modellorganismus für alle Bienenarten bewertet. In den letzten Jahren wurde kontrovers diskutiert, ob Wildbienenarten in der Risikobewertung ebenfalls berücksichtigt werden sollten, um die bisherigen Datenanforderungen für Honigbienen zu erweitern. Dies geht damit einher, etablierte, standardisierte Methoden für die Honigbiene an zusätzliche Wildbienenarten anzupassen und zu verstehen, wie diese Arten auf den verschiedenen Testebenen (Labor-, Halbfreiland- und Freilandtests) eingesetzt werden können. In diesem Artikel gehen wir zunächst auf die Bedeutung von Bienen als Testorganismen ein, diskutieren den derzeitigen Stand der Forschung, die für die Methodenentwicklung und das experimentelle Design für das Arbeiten mit Bienen wichtig ist, um abschließend einen Ausblick auf aktuelle Aktivitäten in der Standardisierung von Testmethoden zu geben.

Stichwörter: Honigbiene, Hummeln, Solitärbienen, Ökotoxikologie, Risikobewertung, Sensitivität, Methodenentwicklung

Bees in agricultural landscapes

Pollinators are an integral part of global biodiversity; insects – primarily bees – are the most prominent pollinator group of many crops and wild plants (Potts et al., 2010). As a domesticated species, European honey bees (*Apis mellifera* L., Hymenoptera: Apidae) are economically important pollinators (Morse and Calderone, 2000; Mwebaze et al., 2010; Bartomeus et al., 2014; Oré Barrios et al., 2017). However, the great majority of bee species

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are *non-Apis* bees that display varying levels of sociality (Michener, 2007). Germany hosts almost 600 wild bee species (Westrich et al., 2011) including colony-building bumble bees as well as ground-nesting and hole-nesting solitary bee species. Wild bee species contribute significantly to crop pollination (Klein et al., 2007; Garibaldi et al., 2013), and many of them forage and nest in agricultural landscapes. An increase in their abundance and diversity can increase crop productivity (Venturini et al., 2017; Catarino et al., 2019 but Bartomeus et al., 2014). The exact number of wild bee species using agricultural landscapes has yet to be estimated for the different regions of Germany (and worldwide). Bees in agricultural landscapes are exposed to a variety of stressors, which are recognized as drivers of wild bee declines and honey bee colony losses (Goulson et al., 2015). It is essential to reduce and regulate these factors in order to maintain or increase ecological and economic benefits.

Bees as model organisms in the registration process

Plant protection products (PPPs) are one of the stressors identified as a major driver for bee declines (Sánchez-Bayo and Wyckhuys, 2019). Depending on the geographical and political region, their legalization and use with respect to their environmental impact is policed by regulatory authorities, e.g. the United States Environmental Protection Agency (USEPA), Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) and Australian Pesticide and Veterinary Medicines Authority (APVMA) (Handford et al., 2015). In this review, we focus on the legislative framework and schemes applied in Europe. Within the European Union, PPPs are regulated by means of the registration process of plant protection products and their active substances (European Parliament, Council of the European Union, 2009). This process involves the evaluation of hazards of PPPs to beneficial insects, including bees, based on a specific use and employing standardized test procedures. So far, these tests are designed to use the European honey bee as a surrogate species for all bee species.

After the publication of the EFSA Bee Guidance Document (European Food Safety Authority, 2013) and of a growing number of studies that showed losses of insect diversity and abundance (Biesmeijer et al., 2006; Hallmann et al., 2017; Seibold et al., 2019), the discussion about other surrogate species in the risk assessment procedure became more intense (e.g. Boyle et al., 2019). First attempts were made to expand and adapt the existing guidelines to other bee species, especially bumble bees and solitary bees (Fischer and Moriarty, 2011; Rortais et al., 2017). While the process has not implemented the use of other bee species on a regular basis, new guidelines were recently adopted to assess risks for bumble bees (Apidae: *Bombus terrestris*) (OECD, 2017b, 2017c) and in the future for solitary bees (Apidae: *Osmia bicornis*) (OECD, 2019b).

Honey bees

As discussed above, testing European honey bees as a model organism for bees in general has been the usual way of evaluation, and test methods for honey bees in the laboratory, under semi-field and field conditions have been well-established (Eppo, 2010). They include acute and chronic exposure tests of adult honey bees (OECD, 1998a, 1998b, 2017a) and honey bee larvae (OECD, 2013, 2016), and a test on honey bee development (OECD, 2007). Honey bees are eusocial insects that form perennial colonies with many thousands of individuals. Consequently, they can be repeatedly sampled for individual bees almost year-round, are commercially available, are widely distributed and are therefore ideally suited for experimental usage (Thompson and Pamminger, 2019).

Non-Apis bees

A proper risk assessment of pesticides to bees must integrate two aspects: (a) the toxicity of the pesticide and (b) the probability of exposure (van der Valk et al., 2013). Toxicity of pesticides to non-*Apis* bees has been suggested to be extrapolatable from data on honey bees (Heard et al., 2017; Lewis and Tzilivakis, 2019; Reid et al., 2020; Thompson and Pamminger, 2019). However, honey bee LD_{50} values may not always be good predictors across different bee species (Mayack and Boff, 2019), and sensitivity among different taxa can be variable (Arena and Sgolastra, 2014; Lewis and Tzilivakis, 2019) and dependent e.g. on body mass (Thompson, 2016). Even if toxicity data can be extrapolated, there might be still a need for higher tier experiments to account for different exposure probabilities in a realistic setting (Thompson, 2016).

The probability of exposure to PPPs depends not only on the intensity of agricultural practice but also on certain aspects of bee biology including nest location and foraging range as well as time, period of day and number of days when foraging (Brittain and Potts, 2011; van der Valk et al., 2013). Exposure risks to non-*Apis* bees from PPPs is assumed to be similar or higher than to the European honey bee (Table 1), but in most cases there are still major data gaps that complicate an assessment (Roubik, 2014).

To account for some of the described differences in life history traits between bee species, impacts of pesticides on some non-*Apis* bee species are considered in some cases of the current registration process even though test procedures have not been established and harmonized for every tier and every species. While inclusion of tests on bee species other than honey bees may be desirable in order to be protective of non-*Apis* bees, it can be challenging to work with them in laboratory or (semi-)field trials. Wild bee species often produce smaller numbers of individuals per population, shorter periods of seasonal activity and restricted food preferences (Table 2, Roubik, 2014).

In order to conduct regular trials with a particular species in the framework of the registration process, a species has to be available in large numbers, standardizable

Table 1. Potential exposure routes and their relative importance to European bees (adopted from Fischer and Moriarty, 2011, extended by additional information (Gradish et al., 2019; Sgolastra et al., 2019)); – = no potential exposure; + = low potential exposure; ++ = medium potential exposure; +++ = high potential exposure

Exposure	European honey bees	Bumble bees	Solitary bees
Nectar	+++	++(+)	+ to +++
Pollen	+ to +++	++ to +++	+ to +++
Honey dew	+ to +++	-/+	–
Water	+ to +++	+(+)	+(+)
Nesting material	+	+	+ to +++
Exposure to soil	-/+	- to ++	- to +++
Foliar residues	+++	+++	+++
Direct spray (at flowering)	+++	+++	+++
Dust drift	++	++	++

Table 2. Life history traits of European bee species (based on information from Prŷs-Jones and Corbet, 1991; Winston, 1995; Goulson, 2003; Michener, 2007; Cueva Del Castillo et al., 2015; Westrich, 2018; Sgolastra et al., 2019)

	European honey bees	Bumble bees	Solitary bees
Sociality	Eusocial (perennial)	Eusocial (annual)	Semi-social, para-social, sub-social, quasi-social or solitary (short-lived)
Casts	Queens, drones, worker bees	Queens, males, worker bees	Females, males
Number of individuals per nest	Up to 50.000	On average 25–150 (species-specific; worldwide mean 20 to 1848)	Single to multiple individuals per nest; in some species aggregations of 10,000 nests and more
Fecundity	Approx. 1,500 eggs per day	Approx. 4 to 16 eggs in batches at a time	Approx. 2 eggs per day (10–40 eggs over entire life span)
Food sources	Polylectic (often mass flowering crops)	Poly- to oligolectic	Poly-, oligo- or monolectic*
Nest location	Epigaic	Epigaic and/or endogaic	Epigaic or endogaic*

* often with specializations or special requirements

and easily measurable in its endpoints[1] and representative in its life history traits of a larger (sub)group of bee species (Sgolastra et al., 2019). Ideally it should also have a prolonged or relatively flexible foraging season so that it can be used in various settings. So far there have been very few species meeting these requirements.

Bumble bees. Like honey bees, bumble bees are eusocial and form colonies housing defined casts (Table 2). Colonies in the temperate zones are founded by a single queen that forages and raises the first generation of workers (Benton, 2009). Workers then take over brood care and forage while the queen lays eggs. The size of a colony increases until reproductive offspring are produced. While the colony then slowly dies, the reproductive offspring leave the nest and mate, and the gynes hibernate and initiate new colonies in the next season. In a few instances, depending on climate and species, an autumn/winter generation may be established (Stelzer et al., 2010), but data on its reproductive success is lacking.

In contrast to the assessment of honey bee colonies, the production of queens and males is a crucial part in a bumble bee colony cycle and an important endpoint in the assessment of colony performance. However, the number of reproductive offspring is often highly variable even within the same species. Factors influencing this

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[1] Measurement Endpoint: a measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint and is a measure of biological effects (e.g. death, reproduction, growth) of particular species, and can include measures of exposure as well as measures of effects; Assessment Endpoint: a qualitative/quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment; an explicit expression of the environmental value that is to be protected

parameter include parasitism, land use context, life span of the founding queen, time of initiation, and growth and size of the colony (Müller and Schmid-Hempel, 1992; Samuelson et al., 2018). This multitude of factors complicates a standardization that has to be ensured for risk assessment trials.

A further essential requirement for conducting standardized risk assessment trials is the availability of colonies. Of the approx. 250 bumble bee species worldwide (Goulson, 2003), there are only a few species that have been successfully established and raised in captivity (Table 3). Although the foundations of bumble bee domestication go back to the 19th century, rearing methods were not fully developed until the late 20th century (Evans, 2017). All (commercially) reared bumble bee species are pollen storers who feed pollen from separate pollen pots to their larvae directly by perforating the cell wall (Sakagami, 1976), in contrast to pocket makers who feed pollen via a pocket at the side of the larval cell (Sladen, 1899). This characteristic is a good example of how life history traits can define exposure probabilities to pesticides (Colla, 2014): pocket makers may feed contaminated pollen to (and only affect) the current cohort of larvae while pollen storers may keep (and mix) it in separate containers and later feed it to all larvae of the colony.

Solitary bees. Of the almost 20.000 known bee species in the world (nearly 2.000 in Europe; Nieto et al., 2014), only a few have been reared in captivity to primarily support the pollination of specific crops in the agricultural landscape (e.g. Bosch and Kemp, 2002). The alfalfa leafcutting bee *Megachile rotundata*, various mason bees *Osmia* spp. and the ground-nesting wild bee species *Nomia melanderi* are some of the few species that have been used in ecotoxicological studies (Table 4, Kopit und Pitts-Singer, 2018). Wild bee species differ in several key traits, including their food and nesting resources (Wcislo und Cane, 1996; Michener, 2007; Roubik, 2014), for which they utilize either one host plant species (monolecty), one host plant family (oligolecty) or more than one host plant family (polylecty) (Cane und Sipes, 2006; Müller und Kuhlmann, 2008).

Sociality is another aspect that is highly variable among wild bees, spanning a wide range that includes solitary, semi-social, para-social, sub-social, quasi-social and kleptoparasitic. Sociality can be an important aspect of life history for assessing risks of PPPs to wild bees. Some solitary bee species, e.g. species within the Halictids, perform regurgitation (trophallaxis) like honey bees; this extends exposure from one individual to many (Martins, 2014) and may increase exposure probabilities on a population rather than an individual level. Within the species that are solitary, individual females serve as reproductive units and have to take care of the offspring themselves rather than being replaceable by a group of workers who care for the brood (as in honey bees and bumble bees). Hence, solitary bee females are more comparable to founding bumble bee queens who provide resources to their offspring on their own (Stoner, 2016). PPP exposure of a single female can directly affect reproductive success over the lifespan of this individual (Straub et al., 2015).

Solitary bees usually occur as only one generation per year (univoltine); a few species with higher degrees of sociality have several generations per year (multivoltinism), correlated to environmental factors such as temperature and food resources (Westrich, 1989). Depending on the period of emergence, exposure probabilities may vary greatly, and early-emerging females with one generation face a different risk compared to late-emerging females or species with two generations. Finally, nesting requirements define exposure and can be different between ground-nesting species that dig their

Table 3. Bumble bee species used in ecotoxicological assays (adopted from Arena and Sgolastra, 2014 and references therein; extended by additional references (Way and Synge, 1948; Wu et al., 2010; BioBest, 2020))

Native range	Species name	Rearing in captivity*
Europe	*Bombus lapidarius*	Yes
	Bombus lucorum	Yes
	Bombus pascuorum	No
	Bombus terrestris	Yes (ca)
	Bombus vestalis	No
North America	*Bombus impatiens*	Yes (ca)
	Bombus occidentalis	Yes
	Bombus terricola	No
Asia	*Bombus ignitus*	Yes (ca)
	Bombus hypocrita	Yes
	Bombus patagiatus	Yes

* ca = commercially available

Table 4. Wild bee species other than *Bombus* sp. used in ecotoxicological assays (adopted from Arena und Sgolastra, 2014 and references therein; extended by information from additional references (e.g. Way und Synge, 1948; Helson et al., 1994; Bosch und Kemp, 2002; Cauich et al., 2004; Cortopassi-Laurino et al., 2006; Nocelli et al., 2012; Quiroga Murcia et al., 2017; Dharampal et al., 2018; Jütte et al., 2019; Padilha et al., 2020))

Native range	Species name	Nesting	Rearing in captivity*
Europe	*Andrena flavipes*†	Ground-nesting	No
	Megachile rotundata	Above-ground	Yes (ca)
	Osmia bicornis	Above-ground	Yes (ca)
	Osmia cornuta	Above-ground	Yes (ca)
North America	*Andrena erythronii*	Ground-nesting	No
	Nomia melanderi	Ground-nesting	Yes (ucp)
	Osmia lignaria	Above-ground	Yes (ca)
	Osmia ribifloris	Above-ground	Yes
Asia	*Osmia cornifrons*	Above-ground	Yes (ca)
	Trigona iridipennis†††	Above-ground	No
Central and South America	*Melipona beecheii*†††	Above-ground	Yes (ucp)
	Melipona quadrifasciata†††	Above-ground	Yes (ucp)
	Melipona scutellaris†††	Above-ground	Yes (ucp)
	Nannotrigona perilampoides†††	Above-ground	Yes (ucp)
	Plebeia emerina†††	Above-ground	Yes (ucp)
	Scaptotrigona postica†††	Above-ground	Yes (ucp)
	Scaptotrigona tubiba†††	Above-ground	No
	Scaptotrigona xanthotricha†††	Above-ground	No
	Tetragonisca angustula†††	Above-ground	Yes (ucp)
	Tetragonisca fiebrigi†††	Above-ground	Yes (ucp)
	Trigona nigra†††	Above-ground	No
	Trigona spinipes†††	Above-ground	No

†also native to parts of Asia and North Africa; ††also native to parts of Asia; †††eusocial species * ca = commercially available, ucp = used for specific commercial purposes but not generally commercially available

own cavities and the species that nest above ground or in existing underground cavities (Sgolastra et al., 2019). Some wild bee species use nesting resources like leaves, soil, resin or fibres to line their brood cells, which may be another source of contaminants (van der Valk et al., 2013).

The high diversity of life-history traits and environmental requirements make solitary bees a group of organisms that are particularly difficult to rear in large numbers. Hence many species are not suitable as model organisms in the risk assessment of registration processes for PPPs. The number of wild bee species used in ecotoxicological tests is therefore limited (Table 4).

Developing methods

Using bee species other than honey bees in the registration process requires not only the knowledge of their characteristics and life history traits but also establishing routines and standards in handling, caring for the bees' specific requirements and reliably measuring endpoints in 1st tier (laboratory) and higher tier (semi-field and field) trials. There have been numerous studies on various species over the last decades that have collected valuable information on feeding, housing, rearing, overwintering and endpoints in different experimental settings (cf. references in EFSA Panel on Plant Protection Products and their Residues, 2012 and Sgolastra et al., 2019). However, a better understanding of the variability of these traits among bees has only complicated the development of standard procedures.

The Bee Protection Group of the International Commission for Plant Pollinator Relationships (ICPPR) provides a forum, in which these aspects are addressed, and coordinates international research and ring tests[2]. Its working groups (e.g. *Apis*, non-*Apis*) focus on the development of suitable test methods and the evaluation of parameters and endpoints related to bee health and effects of PPPs. Regulatory test guidelines and guidance

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[2] Ring test: an inter-laboratory test that allows to evaluate the performance of testing laboratories, and is based on analysis of similar homogeneous samples

documents of the Organization for Economic Cooperation and Development (OECD) are often based on data and information collected and collated by the working groups (OECD, 2019a).

Bumble bees

As mentioned earlier, laboratory (1st tier) test methods for bumble bees have already been standardized and implemented in guidelines for risk assessment processes (OECD, 2017b, 2017c) using *Bombus terrestris* (Europe) and *Bombus impatiens* (North America) as model species. Queenless microcolonies of these two species have been proposed to be a useful tool for evaluating a range of endpoints at colony level (Klinger et al., 2019); however, disadvantages of using only worker bees are likely to outweigh the benefits (cf. Wu-Smart and Spivak, 2018). For example, one limitation of this method is nest initiation by worker bees and subsequent drone production from unfertilized eggs, which is difficult to standardize in the framework of risk assessment.

While laboratory tests have been conducted on more than those two bumble bee species (cf. Table 3), studies on PPPs that include manipulative semi-field and field experiments have so far only utilized the commercially available species *Bombus terrestris* and *Bombus impatiens* (Cutler and Scott-Dupree, 2014; Gill and Raine, 2014; Gradish et al., 2016; Woodcock et al., 2017; Siviter et al., 2018; Dietzsch et al., 2019; Rundlöf and Lundin, 2019). These studies used the weight of a colony, the size/volume of the nest and/or the number of workers (colony strength), males and gynes as proxies for a colony's development and success and thus as endpoints. Rearing colonies from sister queens (queenright) in captivity by commercial suppliers (e.g. Koppert, BioBest) should allow for a way of standardization among colonies that are exposed to the same environmental settings (van der Steen, 2001; Cabrera et al., 2016). While such a restriction of genetic variability in experimental bee colonies neglects a wide range of naturally occurring genetic traits and as a consequence may restrain the generalization of test results (Bakker, 2016), it may not yet have the desired effect of reducing experimental error. Measuring differences in certain endpoints such as gyne production may only be achieved by highly replicating the number of colonies in trials to accomplish an adequate protection goal (e.g. detection of 25% reduction in queen production; cf. Cabrera et al., 2016). Semi-field and field trials have proven different parameters to be significant for colony success, including initial colony strength and its influence on trial duration, colony development and reproductive success in different seasons (Dietzsch et al., 2018). Food availability is another crucial factor for queen production and queen weight (Franke et al., 2018). To further reduce variability in endpoints, colonies should not only contain a similar initial number of workers and brood stages and show an appropriate worker/brood ratio but also develop with a synchronized speed (Klein et al., 2018). This latter criterion is very time-consuming to achieve and requires laboratory space and capacities to conduct assessments for a possibly large number of replicates. While synchronized developmental speed has been shown to work in semi-field trials (Klein et al., 2018) and was included as a criterion in ring tests (Knäbe et al., 2019), it may not be feasible to achieve in field experiments with multiple colonies per site and multiple replicates in each treatment. Other aspects such as colony disturbance during the experimental (semi-) field phase (including removal of wax ceilings for brood nest evaluation) and its influence on endpoints have yet to be experimentally addressed.

Solitary bees

Although establishment of – at least 1st tier – guidelines for solitary bees in the risk assessment process has been initiated (OECD, 2019b), so far standardized methods have not been approved. As for bumble bees, laboratory toxicity tests were performed on many different species (Table 4), yet most experiments on effects of PPPs, particularly higher tier tests, have used only commercially available species, e.g. *Osmia bicornis*, *Osmia lignaria* and *Megachile rotundata* (Abbott et al., 2008; Sandrock et al., 2014; Rundlöf et al., 2015; Becker and Keller, 2016; Nicholls et al., 2017; Woodcock et al., 2017; Dietzsch et al., 2019 but Dharampal et al., 2018). This might be problematic since the three species belong to the same family (Megachilidae) and display relatively similar life history traits, hence may only mirror very few aspects of exposure and behavior of solitary bee species. In addition, availability is not always ensured for all areas of a species' native range in the framework of the registration process. Since imports of such species are restricted, and different regions (e.g. EU authorization zones) within the same registration area may require different native species, the use of a small set of commercially available species can complicate the implementation of adequate tests.

While the difficulty in bumble bee experiments is the handling of high variability within endpoints, problems in experiments with solitary bees occur in relation to standardized feeding of contaminated food under laboratory conditions, general breeding requirements and year-round management/availability of viable individuals. On a laboratory level, methodologies were refined over the last years and standardized ring tests were conducted (Roessink et al., 2018), which led to the above mentioned proposal for a new guideline (OECD, 2019b). Additional 1st tier experiments explored artificial rearing as well as acute and chronic PPP exposure of solitary bee larvae (Sgolastra et al., 2015; Becker and Keller, 2016; Eeraerts et al., 2019). A greater challenge are semi-field and field trials where hatching times and hatching ratios of bee individuals have to be synchronized, and assessment of nest provisioning and mortality rates of adults have to be monitored continuously. Experimental basics such as choice of easily assessable nesting material, hatching times as well as activity patterns and longevity of the solitary bee species over the season were methodologically addressed by some studies (Bosse et al., 2014; Dietzsch et al., 2014; Knäbe et al., 2016; Kondagala et al., 2016). They gave valuable information for semi-field ring tests, which resulted in repeatable and meaningful results of the measured endpoints (Knäbe et al., 2019). Manipulative approaches that combined laboratory methods with field exposure conditions (e.g. experiments on contaminating nesting material; Jütte et al., 2018) highlighted specific exposure routes with little relevance to honey bees and bumble bees. Further experimental aspects such as disturbance during the nesting phase due to assessments (Kunz et al., unpublished data) and establishment of a suitable reference substance for brood studies (Lückmann et al., 2018a) have to be considered in future optimizations of test methodologies.

Across species

Besides experiments that only use one species of bee, recent years saw more – primarily laboratory – experiments involving multiple bee species (Arena and Sgolastra, 2014; Uhl et al., 2019). These studies allow direct comparison of sensitivity rather than relying on meta-analyses of data from multiple studies (like discussed in Lewis and Tzilivakis, 2019; Thompson and Pamminger, 2019). Direct comparisons of bees in laboratory and semi-field studies (honey bee vs. bumble bee, honey bee vs. solitary bee; Heard et al., 2017; Sgolastra et al., 2017; Alkassab et al., 2018; Ansell, 2019; Jütte et al., 2019) show clear differences in the sensitivity of bees and in some cases contradict results from the above mentioned meta-analyses. The direct-comparison approach highlights the need for applying the same laboratory and/or environmental conditions on multiple species to better understand and assess effects. Although standardization of test methods for different solitary bee species is still in progress, experimental efforts (Devillers et al., 2003; Arena and Sgolastra, 2014; Uhl et al., 2016; Jütte et al., 2019) have addressed the question of whether and to what extent honey bees are indeed a suitable surrogate for other bee species in the registration process.

Knowledge gaps and outlook

By explicitly integrating other bee species into standardized protocols, the current revision of the EFSA Bee Guidance Document (European Food Safety Authority, 2013) emphasizes the need for a broader risk assessment in the PPP registration process. Most of the recently developed test methods for bumble bees (higher tier trials) and solitary bees (all tiers approaches) indicated some shortcomings; they highlight the need for further improvements in standardizing toxicity tests. The (submitted) manuscripts of the ICPPR non-*Apis* working group on protocols for bumble bees and mason bees under semi-field conditions (Franke et al., 2020; Klein et al., 2020) as well as the results of laboratory ring tests on oral exposure of solitary bees (Roessink et al., 2018) point to model species and test designs for future risk assessment. Further interdisciplinary research can play an integrative role in evaluating and extrapolating these existing data. For example, experiments on the toxicogenomics (Manjon et al., 2018; Beadle et al., 2019; Troczka et al., 2019) and the phylogenetics of bees (Hayward et al., 2019) clarify mechanisms that correlate with the sensitivity of bee species and hence may facilitate finding model species to extrapolate from. Yet, the goal to identify surrogate species among solitary bee species remains extremely difficult to attain due to the huge variability within important life-history traits. The bee species used so far do not adequately cover this variability (e.g. only hole-nesting solitary bee species and bumble bee pollen storers in higher tier studies). Concepts like the "focal species" approach, which is used in higher tier risk assessment of mammals and birds, could give some directions to choosing and testing appropriate bee species (Lückmann et al., 2018b). A 'focal species' is a real species that uses the crop of interest when a pesticide is applied. It is considered to be representative of all other species of the same feeding guild that may occur in the particular crop (European Food Safety Authority, 2009).

Future research should also cover aspects essential for everyday agricultural practice such as the impact of tank mixtures and additives on bee health (Robinson et al., 2017; Carnesecchi et al., 2019; Wernecke et al., 2019), which has not yet been systematically tested on honey bees and other bee taxa. By expanding risk assessment to a landscape level and incorporating modelling approaches, exposure routes and landscape-scale/landscape-context effects of PPPs are evaluated for bee populations/communities rather than individual bees (Danner et al., 2014; Rortais et al., 2017; Simon-Delso et al., 2017; Uhl and Brühl, 2019). This allows for factors such as spatiotemporal migratory population dynamics that are difficult to detect with single field experiments due to limitations of experimental duration and of spatial scale (Uhl and Brühl, 2019). By considering a multitude of potential stressors at various spatial and temporal scales we may be able to minimize or even exclude risks for bees in anthropogenic landscapes.

Conflict of interest

The authors declare that they do not have any conflicts of interest.

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