FLOWER-VISITING INSECTS IN EUROPE

EVALUATING THE ENVIRONMENTAL CONSEQUENCES OF AGRICULTURAL PESTICIDE USE IN THE CONTEXT OF RISK ASSESSMENT

by

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Ticklish

by *At The Drive-In*

pigeon holed decision | making all my mind's made up | i only colored outside the lines | 'cause i got the knack | stenciled teen initials | that were carved on the roof of my mouth | only to bark the words of so-and-so | fuck so-and-so...

i been dancing in the bathroom stalls | excreting words just for this song | i'm kicking in windows | and it don't make music to me

please get some medication | simple. it's simple... | we must die with dignity

pallbearer we are and all that | we never get and all my little pushes | fall on your deaf ears

kicking in these windows | kicking in these windows | it's on the roof of my mouth | i'm gonna bark the words | on the roof of my mouth

tickling with contusions | paper bag masks hiding infantile music | no pictures, just words | are you afraid of our books? | illiterate cells for the valley of mules

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SUMMARY

The European landscape is dominated by intensive agriculture which leads to widespread impact on the environment. The frequent use of agricultural pesticides is one of the major causes of an ongoing decline in flower-visiting insects (FVIs). The conservation of this ecologically diverse assemblage of mobile, flying insect species is required by international and European policy. To counteract the decrease in species numbers and their abundances, FVIs need to be protected from anthropogenic stressors. European pesticide risk assessment was devised to prevent unacceptable adverse consequences of pesticide use on FVIs. However, there is an ongoing discussion by scientists and policy-makers if the current risk assessment actually provides adequate protection for FVI species.

The first main objective of this thesis was to investigate pesticide impact on FVI species. The scientific literature was reviewed to identify groups of FVIs, summarize their ecology, and determine their habitat. This was followed by a synthesis of studies about the exposure of FVIs in their habitat and subsequent effects. In addition, the acute sensitivity of one FVI group, bee species, to pesticides was studied in laboratory experiments.

The second main objective was to evaluate the European risk assessment for possible deficits and propose improvements to the current framework. Regulatory documents were screened to assess the adequacy of the guidance in place in light of the scientific evidence. The suitability of the honey bee *Apis mellifera* as the currently only regulatory surrogate species for FVIs was discussed in detail.

The available scientific data show that there are far more groups of FVIs than the usually mentioned bees and butterflies. FVIs include many groups of ecologically different species that live in the entire agricultural landscape. Their habitats in crops and adjacent semi-natural areas can be contaminated by pesticides through multiple pathways. Environmentally realistic exposure of these habitats can lead to severe effects on FVI population parameters. The laboratory studies of acute sensitivity in bee species showed that pesticide effects on FVIs can vary greatly between species and pesticides. The follow-up critical evaluation of the European FVI risk assessment revealed major shortcomings in exposure and effect assessment. The honey bee proved to be a sufficient surrogate for bee species in lower tier risk assessment. Additional test species may be chosen for higher tier risk assessment to account for ecological differences.

This thesis shows that the ecology of FVIs should generally be considered to a greater extent to improve the regulatory process. Data-driven computational approaches could be used as alternative methods to incorporate ecological trait data in spatio-temporal scenarios. Many open questions need to be answered by further research to better understand FVI species and promote necessary changes to risk assessment. In general, other FVI groups than bees need to be investigated. Furthermore, comprehensive data on FVI groups and their ecology need to be collected. Contamination of FVI habitat needs to be linked to exposure of FVI individuals and ecologically complex effects on FVI populations should receive increased attention.

In the long term, European FVI risk assessment would benefit from shifting its general principles towards more scientifically informed regulatory decisions. This would require a paradigm shift from arbitrary assumptions and unnecessarily complicated schemes to a substantiated holistic framework.

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1 INTRODUCTION AND OBJECTIVES

1.1 FLOWER-VISITING INSECT DECLINE

There is no general consensus on the meaning of the term "flower-visiting insect" (FVI). The term "pollinator" is much more common in the scientific literature. However, most studies have actually not investigated pollination, i.e. pollen deposition on the stigma of a flower, but only visits to flower heads. In this thesis, FVIs are defined as insect species that directly interact with flowers in at least the flying adult life stage, in accordance with Wardhaugh (2015). They include all pollinating insect species but are a far more diverse assemblage. FVIs are an ecologically complex, functional aggregation, which includes species with very different life strategies, e.g. herbivores, predators, and parasites (Ollerton, 2017).

Global insect biodiversity is in decline and FVIs are among the groups where this ongoing trend is best documented (Goulson et al., 2015; Hallmann et al., 2017; IPBES, 2016; Ollerton, 2017; Potts et al., 2015; Powney et al., 2019; Vray et al., 2019). There have been extensive losses of domestic honey bee (*Apis mellifera*) hives and a simultaneous decline in wild bee diversity in many EU countries and the USA (Goulson et al., 2015; Natural Research Council, 2006; Potts et al., 2015; Potts et al., 2010; Powney et al., 2019; vanEngelsdorp et al., 2008; Vray et al., 2019). Populations of butterfly, moth, and syrphid fly species are also declining in the EU (EASAC, 2015; Gilburn et al., 2015; Potts et al., 2015). This long-term decrease in local abundance and regional distribution can be substantial as shown by Hallmann et al. (2017). This study measured a flying insect biomass decline in German nature reserves by more than 75% over a span of 27 years. Negatively affected taxa included FVI groups such as butterflies, bees, flies, and beetles. The result of this biodiversity deprivation process is notable in the number of endangered species. The German red list of bees assessed 53% of species as threatened (Westrich et al., 2011) and the European red list estimated up to 61% (Nieto et al., 2014).

FVI species are an important part of European ecosystems which needs to be preserved because of its ecological and economical value. They pollinate crops and wild plants which ensures stable food production and conservation of natural flora. Insect pollination is important for agriculture since 35% of the global food production volume comes from crops that increase yield when pollinated by animals. Global economic value of pollination services has been estimated as €100-500 billion (Gallai et al., 2009; IPBES, 2016). Pollination is also important for the preservation of wild plants since 85% of all flowering plants are pollinated by animals (Ollerton et al., 2011). Furthermore, FVIs are relevant in the context of general nature protection because they include a major part of faunal biodiversity (30% of arthropod species; Wardhaugh, 2015).

1.2 AGRICULTURAL PESTICIDE USE

In the second half of the 20th century, European agricultural practices began to change drastically. Driven by technological advancements, industrialization and intensification of agriculture progressed rapidly (Robinson and Sutherland, 2002; Stoate et al., 2001). Heavy farming machinery was introduced to replace manual labor and synthetic agrochemicals such as fertilizers and pesticides were extensively applied to provide optimal nutrient levels and protect crops from pests. As a result, crop yields vastly increased in European countries (Green, 2005; Stoate et al., 2001; Tscharntke et al., 2012).
Nowadays, these cropping measures are common agricultural practice. Considerable amounts of pesticides are applied to agricultural areas. About 4,100,000 t (worldwide) and 480,000 t (Europe) of pesticide active ingredients are used in or sold to the agricultural sector for crops and seeds per year (FAO, 2019). The total number of applied pesticides, application frequency, and overall toxic load is continuously increasing (Goulson et al., 2018; Green, 2005). Currently, there are 479 active ingredients registered for use in the EU (European Commission, 2019). As the dominant land use type, farmland covers about half of the EUs surface area (Stoate et al., 2009; Stoate et al., 2001). Therefore, pesticides are omnipresent chemicals that all biota in the agricultural landscape are potentially exposed to.
Pesticides are applied to reduce pest pressure but they can also inadvertently affect non-target species by their toxic mode of action. These negative consequences of pesticide applications have been determined as a serious threat to biodiversity (Geiger et al., 2010; Kleijn et al., 2009; Stoate et al., 2009). The general decrease in FVI species and their abundances is to a large degree driven by exposure to pesticides (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). Other impact factors are habitat loss and fragmentation, fertilizers and environmental pollution, decreasing resource diversity, invasive species, pathogens, and climate change (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). To ensure the protection of FVIs from the negative impact of pesticides, there is a need for a rigorous assessment of pesticide risk before registration for use in the EU.

1.3 EUROPEAN RISK ASSESSMENT

Many intergovernmental organizations authorities and agencies have set protection goals to ensure the safety of FVI populations. The UN "Convention on Biological Diversity" demands the conservation of biodiversity and the sustainable use of biodiversity components (United Nations, 1992). It commits member states to implement national strategies to ensure these protection goals. At the 13th meeting of the Conference of the Parties to the Convention on Biological Diversity, the "Cancun Declaration on Mainstreaming the Conservation and Sustainable Use of Biodiversity for Well-Being" was passed (United Nations, 2016). According to this declaration, member states are obliged to take effective measures to counteract biodiversity loss. It further includes guidelines for the agricultural sector which state concrete actions to promote the effective management and conservation of pollinators. In addition, a group of twelve EU

countries created the "Coalition of the Willing on Pollinators" which commits to protecting pollinators and their habitat and cooperating towards achieving this goal (Coalition of the Willing on Pollinators, 2016).

In the EU, the "Regulation (EC) No 1107/2009 of the European Parliament and the Council" is in place to ensure that pesticides (active ingredients and formulated products) are only introduced onto the market after it has been determined that they are not harmful to human/animal health and the environment. Pesticides shall have no unacceptable effects on non-target species, ecosystems, and biodiversity in general (European Commission, 2009). Therefore, environmental risk assessment for pesticides is a mandatory measure to ensure that the release of these chemicals on farmland has no detrimental consequences on the environment (Newman, 2014). The EU risk assessment framework for FVIs is set in the guidance document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO, 2002). Bee species are covered under a separate risk assessment scheme because of the economic value of the honey bee (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b) whereas all other FVIs are evaluated as non-target arthropods (NTAs; Candolfi et al., 2001).

However, these current schemes have been criticized by the European Food Safety Authority (EFSA) for major shortcomings in effect and exposure assessment and for not taking the specific ecology of these groups into account (EFSA, 2015; EFSA PPR Panel, 2012). EFSA also proposed improvements for future FVI risk assessment (EFSA, 2013; EFSA, 2015). The fact that FVI decline is still continuing in the EU suggests that the regulatory measures in place may be insufficient to ensure the protection of FVI populations from pesticide effects. Scientific studies agree that pesticides are one of the major causes of FVI decline (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). To improve the European risk assessment, there is a need to summarize the evidence on the impact of pesticides on FVIs. A synthesis of the state of knowledge would enable an evaluation of the suitability of risk assessment schemes, the proposal of changes to regulatory guidance documents, and an identification of open research questions. One topic that has been discussed in this context is the suitability of the currently only surrogate species for FVIs in risk assessment, the honey bee *A. mellifera* (EFSA, 2013). There is a need to compare its sensitivity to other proposed surrogates such as the red mason bee *Osmia bicornis*.

1.4 OBJECTIVES AND THESIS OUTLINE

The overall goal of this thesis was to contribute to a currently much-debated field of research: Consequences of agricultural pesticide applications on FVIs and the adequacy of regulatory prevention measures (e.g. Arena and Sgolastra, 2014; Goulson et al., 2015; Gradish et al., 2019; IPBES, 2016; Peters et al., 2016; Rortais et al., 2017; Rundlöf et al., 2015; Sgolastra et al., 2019; Sterk et al., 2016; Stoner, 2016; Thompson and Pamminger, 2019; Wood and Goulson, 2017). The two main research questions were:

- What is the impact of agricultural pesticide use on FVI species?
- Is the European FVI risk assessment framework suitable to protect FVI species and which improvements may be needed?

Therefore, several research objectives are addressed in the following chapters of this thesis.

(i) Identification of relevant FVI groups:
FVI decline has been mostly noted in bees which have been studied much more frequently than other FVI groups but other FVI taxa are also affected. There is a need to determine the relevant FVI groups that have not been clearly identified (Appendix A).

(ii) Description of FVI habitat and ecology:
It is necessary to investigate the ecology of FVI groups and their habitat to assess their probability of pesticide exposure from application on crops (Appendix A).

(iii) Characterization of FVI exposure to pesticides:
Preferential exposure pathways can be described using ecological and habitat information. Afterwards, the quantitative exposure of FVI habitat compartments can be assessed (Appendix A).

(iv) Summary of pesticide effects on FVIs:
The effects of pesticide exposure at environmentally realistic doses need to be investigated to evaluate consequences for FVI populations (Appendix A). In addition, the interspecific sensitivity of bees and substance-specific toxicity of common insecticides are discussed (Appendix B, C and D).

(v) Discussion of the regulatory effect and exposure evaluation in risk assessment schemes:
The combined information on FVI groups, ecology and habitat, pesticide exposure, and pesticide effects enables a critical examination of regulatory guidance to determine possible deficits (Appendix A). A detailed discussion of the suitability of current and proposed additional surrogate species is performed (Appendix B and C).

(vi) Proposal of data-driven tools to improve risk assessment:
To alleviate shortcomings and improve FVI risk assessment, computational methods that make use of ecological data can be used. Two possible approaches are presented (Appendix A).

After the summary and discussion of the combined results of this thesis, a conclusion of the state of knowledge and general outlook follows.

Fig. 1.1 provides a conceptual overview of this thesis, the relationships of the individual research objectives, and reference to the authors' contributions in peer-reviewed literature (Appendix A, B, C) as well as additional unpublished results (Appendix D) that are attached after the main text.

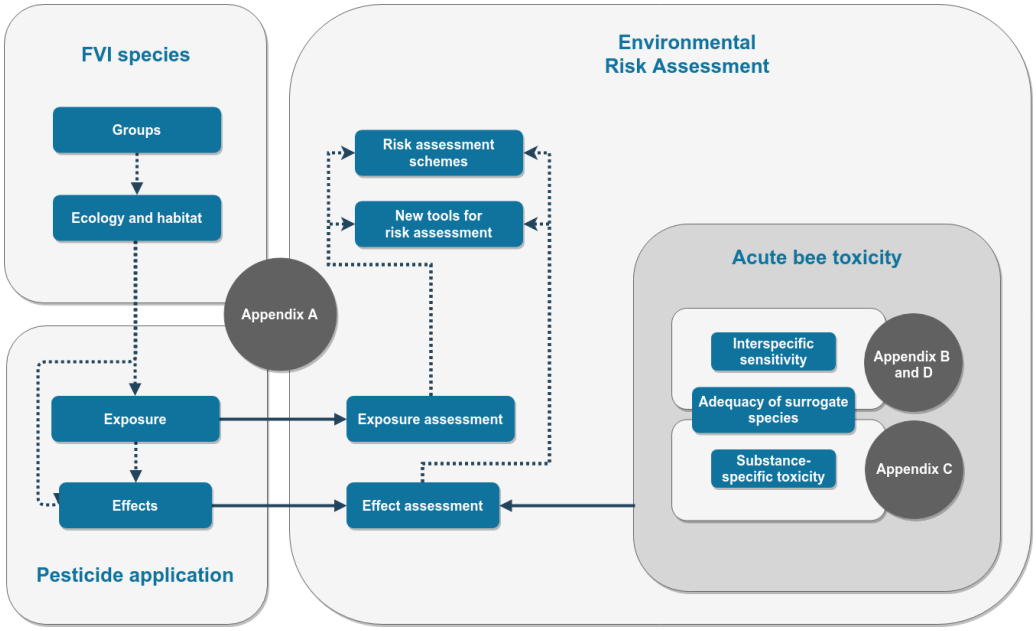

Figure 1.1.: Conceptual overview of the different subjects that were investigated in this thesis.

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2 METHODICAL OVERVIEW

2.1 GENERAL CONCEPT

In this thesis, two approaches have been used to explore the research questions. The available literature on FVI goups, their ecology and habitat, pesticide exposure, and subsequent effects was screened and summarized to evaluate pesticide impact on FVIs. Regulatory documents were synthesized to critically discuss FVI risk assessment in light of the scientific knowledge. In addition, two laboratory acute toxicity studies were conducted to investigate the interspecific sensitivity of the FVI group bee species and the toxic effects of different insecticides on the proposed additional test species *O. bicornis*. These studies contributed information to the slim database of pesticide toxicity on bee species. Results were used to assess the suitability of *A. mellifera* as a surrogate organism by comparing its sensitivity to other bee species.

2.2 REVIEW OF PESTICIDE IMPACT ON FVIS AND RISK ASSESSMENT

The peer-reviewed English-language literature published until the end of 2018 was searched using Google Scholar. Keywords included the following terms and their combinations: "Pollinator", "flower visiting insect", "bee", "butterfly", "moth", "fly", "beetle", "habitat", "trait", "pesticide", "insecticide", "risk assessment", "exposure", "residue", "effect", and "toxicity". Researchgate suggestions (researchgate.net) were also considered as well as results of a continuous Sparrho search (sparrho.com) which used the keywords "pollinator", "pesticide", and "bee". Some papers were selected after recommendations from scientific colleagues and additional papers were obtained from backtracking literature references. Finally, EU regulatory documents were screened to gain detailed knowledge about European risk assessment for bees and non-target arthropods (NTAs). All of the obtained literature was subsequently screened, organized, and synthesized (Appendix A).

2.3 BEE ACUTE TOXICITY EXPERIMENTS

2.3.1 *Testing wild bee species with dimethoate*

Eight different bee species were studied for their sensitivity to a formulation of the organophosphate insecticide dimethoate (Perfekthion®, BASF) in two separate test runs

(Appendix B and D). Dimethoate was chosen because it is used as a toxic standard in regulatory honey bee testing. Tested bee species include *Andrena flavipes* ♀ (Panzer), *Andrena gallica* ♀ (Schmiedeknecht), *Bombus lapidarius* workers (Linneaus), *Bombus terrestris* workers (Linneaus), *Colletes hederae* ♀/♂ (Schmidt & Westrich), *Lasioglossum malachurum* ♀ (Kirby), *Lasioglossum politum* ♀ (Schenck), and *Osmia bicornis* ♀/♂ (Linneaus) (Fig. 2.1, Appendix B and D). Test individuals were either caught in the in the agricultural landscape around Landau, Germany with permission of regional authorities or ordered from a commercial breeder.

Figure 2.1.: Selection of tested bee species. From left to right: *L. malachurum* ♀, *O. bicornis* ♂, *A. flavipes* ♀, *C. hederae* ♀, *O. bicornis* ♀, *B. terrestris* worker. Picture taken by Lea A. Franke.

Body weight of individual bees was measured first. Afterwards, acute, contact toxicity tests were performed with the test species. All tests were conducted according to the ringtest protocol for solitary bee acute contact toxicity developed by the International Commission for Plant-Pollinator Relationships (ICPPR; Roessink, 2014) with minor modifications that are specified in Appendix B. Acute median lethal doses (48h LD50) were calculated for all test species as a proxy for sensitivity. Interspecific differences in sensitivity were investigated using hypothesis testing. Where multiple 48h LD50 values were available, a geometric mean was calculated. A species sensitivity distribution (SSD; Posthuma et al., 2002) was fitted to experimental 48h LD50 values plus additional literature data to derive the 5% hazardous dose (HD5) and 95% lower confidence limit HD5. Fore a detailed account of the experimental methods see Appendix B.

2.3.2 *Testing Osmia bicornis with multiple insecticides*

A total of 16 commercial insecticide formulations were investigated for their toxicity to the red mason bee *O. bicornis*. The majority of tested insecticides were chosen with respect to the application frequency of their commercial products in apple, grapes, and winter oilseed rape (Table 2.1) which represent three of the main cultivation types in Germany according to the Julius Kühn-Institut (2018). The reasons for choosing the remaining formulations are detailed in Appendix C.
Body weight of individual bees was measured first. Afterwards, acute, contact toxicity tests of the insecticides with *O. bicornis* females were performed according to the

revised ICPPR solitary bee acute contact toxicity testing protocol (Roessink et al., 2016) with minor modifications that are specified in Appendix C. Acute median lethal doses (48h LD50) were calculated to measure the toxicity of all tested insecticidal products. Honey bee contact 48h LD50 values were collected from regulatory documents and data inquiries to national and European authorities, manufacturers, and EFSA. *Apis mellifera* and *O. bicornis* endpoints were compared by calculating sensitivity ratios (Arena and Sgolastra, 2014). Fore a detailed account of the experimental methods see Appendix C.

Table 2.1.: Tested insecticides and their usage in German agriculture. The usage share signifies the prominence of a certain compound with regard to all pesticide applications. It is based on the standardised treatment index (STI) which is defined as the number of pesticide applications in a crop in relation to the application rate and cultivated area (Julius Kühn-Institut, 2018; Sattler et al., 2007). Data from Julius Kühn-Institut (2018). Table taken from Appendix C.

Insecticide (a.i.)	Class	Share of application index per culture (2015/2016)			Tested product
		Apple	Grapes	Winter oilseed rape	
alpha-cypermethrin	pyrethroid	/	/	16.8 / 16.1	FASTAC® SC
beta-cyfluthrin	pyrethorid	/	/	12.1 / 13.3	Bulldock®
deltamethrin	pyrethorid	/	/	3.4 /	Decis® Forte
etofenprox	pyrethroid	/	/	12.4 / 18.5	Trebon® 30 EC
lambda-cyhalothrin	pyrethroid	/	/ 3.3	19.5 / 24.6	Karate® Zeon
zeta-cypermethrin	pyrethorid	/	/	2.8 / 4.5	Fury® 10 EW
acetamiprid	neonicotinoid	5.2 / 8.4	/	2.0 /	Mospilan® SG
imidacloprid	neonicotinoid	/	/ 3.0	/	Confidor® WG 70
thiacloprid	neonicotinoid	12.5 / 10.2	/	16.1 / 6.9	Calypso®
dimethoate	organophosphate	/	/	/	PERFEKTHION®
chlorpyrifos	organophosphate	/	/	/	Pyrinex®
chlorantraniliprole	pyridylpyrazole	23.7 / 26.9	/	/	Coragen®
flupyradifurone	unclassified	/	/	/	Sivanto® SL 200 G
indoxacarb	oxadiazine	3.8 / 3.3	44.3 / 34.6	2.3 / 2.9	AVAUNT® 150 EC
pirimicarb	carbamate	19.5 / 15.0	/	/	Pirimor®
spinosad	spinosyn	/	/ 27.7	/	SpinTor®

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3 SUMMARY OF FINDINGS AND GENERAL DISCUSSION

3.1 DIVERSITY OF FVI COMMUNITIES

The particular groups of FVIs are sparsely identified in the literature. Bee species are usually mentioned as the main group as well as lepidopterans (moths and butterflies), and flies (mainly hover flies) (Winfree et al., 2011). However, this classification is only partially substantiated by data and rather a result of research bias towards these taxa (Ollerton, 2017; Wardhaugh, 2015). Theoretical studies suspected that FVIs are a much more complex aggregation of species that includes bees, lepidopterans, and flies but also less prominent groups such as beetles, wasps, and ants. Even unexpected taxa such as thrips, true bugs, springtails, termites, and cockroaches may be relevant flower visitors (Ollerton, 2017; Wardhaugh, 2015). To perform an impact assessment of pesticides, FVI groups need to be identified first. Relevant groups in the context of this thesis are those that frequently visit flowers and are abundant in the agricultural landscape (Appendix A).

Recent field research has provided additional evidence that FVI communities in the agricultural landscape are much more diverse than previously assumed. A study in wildflower plantings in central Germany found a diverse assemblage of FVI species (Grass et al., 2016). Half of the individuals and 75% of the flower visiting species were from groups other than bees or hover flies (Fig. 3.1). Flowers were most frequently visited by dipterans and only to a small degree by butterflies. Beetles and non-hover fly dipterans were detected in similar individual numbers as the presumably abundant honey bee. The importance of non-syrphid flies as flower visitors was emphasized in a meta-analysis of pollinator networks in agricultural, semi-natural, and natural habitats (Orford et al., 2015). Syrphids and non-syrphids mainly visited the same flowers but non-syrphids made up 82% of dipteran abundance and 73% of dipteran species on farmland. A similarly diverse community of FVI species was also found in the Biodiversity Exploratories; a large-scale, long term project in three German regions (Fischer et al., 2010; Nico Blüthgen, personal communication, 2012). Flower visits on the widely distributed common buttercup *Ranunculus acris* were dominated by fly species (Fig. 3.2). Beetle visits were detected in comparable species and individual numbers to bees.

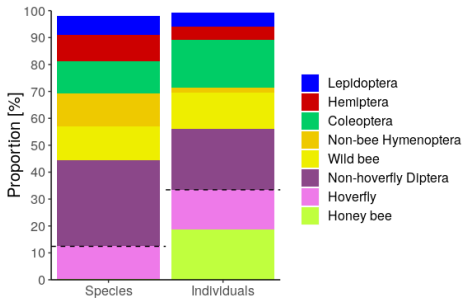

Figure 3.1.: Wildflower planting flower visits in central Germany. The dashed line shows the cumulative fraction of honey bee and hover fly flower visits. Figure adapted from Grass et al. (2016) and taken from Appendix A.

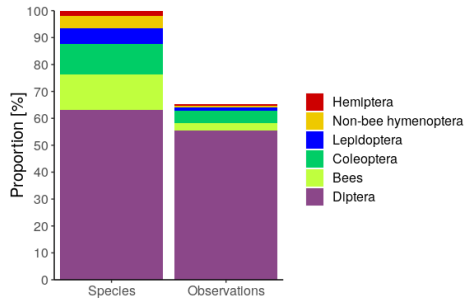

Figure 3.2.: *Ranunuculus acris* visits on Biodiversity Exploratories sites. Data from Nico Blüthgen (personal communication, 2012). Figure taken from Appendix A

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FVI communities are not only diverse on (semi-)natural flora but also on crops. Non-bee species accounted for 38% of flower visits in an extensive meta-analysis of field studies from five continents (Fig. 3.3; Rader et al., 2015). Non-bee flower visits of a typical European mass-flowering crop (oilseed rape *Brassica napus*) varied considerably between studies (5-80%) and even within countries (5-60%).

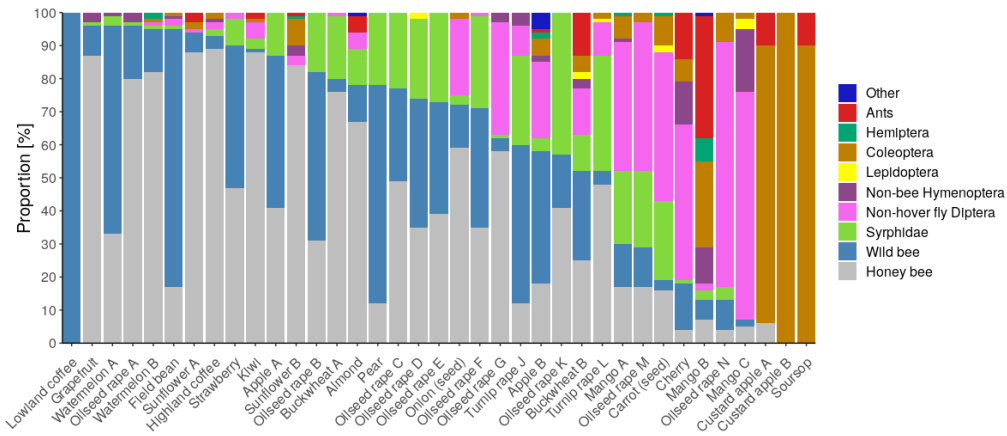

Figure 3.3.: Flower visits by insect taxa in different worldwide crop systems. Figure adapted from (Rader et al., 2015).

This collective information shows that FVI community diversity was underestimated in the past. However, visit rates vary between cropping systems, natural habitats, and geographic locations (Grass et al., 2016; Orford et al., 2015; Rader et al., 2015). Bees, flies (non-syrphids and syrphids), lepidopterans (moths and butterflies), and beetles can clearly be identified as relevant FVI groups in Europe. There are several possible groups

that cannot currently be evaluated due to limited available information such as non-bee hymenopterans and hemipterans, let alone more unexpected groups (Appendix A).

3.2 ECOLOGICAL PROFILE AND HABITAT REQUIREMENTS

3.2.1 *Ecology*

The ecological properties of FVIs differ substantially between species (Ollerton, 2017). These traits determine the potential for exposure to pesticides and subsequent effects on FVI populations (see section 3.6). Exposure incidence is influenced by the spatio-temporal movements of FVIs, preferred food plants, choice of nesting grounds, and the level of social organisation (Brittain and Potts, 2011; de Palma et al., 2015; Sgolastra et al., 2019; Thompson, 2001). Vulnerability of FVI species to environmental stressors depends on the breadth of the ecological niche. Populations of specialist FVI species with low mobility are more sensitive to pesticide effects than populations of mobile generalists (de Palma et al., 2015; Forrest et al., 2015; Hofmann et al., 2019; Williams et al., 2010).

A complete summary of FVI ecology would go beyond the scope of this thesis. For many FVI groups, there also is no comprehensive ecological information available for their entire group. Therefore, FVI ecology is introduced using the example of the well-researched group of bee species. This group is generally representative for many FVI groups in its ecological properties (Appendix A). Specific traits of other groups that are relevant for pesticide exposure and population vulnerability are additionally noted.

All bee larvae and adults feed solely on floral resources. This is unusual for FVIs because in most other relevant taxa only a fraction of species are flower visitors (except lepidopterans). In addition, adults are usually the only florivore life stage of other FVI groups. Adults bees feed almost exclusively on nectar while larvae consume pollen or pollen bread (Michener, 2007). FVI groups such as moths, butterflies, and beetles also include numerous species with herbivore life stages (Ebert, 1994; Koch and Freude, 1992; Scoble, 1995). There are about 2000 bee species in Europe with varying ecological properties. Most species are solitary but some species also live in social colonies or aggregations such as bumble bees. About one fifth of European bee species are parasites that feed on brood rations of other bee species or subdue colonies to tend to their offspring (Michener, 2007; Westrich, 1990). Most bee species build their nest in the soil whereas others excavate deadwood, occupy preexisting cavities in soil or deadwood, or construct nests from collected material (Michener, 2007). Soil is also important for many fly and beetle species whose larvae dwell in the ground (Frouz, 1999; Koch and Freude, 1992). Bees and FVIs in general vary in their food specialization. Polylectic species are generalists but there are also oligolectic species that forage only on a specific plant genus or in extreme cases just one specific plant species (Michener, 2007; Westrich, 1990). Most European bee species begin mating and foraging in spring while some begin their adult stage only as early as summer. Length of active flight period also differs (Westrich, 1990). The major part of European bee species has one brood per

year (univoltine). Only a few species produce offspring throughout the whole year (multivoltine) (Michener, 2007). The daily activity peak of the majority of bee species is around midday but some species also fly in the morning and evening hours (Steen, 2016; Thompson, 2001). The foraging range of bees is extremely variable. Small species can only fly a few meters whereas the biggest species can cover more than ten kilometes (Zurbuchen et al., 2010).
The ecological profile of FVI species influences their potential for pesticide exposure and the vulnerability of their population to pesticide stress (Brittain and Potts, 2011; de Palma et al., 2015; Williams et al., 2010). It also determines the composition of habitats that can sustain FVI populations.

3.2.2 *Habitat*

Knowledge about FVI habitats is essential to assess potential exposure to pesticides. FVIs will only be exposed to pesticides if their habitat is exposed. FVI species require different compartments in an optimal habitat (Appendix A). FVI habitats need to provide food, water, shelter, mating space, and nesting grounds (Table 3.1). There are suitable habitats in the agricultural landscape in crop and non-target areas, e.g. field edge structures or flower strips (Hahn et al., 2015; Marshall and Moonen, 2002; Tschumi et al., 2015). These habitat types differ in structure, plant species, food resource availability, natural enemies, and anthropogenic stressors (Hahn et al., 2015; Marshall and Moonen, 2002; Tschumi et al., 2015). This variation in habitat configuration leads to differences in the attractiveness of habitats for FVI species. However, FVIs preference of certain habitats has usually not been specified by research.

Table 3.1.: Habitat compartments that are used by FVIs. Table taken from Appendix A.

Compartment	Life stages	Function
Airspace	Adults	Food search (foraging), mate search, nest search
Flowers	Adults and florivore larvae	Food collection (foraging), shelter, mating, nesting, nest material collection
Stem/leaves	Adults and herbivore larvae	Food collection (foraging), shelter, mating, nesting, nest material collection
Soil	Adults and soil-dwelling larvae	Nesting, shelter
Water sources	Adults	Water collection / consumption

Many crops have been determined to be attractive habitats for bees and might therefore also be suitable for other FVI species (Appendix A Supporting Information Table A.S1; EFSA, 2013). Mass-flowering crops such as oilseed rape *Brassica napus* and sunflower *Helianthus annuus* are used by wild and managed bees as food supply (Coudrain et al., 2015; Holzschuh et al., 2013; Requier et al., 2015). They provide great amounts of floral resources which are relevant food sources even if these crops are not preferred food plants. Even plantings of crops that are not attractive as food sources can contain undergrowth of associated attractive weeds, e.g. cornflower and poppy species, or

provide other habitat functions such as nesting grounds or temporary refuge (Balmer et al., 2014; Manandhar and Wright, 2016; Storkey and Westbury, 2007). Unfortunately, it is currently not possible to quantitatively evaluate the suitability of particular crops as FVI habitat.

Non-target areas generally present valuable habitats for FVIs. Field edge structures are common semi-natural habitats in agricultural areas, e.g. field margin or hedgerows, which provide many FVI habitat functions (Denisow and Wrzesień, 2015; Marshall and Moonen, 2002; Marshall et al., 2006). Managed flower strips are designed to facilitate insect conservation and specifically sustain pollinator populations which also makes them attractive FVI habitats (Feltham et al., 2015; Haaland et al., 2011; Tschumi et al., 2015). However, there is also not enough information available to further evaluate of the attractiveness of non-target areas.

It is therefore not possible to quantify the suitability of individual habitats (Appendix A). To exercise the precautionary principle, the entire agricultural landscape is considered as FVI habitat in this thesis.

3.3 PESTICIDE EXPOSURE OF FVI SPECIES

3.3.1 *Exposure pathways*

All compartments of FVI habitats are potentially exposed to pesticides (Gradish et al., 2019; Sgolastra et al., 2019; Appendix A). After the direct application to the in-crop areas (primary processes), pesticides can also be unintentionally transported into off-crop, non-target areas (secondary process; Fig. 3.4). Primary processes are usually spray and solid application, e.g. seed treatment or granules (Nuyttens et al., 2013; Walker et al., 2016). Secondary processes include spray drift, field-edge overspray, dust dispersion, and run-off (Schmitz et al., 2015; Walker et al., 2016). This exposure of FVI habitat can lead to exposure of FVI individuals. It is therefore necessary to quantify the contamination of analytic matrices of these habitat compartments and to link this habitat exposure to FVI exposure to identify relevant exposure pathways.

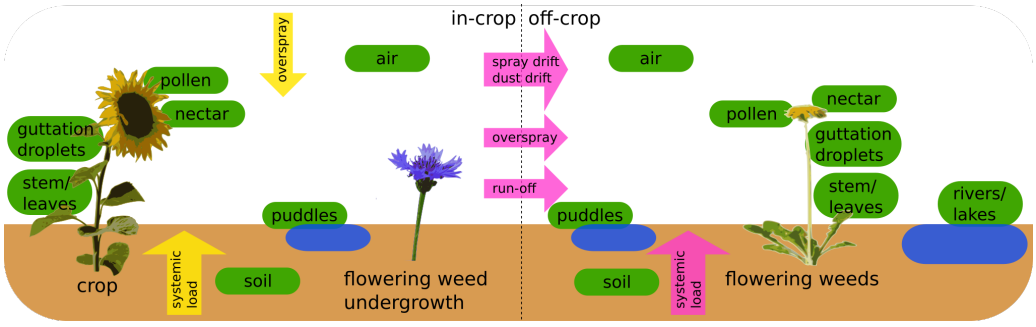

Figure 3.4.: Exposure pathways from application to compartments in in- and off-crop habitats. Yellow up-/downwards arrows indicate primary and pink side-/upwards arrows secondary transport processes. Figure taken from Appendix A.

3.3.2 *Residue studies*

Analytic studies show that FVI individuals in the agricultural landscape are often contaminated with pesticides (Appendix A). There have been numerous investigations in recent years which measured residues of neonicotinoid insecticides in bee species in particular (Blacquière et al., 2012; Bonmatin et al., 2015; Godfray et al., 2014; Godfray et al., 2015; Wood and Goulson, 2017). FVIs are usually exposed to a multitude of pesticides at the same time, as honey bee studies show. All major pesticide classes, i.e. insecticides, fungicides, and herbicides, have been detected in honey bees (Botías et al., 2017; Chauzat et al., 2011; EFSA PPR Panel, 2012; Mullin et al., 2010).

Matrices of different FVI habitat compartments, e.g. nectar/pollen, stem/leave material, soil, and water sources are also contaminated with pesticides (Appendix A). Nectar and pollen are frequently exposed to a number of pesticides (Chauzat et al., 2011; Mullin et al., 2010; Tosi et al., 2018). Residues fluctuate between crops but levels in pollen are consistently higher than nectar doses (Table 3.2; Gierer et al., 2019; Wood and Goulson, 2017). Wild plants in crop-adjacent non-target areas are often exposed to pesticides. Residue doses vary but can be comparable to crop residues (Botías et al., 2015; Mogren and Lundgren, 2016; Wood and Goulson, 2017).

Aside from floral resources, the rest of the plant can also be contaminated with pesticides. Systemic pesticides are especially designed to be taken up by the plant body. Stem and leaf material have been shown to contain levels of neonicotinoid pesticides that are similar to nectar and pollen (Table 3.2; Botías et al., 2016; Mogren and Lundgren, 2016; Pecenka and Lundgren, 2015).

Only a small fraction of applied systemic pesticides are actually taken up by the crop whereas the major part remains in the soil (Alford and Krupke, 2017; Sur and Stork, 2003). Consequently, agricultural soils are frequently contaminated with pesticides. Varying doses of neonicotinoids have been detected in multiple analytic studies (Table 3.2; Botías et al., 2015; Heimbach et al., 2016; Jones et al., 2014; Wood and Goulson, 2017). Neonicotinoid residues can persist in the soil for over one year which can lead to accumulation with doses that are applied in successive years (Bonmatin et al., 2005; Goulson, 2013; Jones et al., 2014).

Water sources of FVI species may also contain pesticides. The detected maximum neonicotinoid concentrations in ephemeral puddles and guttation water are extremely variable but can reach levels that present a risk to bees (Table 3.2; Godfray et al., 2014; Samson-Robert et al., 2014; Schaafsma et al., 2015; Schmolke et al., 2018; Wirtz et al., 2018). Bigger water bodies, e.g. rivers and lakes, next to fields are also frequently contaminated with many pesticides that may be toxicologically relevant for FVI species (Sánchez-Bayo et al., 2016; Stehle and Schulz, 2015).

Table 3.2.: Measured neonicotinoid residues in matrices of FVI habitat compartments. Accumulated data from several publications. See Appendix A for details.

Matrix	Range of measured residues
Nectar	10^{0} ng/g
Pollen	10^{0}–10^{1} ng/g
Stem/leaf	10^{0}–10^{1} ng/g
Soil	10^{-1}–10^{1} ng/g
Water sources	
Puddles	10^{-1}–10^{1} ng/mL
Guttation water	10^{1}–10^{5} ng/mL

3.3.3 *Link of FVI habitat to FVI individual exposure*

With the current knowledge, it is difficult to determine which exposure pathways preferentially lead to FVI exposure or quantify the actual amounts that are taken up from specific matrices (Appendix A). Since many FVIs collect pollen and nectar from a wide variety of plants, the dietary spectrum determines their contamination to a large degree. This also applies to herbivore FVIs that feed on stem/leaf material. Without reliable information of food plant spectrum and quantitative estimates of food uptake it is not possible to identify a clear connection to FVI exposure. There is a trend that pesticide levels in bee-collected pollen are higher when a large proportion of crop pollen is collected (Botías et al., 2015; Cutler et al., 2014; David et al., 2016; Pohorecka et al., 2013; Rundlöf et al., 2015). On the other hand, chronic exposure over the active flight season might be driven by wildflower foraging since crops only flower for a limited time but field-adjacent weeds provide flower supply for the whole season (Botías et al., 2015; Wood and Goulson, 2017). There is also no explicit link of soil to FVI exposure. Although many bee and other FVI species build their nest in the soil or dwell in the soil as larvae, this pathways has been previously overlooked by research and risk assessment (Gradish et al., 2019; Sgolastra et al., 2019). It is mostly unknown to what degree soil residues are bioavailable and if they are taken up by FVIs (Gevao et al., 2000; Gradish et al., 2019; Semple et al., 2003; Sgolastra et al., 2019). The quantitative water uptake of FVI species is usually unknown. In addition, it has been questioned if puddle and guttation water contamination occur frequently enough, especially at maximum levels, to be relevant for FVI exposure (Schaafsma et al., 2015; Schmolke et al., 2018; Wirtz et al., 2018). In general, pesticide residue information is available but it is currently not possible to establish clear connections between habitat exposure and the exposure of FVI individuals (Appendix A).

3.4 EFFECTS OF PESTICIDES ON FVI SPECIES

3.4.1 *Laboratory sensitivity*

For an assessment of pesticide impact on FVIs, the sensitivity of a representative amount of species needs to be determined. Honey bee acute toxicity data is available for all registered pesticides since it is the only FVI test organism in EU risk assessment (Candolfi et al., 2001; OEEP/EPPO, 2010a; OEEP/EPPO, 2010b). Sensitivity of other FVI species is mostly unknown. A review of the few available wild bee acute toxicity data revealed no general pattern of interspecific sensitivity (Arena and Sgolastra, 2014). Relative susceptibilities of bee species are specific for every pesticide which makes an extrapolation of toxicity data between bee species unreliable (Biddinger et al., 2013; Heard et al., 2017). However, dividing the honey bee LD50 by an assessment factor of 10 covered wild bee species sensitivity in 95% of all cases in the meta-analysis by Arena and Sgolastra (2014). This approach was also proposed by EFSA (2013) to account for interspecific acute sensitivity differences of bees in regulatory testing.

To expand the bee toxicity dataset, this thesis investigated the acute contact toxicity of additional wild bee species to determine their sensitivity to the toxic reference dimethoate (Appendix B). Additional data from continued investigations was added to this dataset (Appendix D) and a species sensitivity distribution (SSD) was modeled to calculate a hazardous dose (HD5). The HD5 95% lower confidence limit was comparable to the honey bee 48h contact LD50 when applying an assessment factor of 10 (0.01 and 0.02 µg a.i/bee respectively; Fig. 3.5). Sensitivity to dimethoate varied considerably between bee species by a maximum of two orders of magnitude. Body weight was identified as a partial predictor for bee sensitivity to dimethoate. Smaller species were generally more sensitive than larger species (Appendix B).

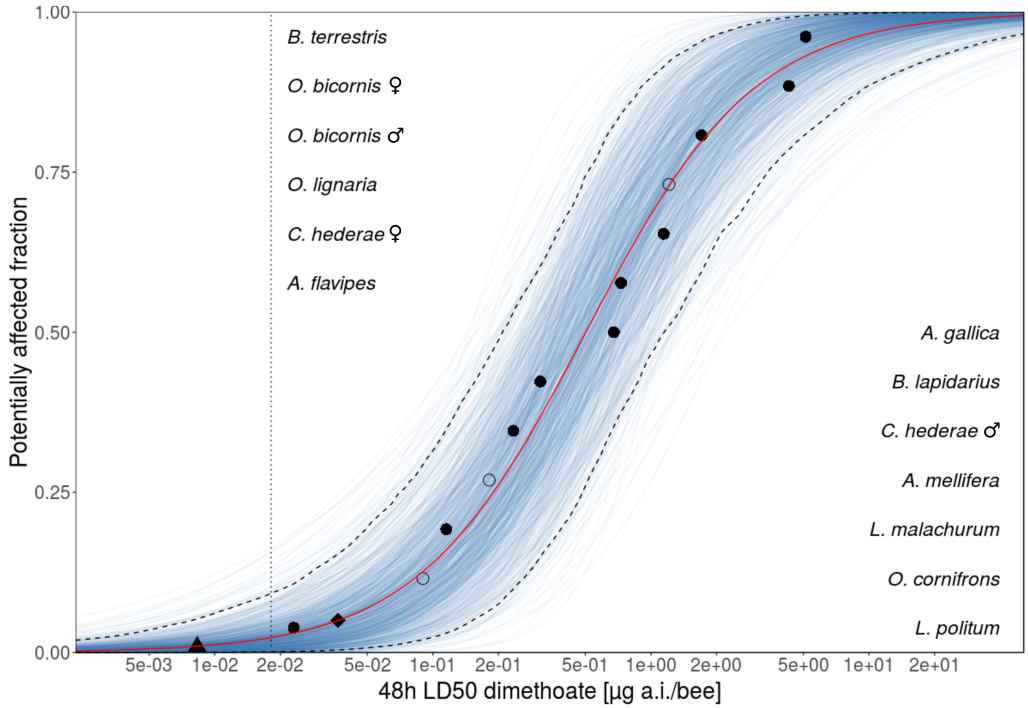

Figure 3.5.: Species sensitivity distribution of dimethoate calculated from multiple bee species' acute sensitivity. ● and ○ denote 48 h LD50 values of bee species (○ are literature values). Species names are aligned by sensitivity in ascending order from bottom to top on the same y-axis coordinate as their respective ●/○. Dashed lines enclose parametric bootstrap 95% CI (1000 iterations). Blue, transparent lines display all parametric bootstrap samples. ◆ marks the HD5 value, ▲ the lower limit HD5. The proposed regulatory threshold of honey bee LD50/10 is indicated by the dotted line. Figure taken from Bereswill et al. (2019). Adapted from Appendix B and complemented with additional data (Appendix D).

In addition, the acute toxicity of several common insecticides to the red mason bee *O. bicornis* was compared with the honey bee, in this thesis (Appendix C). Insecticide toxicity varied substantially between the tested substances. When comparing the most and least toxic insecticides, imidacloprid was 3679 times more toxic than pirimicarb. 69% of all tested substances had 48h LD50 values below 2 µg a.i./bee. For two thirds of the evaluated 15 substances, *O. bicornis* was less sensitive than the honey bee (Fig. 3.6). In 87% off all cases, dividing the regulatory honey bee endpoint by a factor of 10 covered *O. bicornis*' sensitivity (Appendix C).

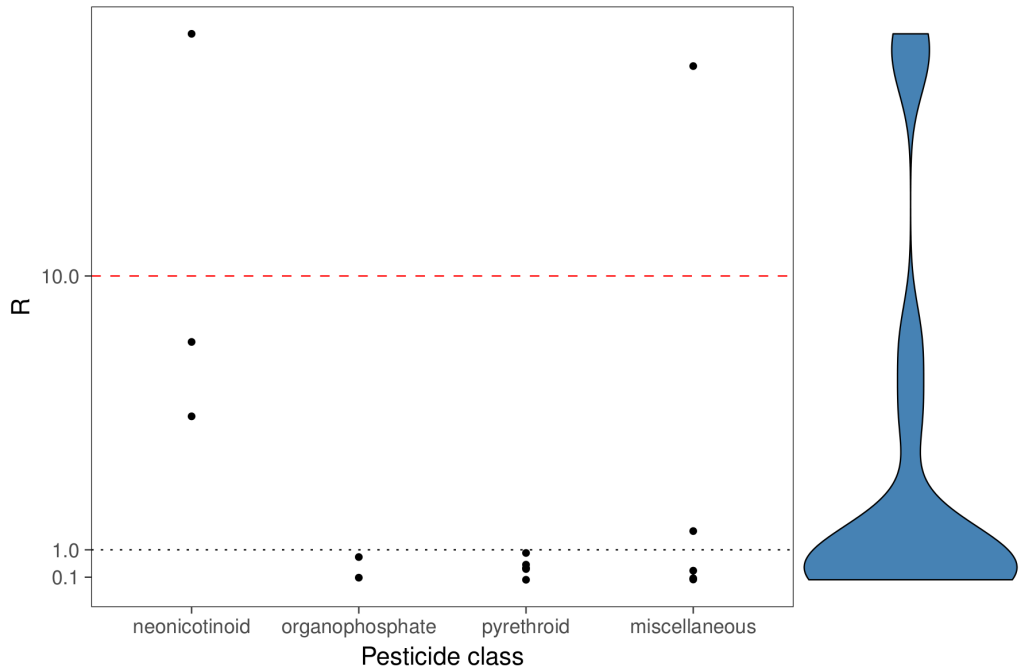

Figure 3.6.: Sensitivity ratio (R) of all tested insecticides grouped by insecticide class. The dotted, grey line signifies equal sensitivity of *O. bicornis* and *A. mellifera*. The dashed, red line indicates the insecticides whose toxicity towards *O. bicornis* would be covered when dividing the honey bee endpoint by an assessment factor of 10. The violin plot on the right shows the distribution of data points. R is the sensitivity ratio defined as $LD50_{A.\ mellifera}/LD50_{O.\ bicornis}$. $n_{neonicotinoid}=3$, $n_{organophospahte}=2$, $n_{pyrethroid}=5$, $n_{miscellaneous}=5$. Figure taken from Appendix C.

3.4.2 *Semi-field and field studies*

Since the restriction of the use of the neonicotinoids imidacloprid, clothianidin, and thiamethoxam in 2013, there have been numerous semi-field and field experiment to further investigate the effects of environmentally realistic neonicotinoid exposure on bee species (Wood and Goulson, 2017). Information for other FVI groups or other pesticide classes is still scarce (Appendix A). Effects of neonicotinoids on honey bees are small to negligible as shown in several colony-level studies (Cutler et al., 2014; Dively et al., 2015; Pilling et al., 2013; Rundlöf et al., 2015). Due to ecological differences to the honey bee, e.g. social structure, individuals in a colony/population, most other European bee species are more prone to population-level pesticide effects (Stoner, 2016; Wood and Goulson, 2017). Honey bee (semi-)field effects have been summarized in multiple review articles (e.g. Blacquière et al., 2012; Godfray et al., 2014; Godfray et al., 2015; Goulson, 2013; Pisa et al., 2015; Pisa et al., 2017) and will not be further discussed in this thesis.

Multiple studies detected reproduction and colony growth effects of neonicotinoid insecticides in non-*Apis* bee species (Appendix A). In most cases these studies investi-

gated the buff-tailed bumble bee *Bombus terrestris*, but also other bumble bee species, as well as the solitary bee *O. bicornis*. A wide array of effects on reproductive parameters such as a reduced number of constructed brood cells, reduced worker, male and queen offspring (colony growth), reduced individual growth, and skewed sex ratio has been shown for these species (Cutler and Scott-Dupree, 2014; Ellis et al., 2017; Gels et al., 2002; Main et al., 2018; Moffat et al., 2016; Moffat et al., 2015; Rundlöf et al., 2015; Whitehorn et al., 2012). However, there are also a few studies that found no adverse effects (Peters et al., 2016; Ruddle et al., 2018; Sterk et al., 2016) which to a large degree results from differences in the chosen exposure scenarios and subsequently lower exposure levels.
Neonicotinoids also cause altered foraging behavior. Bumble bee experiments generally resulted in increased number of foraging trips to flowers but decreased foraging efficiency. Several studies noted increases in forage trip length or a reduction in the number of successful trips (Feltham et al., 2014; Gill and Raine, 2014; Gill et al., 2012; Stanley et al., 2015; Stanley and Raine, 2016).
Neonicotinoids have been connected to immune system effects in several honey bee experiments (e.g. Alburaki et al., 2015; Dively et al., 2015; Pettis et al., 2012; Vidau et al., 2011). It is suspected that pesticides also make other bee species more susceptible to diseases and parasites (Wood and Goulson, 2017).

3.4.3 *Ecologically complex issues*

Many ecologically relevant but complex effects of pesticides on FVIs have been overlooked by research (Appendix A). These effects result from intra- and interspecific interactions and only become visible at the population and community level (EFSA, 2015).
FVIs are mobile species that can move freely between multiple habitats within the landscape. Their advanced spatial movement can lead to migratory effects. Migration from off-field habitats to pesticide-treated crops can result in adverse source-sink dynamics: Individuals from a sustaining habitat migrate to a non-sustaining habitat and subsidize the sink population but also deplete the source population (Topping et al., 2015). On a small scale this overall adverse effect can seem like local recovery (Topping et al., 2014). Therefore, migratory population dynamics in space and time can only be detected by landscape-scale modeling and monitoring approaches (Topping et al., 2015).
Trophic interactions in farmland ecosystems can lead to an indirect propagation of pesticide effects. FVI habitat quality can decrease because food and nesting resources are reduced or modified (Relyea and Hoverman, 2006; Rohr et al., 2006). In the worst case, this can lead to habitat loss which is one of the major causes of FVI decline and also a byproduct of widespread herbicide use (Forister et al., 2016; Goulson et al., 2015; Ollerton, 2017). There is currently no information available to assess the severity of herbicide-induced indirect effects on FVIs.
FVI species are supposed to be protected from pesticide effects to preserve their ecosystem services pollination and biodiversity, amongst other things (European Commission, 2009; United Nations, 1992). However, nearly nothing is known about the effects of pes-

ticides on FVI pollination services. The only available study on this subject found a negative effect of a neonicotinoid on the pollination service of *B. terrestris* on apple (Stanley et al., 2015). Long-term pesticide effects on FVI diversity have also not been investigated. It is very difficult to detect such effects because that would take enormous sampling efforts over multiple years. There is only correlative evidence of effects on bee species' population dynamics at this point (Woodcock et al., 2016).

3.5 DISCUSSION OF FVI RISK ASSESSMENT

3.5.1 *Bee species*

The collective information on the relevant FVI groups, their ecology and habitat, exposure to pesticides, and subsequent effects, allows for a critical evaluation of the European exposure and effect assessment (Appendix A). The current bee risk assessment scheme has been reviewed by EFSA PPR Panel (2012) and EFSA (2013) who identified major deficits regarding the protection of this FVI group (Table 3.3). Bee ecology is not sufficiently considered at several points in the effectual guidance documents (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b). There is no exposure assessment for off-field areas which are FVI habitats (EFSA, 2013; EFSA PPR Panel, 2012). Possible contamination of bees from abrasion dust after solid applications or from water sources is not evaluated. The entire group of bees is not sufficiently represented by the only surrogate species, the honey bee *A. mellifera*. Furthermore, current semi-field and field testing designs have been criticized by EFSA PPR Panel (2012) for their insufficiency to detect statistical effects because they allow too much variance, e.g. small sample size, low number of replicates at site levels, and short study duration.

EFSA (2013) drafted an improved guidance document which has yet to be ratified by the EU member states (Table 3.3). They suggested an expanded assessment of additional exposure pathways for in-field and off-field scenarios. Two additional test species were proposed for lower and higher tier effect assessment (*B. terrestris* and *O. bicornis*/*O. cornuta*). Chronic oral and larval toxicity tests were added to first tier assessment and they proposed enhanced higher tier test designs to increase statistical power (Appendix A).

Notwithstanding these improvements, there still remain several issues that have not been addressed by EFSA (2013). Soil exposure evaluation is not included in the proposed guidance although it has been determined as an underestimated source of contamination (Gradish et al., 2019; Sgolastra et al., 2019). Honey dew and extrafloral nectars are discussed by EFSA (2013) as exposure sources but their relevance for FVIs is not justified by available data. There are more general issues that have not yet been accounted for in bee risk assessment (Table 3.3). Ecologically complex consequences of pesticide use such as source-sink effects, indirect effects through trophic interactions and ecosystem service effects (pollination, biodiversity) are not considered. There is also no framework to assess the risk of simultaneous exposure to multiple pesticides as a result of sequential application or tank mixtures (Appendix A).

The adequacy of current and proposed additional test species has been extensively discussed in the scientific community (Arena and Sgolastra, 2014; EFSA PPR Panel, 2012; Heard et al., 2017; Hinarejos et al., 2019; Thompson, 2016; Thompson and Pamminger, 2019; Appendix B; Appendix C). There is reasonable doubt that the new test species are suitable to decrease uncertainty in lower tier effect assessment. A meta-analysis compared honey bee with wild bee toxicity studies and concluded that dividing the honey bee acute toxicity endpoint by an assessment factor of 10 would cover wild bee sensitivity (Arena and Sgolastra, 2014). EFSA (2013) proposed this approach to account for interspecific acute sensitivity differences of bees in regulatory testing. Two acute contact toxicity laboratory studies that were conducted as part of this thesis reaffirmed these results. The first study showed that *Bombus terrestris* and *O. bicornis* are less sensitive to the toxic standard dimethoate than the honey bee (Appendix B; Appendix D). Applying the assessment factor of 10 on the honey bee endpoint lead to a value comparable to the 5% hazardous dose (from SSD) that indicates a sufficient level of protection for bee communities. The second study found that the assessment factor also covers the sensitivity of the proposed test species *O. bicornis* to nearly all of tested common insecticide formulations (Appendix C). *Osmis bicornis* was also less sensitive than the honey bee in the majority of cases. An oral acute toxicity laboratory study also showed that the honey bee can cover *B. terrestris*' and *O. bicornis*' if a reasonable assessment factor is applied (Heard et al., 2017). Therefore, it is unnecessary to include additional test species in lower tier effect assessment (Thompson and Pamminger, 2019; Appendix B; Appendix C). However, additional test species should be used in higher tier effect assessment where interspecific differences in ecological properties such as sociality, life cycle, or behavior have a substantial impact on pesticide effects (Gradish et al., 2019; Rundlöf et al., 2015; Sgolastra et al., 2019; Appendix B; Appendix C). Semi-field and field test protocols for non-*Apis* bee species need to be established to that end. Furthermore, the ambitious higher tier study designs that EFSA (2013) proposed may need to be revised because they are difficult to practically implement in the field, e.g. excessive distances between sites, too many replicates (Bakker, 2016).

3.5.2 *Non-bee FVI species*

The NTA risk assessment scheme that incorporates all non-bee FVIs (Candolfi et al., 2001) was also criticized by EFSA (2015) in a scientific opinion. They identified several deficits because FVI ecology was not sufficiently taken into account (Table 3.3). There is no exposure assessment for seed coating dust. Oral testing is not mandatory in tier one effect assessment and no FVI surrogate species is included. Current test species are all beneficial insects, e.g. *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Orius laevigatus*, *Chrysoperla carnea*, *Coccinella septempunctata*, *Aleochara bilineata*. Several adjustments were suggested (Table 3.3; EFSA, 2015). Pesticide contamination through food uptake should be evaluated by measuring residues in food items. A soil surface exposure evaluation was included. Furthermore, an additional FVI test species (lepidopteran larvae) was proposed (Appendix A).

Aside from these improvements of details, EFSA (2015) called for the development of a landscape-scale risk assessment to ensure that mobile species such as FVIs are sufficiently protected from in-field and off-field effects. They also discuss previously neglected issues such as indirect effects, source-sink dynamics, and ecosystem service effects and recommend an evaluation of sequential and simultaneous pesticide use.
Some deficits still remain unaddressed such as an in-soil or guttation water exposure assessment. It is unclear if the proposed additional lepidopteran test species would be representative for all non-bee FVIs due to their differences in ecological properties. In general, there are no protocols available in the scientific opinion by EFSA (2015) to specify effect and exposure assessment which should be addressed in an upcoming NTA guidance document (Appendix A).

Table 3.3.: FVI risk assessment guidelines, regulatory deficits and development. + indicates an addition to the existing guidance. Table taken from Appendix A.

	Regulatory documents	FVI test organisms	Effect assessment	Exposure assessment	Exposure scenarios	Deficits
Bees	Current guidance document (OEEP/EPPO, 2010b; OEEP/EPPO, 2010a)	*A. mellifera*	First tier: Acute contact/oral, brood feeding Higher tier: chronic oral, semi-field, field	Spray, nectar, pollen	In-field	Test organism not representative, insufficient exposure assessment
	New guidance document (EFSA, 2013)	+ *O. bicornis*, *B. terrestris*	First tier: + Chronic oral, chronic larvae Higher tier: + improved quality in semi-field, field protocols	+ Dust, guttation water, surface waters, puddles	+ Off-field	No soil exposure assessment, uncertainty about suitability of additional test species, missing or unvalidated semi-field and field test protocols, ecologically complex issues not incorporated
Non-bee FVIs	Current guidance document (Candolfi et al., 2001)	None	First tier: Acute/chronic contact Higher tier: Extended lab, aged residue, semi-field, field	Spray/residue	In-field, off-field	No FVI species, insufficient exposure assessment
	New scientific opinion (EFSA, 2015)	+ 1 lepidopteran species	Higher tier: + Landscape-scale assessment	+ Nectar, pollen, plant material, dust	No change	No in-soil & guttation water exposure assessment, uncertainty if additional test species is adequate surrogate for FVIs, lacking concrete guidance

3.6 FUTURE DATA-DRIVEN APPROACHES

3.6.1 *Effect and exposure assessment based on trait data*

Ecological information can be used to improve the risk assessment by eliminating many of the existing shortcomings (Appendix A). It is currently not possible to determine consequences of pesticide applications for FVI populations and communities. In theory, the susceptibility of populations to environmental stressors is influenced by the breadth of their ecological niche (de Palma et al., 2015; Forrest et al., 2015; Hofmann et al., 2019; Williams et al., 2010). Ecological trait data can be utilized to assess vulnerability of FVI populations and assign them to threat categories (Fig. 3.7; Brittain and Potts, 2011; Hofmann et al., 2019; Sponsler et al., 2019). Trait-based approaches have also already been discussed for in the inclusion into aquatic risk assessment (Rubach et al., 2011; Van den Brink et al., 2011). The developed concepts can be adapted for FVI species and their ecological profile. There are multiple traits that influence FVI vulnerability. For bee species, identified traits include mobility, sociality, nesting, lecty (level of food plant preference), flight season/duration, and voltinism (Appendix A Supporting Information Table A.S2). When trait and toxicity data are connected, it is possible to predict impacts of pesticides on FVI communities.

Similarly, trait data can be analyzed for the use in exposure assessment. This enables an evaluation of the probability of pesticide uptake from different habitat matrices (Brittain and Potts, 2011; de Palma et al., 2015; Sgolastra et al., 2019; Sponsler et al., 2019). Several relevant traits have been identified for bee species, e.g. flight activity throughout the year, daily flight activity, lecty, nesting (location and construction), sociality, and mobility (Brittain and Potts, 2011; de Palma et al., 2015; Sgolastra et al., 2019; Thompson, 2001). The proportion of potentially exposed FVI species over the course of the season can be assessed by combining active flight period data with pesticide application dates (Fig. 3.7). The connection of trait and application data with residues measurements from habitat matrices allows for an estimation of FVI contamination through individual pathways.

All trait-based analyses require extensive amounts of ecological data and additional monitoring data for validation. Unfortunately, a comprehensive trait database is currently only available for bee species (Roberts et al., 2016). However, if data requirements are met, it would be possible to create a holistic framework that combines effect and exposure assessment as proposed for aquatic organisms (Rubach et al., 2011; Van den Brink et al., 2011).

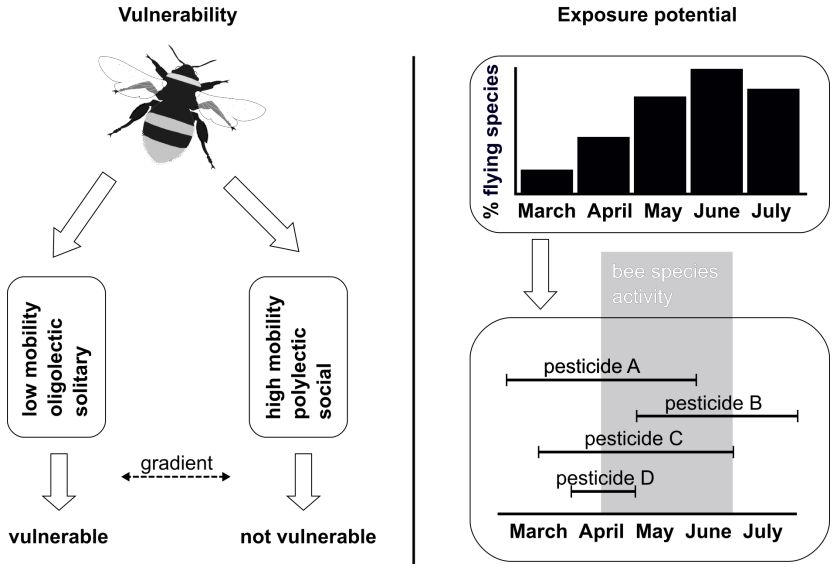

Figure 3.7.: Potential applications of trait data to estimate FVI vulnerability to stressors and likelihood of pesticide exposure. Figure taken from Appendix A.

3.6.2 *Population modeling on the landscape-scale*

Spatio-temporal population dynamics of FVIs and the effects of stressors such as pesticides can be simulated and assessed with modeling approaches (Rortais et al., 2017; Appendix A). A suitable modeling framework is provided by the animal, landscape, and man simulation system (ALMaSS) that can be implemented to assess pesticide effects on selected representative species (Topping et al., 2003). It includes individual-based animal population models in a detailed, dynamic landscape simulation in space and time. These models predict exposure and subsequent effects of pesticides on individuals using animal behavior parameters and pesticide use patterns. Modeling the impact of pesticides on FVI individuals enables a subsequent prediction of consequences for FVI populations. However, this complex suite of models requires extensive data on FVI species' ecology, landscape composition and land use (Topping et al., 2003).
Another available model is BEEHAVE which might be easier to implement for several reasons (Becher et al., 2014; Rortais et al., 2017; Thompson and Pamminger, 2019). This model is specifically designed to simulate pesticide impact on honey bee colonies. An adapted version for bumble bees has already been developed (Becher et al., 2018). Predictive capabilities at the landscape-scale are lower compared to the ALMaSS system but data requirements are also reduced (Rortais et al., 2017). An implementation of other FVI groups is not readily available since the modeling approach is very specific to bees.

3

4 CONCLUSION AND OUTLOOK

Agriculture dominates the European landscape (Stoate et al., 2009; Stoate et al., 2001). The increasing frequency of pesticide applications and overall toxic load necessitates adequate regulatory measures to protect FVI species from adverse long-term impact (IPBES, 2016; Wood and Goulson, 2017). Therefore, policy makers should revise the risk assessment framework with regard to latest research and make use of scientific advances.

This thesis summarized the available knowledge on FVIs and how they are affected by agricultural pesticide use. FVIs are an assemblage of ecologically diverse species that includes many taxa, e.g. bees, flies (non-syrphids and syrphids), lepidopterans (moths and butterflies), and beetles. These species live in crops and crop-adjacent semi-natural habitats which may result in their unintentional contamination with pesticides. FVI individuals and several of their habitat compartments are frequently exposed to varying doses of numerous pesticides in in-field and off-field areas. Environmentally realistic exposure levels can cause a wide array of population-relevant adverse effects in FVI species. This thesis further showed that the acute sensitivity of one FVI group, bee species, varies greatly between species and pesticides. A critical evaluation of the current risk assessment revealed major shortcomings that result from the insufficient incorporation of ecological properties of FVIs. There is an ongoing development to improve the regulatory process which should be continuously pursued to achieve protective measures. The honey bee *A. mellifera* proved to be a suitable surrogate for other bee in lower tier risk assessment. Additional test species might be necessary for higher tier risk assessment where ecological differences are more relevant. FVI ecology could generally be incorporated to a greater extent into risk assessment. Data-driven computational approaches are potential tools that can be used to predict the outcome of pesticide applications for FVI populations by using ecological trait data.

However, there are many open research questions and data gaps that need to be addressed to facilitate a revision of the current risk assessment. FVI research usually concentrates on bees species. All relevant FVI groups need to be identified and their ecology and habitat should be thoroughly investigated. There is a need for a comprehensive FVI trait database. Extensive monitoring campaigns of FVI populations should be implemented to determine the threat level of FVI groups. Exposure of all relevant FVI habitat matrices should be investigated and collated in a pesticide residues database. Habitat exposure needs to be connected to exposure of FVI individuals. Pesticide effects on FVI population should be further assessed, especially considering non-bee FVIs and pesticide classes other than neonicotinoid insecticides. Previously neglected ecological complex issues such as source-sink effects, indirect effects through trophic interactions, and ecosystem service (pollination, biodiversity) effects need extensive research. State-

of-the-art trait-based approaches should be advanced to promote a landscape scale-risk assessment.

In addition to a revision of the current risk assessment schemes with regard to scientific input, pesticide regulation needs a paradigm shift from arbitrary assumptions and unnecessarily complicated schemes to a substantiated holistic framework. Furthermore, it would be reasonable to integrate pesticide risk assessment into broader evaluation approaches of anthropogenic impact. The Group of Chief Scientific Advisors (2018) to the European Commission formulated a long-term vision of an impact assessment of food production as a whole. This would mean an evaluation of the effects of the entire food production chain on humans and the environment to create a sustainable food industry. From a conservation standpoint, this notion should go even further. A collective anthropogenic impact assessment could be conceptualized which would evaluate the consequences of all combined man-made stressors on the environment. FVI decline and species decline in general are caused by the interaction of multiple anthropogenic factors such as pesticides and fertilizers, environmental pollution, land use (habitat loss, habitat change), and climate change (Goudie, 2018; Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). Addressing all of these environmental issues in one framework would be a significant step towards a sustainable coexistence of humans and the environment and the protection of European flora, fauna, and ecosystems.

Aside from regulatory measures, a less intensive agriculture based on an ecological understanding of farmland ecosystems and their surroundings would greatly benefit insect biodiversity (Altieri, 1995; Gliessman, 2014). Ecosystem services of beneficial insects such as pest control can be facilitated by different management practices (Wezel et al., 2014). Promotion of biological pest control by beneficial insects reduces pest pressure which decreases the amount of pesticides that needs to be applied. Integration and management of semi-natural structures, e.g. field edge structures and flower strips, in crops and adjacent areas creates viable habitates for natural enemies of pest species (Haaland et al., 2011; Tscharntke et al., 2007; Tschumi et al., 2016; Wezel et al., 2014). These structures also contribute to FVI conservation and improve crop pollination (Feltham et al., 2015; Haaland et al., 2011; Wezel et al., 2014). Additional measures such as agroforestry and intercropping could further reduce pesticide input. There already is a system in place to encourage farmers to incorporate agroecological management. The second pillar of the EU Common Agricultural Policy (CAP) awards payments for the provision of environmental services in the form of agri-environment measures (European Commission, 2005; European Commission, 2013). To counteract agricultural intensification and biodiversity decline, this development should be promoted by further increasing incentives and providing implementation advice for farmers. Efforts to preserve European FVI species should be generally intensified to stop FVI decline and promote stable FVI populations in the future.

5 REFERENCES

Alburaki, M., S. Boutin, P.-L. Mercier, Y. Loublier, M. Chagnon, and N. Derome (2015). "Neonicotinoid-Coated Zea mays Seeds Indirectly Affect Honeybee Performance and Pathogen Susceptibility in Field Trials". *PloS one* 10 (5), e0125790.

Alford, A. and C. H. Krupke (2017). "Translocation of the neonicotinoid seed treatment clothianidin in maize". *PLOS ONE* 12 (3), e0173836.

Altieri, M. A. (1995). *Agroecology: the science of sustainable agriculture*. 2nd ed. Boulder, Colo. : London: Westview Press ; IT Publications.

Arena, M. and F. Sgolastra (2014). "A meta-analysis comparing the sensitivity of bees to pesticides". *Ecotoxicology* 23 (3), 324–334.

Bakker, F. (2016). "Design and analysis of field studies with bees: A critical review of the draft EFSA guidance: Critical Review of Draft EFSA Guidance on Bees (Field)". *Integrated Environmental Assessment and Management* 12 (3), 422–428.

Balmer, O., C. E. Géneau, E. Belz, B. Weishaupt, G. Förderer, S. Moos, N. Ditner, I. Juric, and H. Luka (2014). "Wildflower companion plants increase pest parasitation and yield in cabbage fields: Experimental demonstration and call for caution". *Biological Control* 76, 19–27.

Becher, M. A., V. Grimm, P. Thorbek, J. Horn, P. J. Kennedy, and J. L. Osborne (2014). "BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure". *Journal of Applied Ecology* 51 (2), 470–482.

Becher, M. A., G. Twiston-Davies, T. D. Penny, D. Goulson, E. L. Rotheray, and J. L. Osborne (2018). "*Bumble* -BEEHAVE: A systems model for exploring multifactorial causes of bumblebee decline at individual, colony, population and community level". *Journal of Applied Ecology* 55.

Bereswill, R., K. Krichbaum, M. Meller, K. Schmidt, C. A. Brühl, P. Uhl, and C. J. Topping (2019). *Protection of wild pollinators in the pesticide risk assessment and management. Final Report. Project No. (FKZ) 3715 64 409 0.* Texte 54/2019. German Environment Agency.

Biddinger, D. J., J. L. Robertson, C. Mullin, J. Frazier, S. A. Ashcraft, E. G. Rajotte, N. K. Joshi, and M. Vaughn (2013). "Comparative toxicities and synergism of apple orchard pesticides to Apis mellifera (L.) and Osmia cornifrons (Radoszkowski)". *PloS one* 8 (9), e72587.

Blacquière, T., G. Smagghe, C. A. M. Gestel, and V. Mommaerts (2012). "Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment". *Ecotoxicology* 21 (4), 973–992.

Bonmatin, J.-M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, Mitchell, E A D, D. A. Noome, N. Simon-Delso, and A. Tapparo (2015). "Environmental fate and exposure; neonicotinoids and fipronil". *Environmental science and pollution research international* 22 (1), 35–67.

Bonmatin, J. M., P. A. Marchand, R. Charvet, I. Moineau, E. R. Bengsch, and M. E. Colin (2005). "Quantification of imidacloprid uptake in maize crops". *Journal of agricultural and food chemistry* 53 (13), 5336–5341.

Botías, C., A. David, E. M. Hill, and D. Goulson (2016). "Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects". *Science of The Total Environment* 566-567, 269–278.

Botías, C., A. David, E. M. Hill, and D. Goulson (2017). "Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes". *Environmental Pollution* 222, 73–82.

Botías, C., A. David, J. Horwood, A. Abdul-Sada, E. Nicholls, E. Hill, and D. Goulson (2015). "Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees". *Environmental science & technology* 49 (21), 12731–12740.

Brittain, C. and S. G. Potts (2011). "The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination". *Basic and Applied Ecology* 12 (4), 321–331.

Candolfi, M., K. Barrett, P. Campbell, R. Forster, N. Grandy, M. Huet, G. Lewis, P. Oomen, R. Schmuck, and H. Vogt (2001). *Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods: From the ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropods Regulatory Testing), a joint BART, EOOP/CoE, OECD and IOBC Workshop organized in conjunction with SETAC-Europe and EC: Held at Wageningen International Conference Centre; Wageningen, The Netherlands; 21-23 March 2000*. Pensacola FL (USA): Setac Office.

Chauzat, M.-P., A.-C. Martel, N. Cougoule, P. Porta, J. Lachaize, S. Zeggane, M. Aubert, P. Carpentier, and J.-P. Faucon (2011). "An assessment of honeybee colony matrices, Apis mellifera (Hymenoptera: Apidae) to monitor pesticide presence in continental France". *Environmental Toxicology and Chemistry* 30 (1), 103–111.

Coalition of the Willing on Pollinators (2016). *Declaration on the Coalition of the Willing on Pollinators*. Cancun (Mexico).

Coudrain, V., S. Rittiner, F. Herzog, W. Tinner, and M. H. Entling (2015). "Landscape distribution of food and nesting sites affect larval diet and nest size, but not abundance of Osmia bicornis". *Insect Science* 23 (5), 746–753.

Cutler, G. C. and C. D. Scott-Dupree (2014). "A field study examining the effects of exposure to neonicotinoid seed-treated corn on commercial bumble bee colonies". *Ecotoxicology* 23 (9), 1755–1763.

Cutler, G. C., C. D. Scott-Dupree, M. Sultan, A. D. McFarlane, and L. Brewer (2014). "A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success". *PeerJ* 2, e652.

David, A., C. Botías, A. Abdul-Sada, E. Nicholls, E. L. Rotheray, E. M. Hill, and D. Goulson (2016). "Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops". *Environment international* 88, 169–178.

de Palma, A., M. Kuhlmann, S. P. Roberts, S. G. Potts, L. Börger, L. N. Hudson, I. Lysenko, T. Newbold, A. Purvis, and I. Kaplan (2015). "Ecological traits affect the sensitivity of bees to land-use pressures in European agricultural landscapes". *Journal of Applied Ecology* 52 (6), 1567–1577.

Denisow, B. and M. Wrzesień (2015). "The Importance of Field-Margin Location for Maintenance of Food Niches for Pollinators". *Journal of Apicultural Science* 59 (1), 27–37.

Dively, G. P., M. S. Embrey, A. Kamel, D. J. Hawthorne, and J. S. Pettis (2015). "Assessment of chronic sublethal effects of imidacloprid on honey bee colony health". *PLoS ONE* 10 (3), e0118748.

EASAC (2015). *Ecosystem services, agriculture and neonicotinoids: EASAC policy report 26*. Vol. 26. Halle (Saale): German National Academy of Sciences.

Ebert, G., ed. (1994). *Die Schmetterlinge Baden-Württembergs, Bd.3, Nachtfalter 1: Allgemeiner Teil: Benutzerhinweise, Beobachtungsmethoden bei Nachtfaltern, Aspekte zur Nahrungskonkurrenz unter Nachtfaltern, Ergebnisse. Spezieller Teil: Hepialidae, Cossidae, Zygaenidae, Limacodidae, Psychidae, Thyrididae*. Vol. 3. Die Schmetterlinge Baden-Württembergs. Stuttgart: Ulmer Verlag.

EFSA (2013). "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 11 (7), 3295.

EFSA (2015). "Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods". *EFSA Journal* 13 (2).

EFSA PPR Panel (2012). "Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 10 (5), 2668 pp.

Ellis, C., K. J. Park, P. Whitehorn, A. David, and D. Goulson (2017). "The Neonicotinoid Insecticide Thiacloprid Impacts upon Bumblebee Colony Development under Field Conditions". *Environmental Science & Technology* 51 (3), 1727–1732.

European Commission (2005). "Agri-environment Measures - Overview on General Principles, Types of Measures, and Application", 24.

European Commission (2009). "Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009: concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC". *Official Journal of the European Union*.

European Commission (2013). "Regulation (EU) No 1305/2013 of the European Parliament and of the Council of 17 December 2013 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD) and repealing Council Regulation (EC) No 1698/2005". *Official Journal of the European Union*, 62.

European Commission (2019). *EU Pesticides database*. Last accessed 2019-04-06. URL: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database.

FAO (2019). *FAOSTAT: Pesticides Use [database]*. Last accessed 2019-04-06. URL: http://www.fao.org/faostat/en/#data/RP.

Feltham, H., K. Park, and D. Goulson (2014). "Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency". *Ecotoxicology* 23 (3), 317–323.

Feltham, H., K. Park, J. Minderman, and D. Goulson (2015). "Experimental evidence that wildflower strips increase pollinator visits to crops". *Ecology and evolution* 5 (16), 3523–3530.

Fischer, M., O. Bossdorf, S. Gockel, F. Hänsel, A. Hemp, D. Hessenmöller, G. Korte, J. Nieschulze, S. Pfeiffer, D. Prati, S. Renner, I. Schöning, U. Schumacher, K. Wells, F. Buscot, E. K. Kalko, K. E. Linsenmair, E.-D. Schulze, and W. W. Weisser (2010). "Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories". *Basic and Applied Ecology* 11 (6), 473–485.

Forister, M. L., B. Cousens, J. G. Harrison, K. Anderson, J. H. Thorne, D. Waetjen, C. C. Nice, M. De Parsia, M. L. Hladik, R. Meese, H. van Vliet, and A. M. Shapiro (2016). "Increasing neonicotinoid use and the declining butterfly fauna of lowland California". *Biology Letters* 12 (8), 20160475.

Forrest, J. R. K., R. W. Thorp, C. Kremen, N. M. Williams, and Y. Clough (2015). "Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape". *Journal of Applied Ecology* 52 (3), 706–715.

Frouz, J. (1999). "Use of soil dwelling Diptera (Insecta, Diptera) as bioindicators: a review of ecological requirements and response to disturbance". *Agriculture, Ecosystems & Environment* 74 (1-3), 167–186.

Gallai, N., J.-M. Salles, J. Settele, and B. E. Vaissière (2009). "Economic valuation of the vulnerability of world agriculture confronted with pollinator decline". *Ecological Economics* 68 (3), 810–821.

Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, J. Liira, T. Tscharntke, C. Winqvist, S. Eggers, R. Bommarco, T. Pärt, V. Bretagnolle, M. Plantegenest, L. W. Clement, C. Dennis, C. Palmer, J. J. Oñate, I. Guerrero, V. Hawro, T. Aavik, C. Thies, A. Flohre, S. Hänke, C. Fischer, P. W. Goedhart, and P. Inchausti (2010). "Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland". *Basic and Applied Ecology* 11 (2), 97–105.

Gels, J. A., D. W. Held, and D. A. Potter (2002). "Hazards of insecticides to the bumble bees Bombus impatiens (Hymenoptera: Apidae) foraging on flowering white clover in turf". *Journal of Economic Entomology* 95 (4), 722–728.

Gevao, B., K. Semple, and K. Jones (2000). "Bound pesticide residues in soils: a review". *Environmental Pollution* 108 (1), 3–14.

Gierer, F., S. Vaughan, M. Slater, H. M. Thompson, J. S. Elmore, and R. D. Girling (2019). "A review of the factors that influence pesticide residues in pollen and nectar: Future research requirements for optimising the estimation of pollinator exposure". *Environmental Pollution* 249, 236–247.

Gilburn, A. S., N. Bunnefeld, J. M. Wilson, M. S. Botham, T. M. Brereton, R. Fox, and D. Goulson (2015). "Are neonicotinoid insecticides driving declines of widespread butterflies?" *PeerJ* 3, e1402.

Gill, R. J. and N. E. Raine (2014). "Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure". *Functional Ecology* 28 (6), 1459–1471.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine (2012). "Combined pesticide exposure severely affects individual-and colony-level traits in bees". *Nature* 491 (7422), 105–108.

Gliessman, S. R. (2014). *Agroecology: the ecology of sustainable food systems*. CRC press.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, G. Petrokofsky, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean (2014). "A restatement of the natural

science evidence base concerning neonicotinoid insecticides and insect pollinators". *Proceedings. Biological sciences / The Royal Society* 281 (1786), 20140558.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean (2015). "A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators". *Proceedings. Biological sciences / The Royal Society* 282 (1818), 20151821.

Goudie, A. S. (2018). *Human impact on the natural environment. Past, present and future*. 8th. John Wiley & Sons.

Goulson, D. (2013). "An overview of the environmental risks posed by neonicotinoid insecticides". *Journal of Applied Ecology* 50 (4), 977–987.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray (2015). "Bee declines driven by combined stress from parasites, pesticides, and lack of flowers". *Science* 347 (6229), 1255957.

Goulson, D., J. Thompson, and A. Croombs (2018). "Rapid rise in toxic load for bees revealed by analysis of pesticide use in Great Britain". *PeerJ* 6, e5255.

Gradish, A. E., J. van der Steen, C. D. Scott-Dupree, A. R. Cabrera, G. C. Cutler, D. Goulson, O. Klein, D. M. Lehmann, J. Lückmann, B. O'Neill, N. E. Raine, B. Sharma, and H. Thompson (2019). "Comparison of Pesticide Exposure in Honey Bees (Hymenoptera: Apidae) and Bumble Bees (Hymenoptera: Apidae): Implications for Risk Assessments". *Environmental Entomology* 48 (1), 12–21.

Grass, I., J. Albrecht, F. Jauker, T. Diekötter, D. Warzecha, V. Wolters, and N. Farwig (2016). "Much more than bees—Wildflower plantings support highly diverse flower-visitor communities from complex to structurally simple agricultural landscapes". *Agriculture, Ecosystems & Environment* 225, 45–53.

Green, R. E. (2005). "Farming and the Fate of Wild Nature". *Science* 307 (5709), 550–555.

Group of Chief Scientific Advisors (2018). *EU authorisation processes of Plant Protection Products*. Scientific Opinion 5.

Haaland, C., R. E. Naisbit, and L.-F. Bersier (2011). "Sown wildflower strips for insect conservation: a review". *Insect Conservation and Diversity* 4 (1), 60–80.

Hahn, M., A. Schotthöfer, J. Schmitz, L. A. Franke, and C. A. Brühl (2015). "The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats". *Agriculture, Ecosystems & Environment* 207, 153–162.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. de Kroon (2017). "More than 75

percent decline over 27 years in total flying insect biomass in protected areas". *PLOS ONE* 12 (10), e0185809.

Heard, M. S., J. Baas, J.-. L. Dorne, E. Lahive, A. G. Robinson, A. Rortais, D. J. Spurgeon, C. Svendsen, and H. Hesketh (2017). "Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species?" *Science of The Total Environment* 578, 357–365.

Heimbach, F., A. Russ, M. Schimmer, and K. Born (2016). "Large-scale monitoring of effects of clothianidin dressed oilseed rape seeds on pollinating insects in Northern Germany: implementation of the monitoring project and its representativeness". *Ecotoxicology* 25 (9), 1630–1647.

Hinarejos, S., J. Abbott, A. Alix, S. Bibek, A. Cabrera, T. Joseph, B. O'Neill, R. Singh, and H. Thompson (2019). "Non- *Apis* Bee Exposure Workshop: Industry Participants' View". *Environmental Entomology* 48 (1), 49–52.

Hofmann, M. M., C. M. Zohner, and S. S. Renner (2019). "Narrow habitat breadth and late-summer emergence increases extinction vulnerability in Central European bees". *Proceedings of the Royal Society B: Biological Sciences* 286 (1898), 20190316.

Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter (2013). "Mass-flowering crops enhance wild bee abundance". *Oecologia* 172 (2), 477–484.

IPBES (2016). *The assessment report on pollinators, pollination and food production of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*.

Jones, A., P. Harrington, and G. Turnbull (2014). "Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years". *Pest management science* 70 (12), 1780–1784.

Julius Kühn-Institut (2018). *PAPA - Panel Pflanzenschutzmittel-Anwendungen*. Last accessed 2018-03-19. URL: http://papa.julius-kuehn.de/.

Kleijn, D., F. Kohler, A. Báldi, P. Batáry, E. Concepción, Y. Clough, M. Díaz, D. Gabriel, A. Holzschuh, E. Knop, A. Kovács, E. Marshall, T. Tscharntke, and J. Verhulst (2009). "On the relationship between farmland biodiversity and land-use intensity in Europe". *Proceedings of the Royal Society B: Biological Sciences* 276 (1658), 903–909.

Koch, K. and H. Freude (1992). *Die Käfer Mitteleuropas: Ökologie*. Vol. 3. Goecke & Evers.

Main, A. R., E. B. Webb, K. W. Goyne, and D. Mengel (2018). "Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis". *Ecological Applications* 28 (5), 1232–1244.

Manandhar, R. and M. G. Wright (2016). "Effects of Interplanting Flowering Plants on the Biological Control of Corn Earworm (Lepidoptera: Noctuidae) and Thrips

(Thysanoptera: Thripidae) in Sweet Corn". *Journal of Economic Entomology* 109 (1), 113–119.

Marshall, E. J. and A. C. Moonen (2002). "Field margins in northern Europe: their functions and interactions with agriculture". *Agriculture, Ecosystems & Environment* 89 (1-2), 5–21.

Marshall, E., T. M. West, and D. Kleijn (2006). "Impacts of an agri-environment field margin prescription on the flora and fauna of arable farmland in different landscapes". *Agriculture, Ecosystems & Environment* 113 (1-4), 36–44.

Michener, C. D. (2007). *The bees of the world*. 2. ed. Baltimore: Johns Hopkins Univ. Press.

Moffat, C., S. T. Buckland, A. J. Samson, R. McArthur, V. Chamosa Pino, K. A. Bollan, J. T.-J. Huang, and C. N. Connolly (2016). "Neonicotinoids target distinct nicotinic acetylcholine receptors and neurons, leading to differential risks to bumblebees". *Scientific Reports* 6, 24764.

Moffat, C., J. G. Pacheco, S. Sharp, A. J. Samson, K. A. Bollan, J. Huang, S. T. Buckland, and C. N. Connolly (2015). "Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (Bombus terrestris)". *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 29 (5), 2112–2119.

Mogren, C. L. and J. G. Lundgren (2016). "Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status". *Scientific Reports* 6, 29608.

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp, and J. S. Pettis (2010). "High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health". *PloS one* 5 (3), e9754.

Natural Research Council, ed. (2006). *Status of Pollinators in North America*. National Academic Press.

Newman, M. C. (2014). *Fundamentals of ecotoxicology: the science of pollution*. CRC press.

Nieto, A., S. Roberts, J. Kemp, P. Rasmont, M. Kuhlmann, García Criado, M., J. C. Biesmeijer, P. Bogusch, H. H. Dathe, De la Rúa, P., De Meulemeester, T., M. Dehon, A. Dewulf, F. J. Ortiz-Sánchez, P. Lhomme, A. Pauly, S. G. Potts, C. Praz, M. Quaranta, V. G. Radchenko, E. Scheuchl, J. Smit, J. Straka, M. Terzo, B. Tomozii, J. Window, and D. Michez (2014). *European Red List of Bees*. Luxembourg: Publication Office of the European Union.

Nuyttens, D., W. Devarrewaere, P. Verboven, and D. Foqué (2013). "Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review". *Pest Management Science* 69 (5), 564–575.

OEEP/EPPO (2010a). "Efficacy evaluation of plant protection products. Side-effects on honeybees". *Bulletin OEPP/EPPO Bulletin* 40, 313–319.

OEEP/EPPO (2010b). "Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees". *Bulletin OEPP/EPPO Bulletin* 40, 323–331.

Ollerton, J. (2017). "Pollinator Diversity: Distribution, Ecological Function, and Conservation". *Annual Review of Ecology, Evolution, and Systematics* 48, 353–76.

Ollerton, J., R. Winfree, and S. Tarrant (2011). "How many flowering plants are pollinated by animals?" *Oikos* 120 (3), 321–326.

Orford, K. A., I. P. Vaughan, and J. Memmott (2015). "The forgotten flies: the importance of non-syrphid Diptera as pollinators". *Proceedings of the Royal Society B: Biological Sciences* 282 (20142934).

Pecenka, J. R. and J. G. Lundgren (2015). "Non-target effects of clothianidin on monarch butterflies". *The Science of Nature* 102 (3-4).

Peters, B., Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (Osmia bicornis)". *Ecotoxicology* 25 (9), 1679–1690.

Pettis, J. S., D. vanEngelsdorp, J. Johnson, and G. Dively (2012). "Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema". *Naturwissenschaften* 99 (2), 153–158.

Pilling, E., P. Campbell, M. Coulson, N. Ruddle, and I. Tornier (2013). "A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam". *PLoS ONE* 8 (10), e77193.

Pisa, L. W., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, M. McField, C. A. Morrissey, D. A. Noome, J. Settele, N. Simon-Delso, J. D. Stark, Van der Sluijs, J P, H. van Dyck, M. Wiemers, and D. Goulson (2015). "Effects of neonicotinoids and fipronil on non-target invertebrates". *Environmental science and pollution research international* 22 (1), 68–102.

Pisa, L., D. Goulson, E.-C. Yang, D. Gibbons, F. Sánchez-Bayo, E. Mitchell, A. Aebi, J. van der Sluijs, C. J. K. MacQuarrie, C. Giorio, E. Y. Long, M. McField, M. Bijleveld van Lexmond, and J.-M. Bonmatin (2017). "An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems". *Environmental Science and Pollution Research*.

Pohorecka, K., P. Skubida, P. Semkiw, A. Miszczak, D. Teper, P. Sikorski, K. Zagibajło, M. Skubida, D. Zdańska, and A. Bober (2013). "Effects of exposure of honey bee colonies to neonicotinoid seed–treated maize crops". *Journal of Apicultural Science* 57 (2).

Posthuma, L., G. W. Suter II, and T. P. Traas (2002). *Species sensitivity distributions in ecotoxicology*. CRC Press.

Potts, S. G., K. Biesmeijer, R. Bommarco, T. Breeze, L. Carvalheiro, M. Franzén, J. P. González-Varo, A. Holzschuh, D. Kleijn, A.-M. Klein, B. Kunin, T. Lecocq, O. Lundin, D. Michez, P. Neumann, A. Nieto, L. Penev, P. Rasmont, O. Ratamäki, V. Riedinger, Roberts, S. P. M., M. Rundlöf, J. Scheper, P. Sørensen, I. Steffan-Dewenter, Stoev, P., Vilà, M., and O. Schweiger (2015). *Status and trends of European pollinators. Key findings of the STEP project.* Ed. by Pensoft Publishers. 74 pp. Sofia.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin (2010). "Global pollinator declines: trends, impacts and drivers". *Trends in ecology & evolution* 25 (6), 345–353.

Powney, G. D., C. Carvell, M. Edwards, R. K. A. Morris, H. E. Roy, B. A. Woodcock, and N. J. B. Isaac (2019). "Widespread losses of pollinating insects in Britain". *Nature Communications* 10 (1).

Rader, R., I. Bartomeus, L. A. Garibaldi, Garratt, Michael P. D., B. G. Howlett, R. Winfree, S. A. Cunningham, M. M. Mayfield, A. D. Arthur, G. K. S. Andersson, R. Bommarco, C. Brittain, L. G. Carvalheiro, N. P. Chacoff, M. H. Entling, B. Foully, B. M. Freitas, B. Gemmill-Herren, J. Ghazoul, S. R. Griffin, C. L. Gross, L. Herbertsson, F. Herzog, J. Hipólito, S. Jaggar, F. Jauker, A.-M. Klein, D. Kleijn, S. Krishnan, C. Q. Lemos, S. A. M. Lindström, Y. Mandelik, V. M. Monteiro, W. Nelson, L. Nilsson, D. E. Pattemore, N. de O. Pereira, G. Pisanty, S. G. Potts, M. Reemer, M. Rundlöf, C. S. Sheffield, J. Scheper, C. Schüepp, H. G. Smith, D. A. Stanley, J. C. Stout, H. Szentgyörgyi, H. Taki, C. H. Vergara, B. F. Viana, and M. Woyciechowski (2015). "Non-bee insects are important contributors to global crop pollination". *Proceedings of the National Academy of Sciences* 113 (1), 146–151.

Relyea, R. and J. Hoverman (2006). "Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems". *Ecology Letters* 9 (10), 1157–1171.

Requier, F., J.-F. Odoux, T. Tamic, N. Moreau, M. Henry, A. Decourtye, and V. Bretagnolle (2015). "Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds". *Ecological Applications* 25 (4), 881–890.

Roberts, S. P. M., S. G. Potts, and M. Kuhlmann (2016). *Trait database of European bees*. unpublished. Excerpt provided 2016-07-14.

Robinson, R. A. and W. J. Sutherland (2002). "Post-war changes in arable farming and biodiversity in Great Britain". *Journal of Applied Ecology* 39 (1), 157–176.

Roessink, I., ed. (2014). *Ringtest Solitary bee acute contact toxicity*. ICPPR workgroup non-Apis bees.

Roessink, I., van der Steen, Jozef J. M., and N. Hanewald (2016). *Solitary bee, Acute Contact Toxicity Test*. ICPPR workgroup non-Apis bees.

Rohr, J. R., J. L. Kerby, and A. Sih (2006). "Community ecology as a framework for predicting contaminant effects". *Trends in ecology & evolution* 21 (11), 606–613.

Rortais, A., G. Arnold, J.-L. Dorne, S. J. More, G. Sperandio, F. Streissl, C. Szentes, and F. Verdonck (2017). "Risk assessment of pesticides and other stressors in bees: Principles, data gaps and perspectives from the European Food Safety Authority". *Science of The Total Environment* 587-588, 524–537.

Rubach, M. N., R. Ashauer, D. B. Buchwalter, H. De Lange, M. Hamer, T. G. Preuss, K. Töpke, and S. J. Maund (2011). "Framework for traits-based assessment in ecotoxicology". *Integrated Environmental Assessment and Management* 7 (2), 172–186.

Ruddle, N., C. Elston, O. Klein, A. Hamberger, and H. Thompson (2018). "Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee *Osmia bicornis*: Thiamethoxam-treated oilseed rape and *Osmia bicornis* reproduction". *Environmental Toxicology and Chemistry* 37 (4), 1071–1083.

Rundlöf, M., Andersson, Georg K S, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone, and H. G. Smith (2015). "Seed coating with a neonicotinoid insecticide negatively affects wild bees". *Nature* 521, 77–80.

Samson-Robert, O., G. Labrie, M. Chagnon, and V. Fournier (2014). "Neonicotinoid-Contaminated Puddles of Water Represent a Risk of Intoxication for Honey Bees". *PLoS ONE* 9 (12), e108443.

Sánchez-Bayo, F., K. Goka, and D. Hayasaka (2016). "Contamination of the Aquatic Environment with Neonicotinoids and its Implication for Ecosystems". *Frontiers in Environmental Science* 4 (71).

SANCO (2002). *Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC*. European Commission Health & Consumer Protection Directorate-General and Directorate E-Food Safety, plant health animal health and welfare and international questions and E1 - Plant health.

Sattler, C., H. Kächele, and G. Verch (2007). "Assessing the intensity of pesticide use in agriculture". *Agriculture, Ecosystems & Environment* 119 (3-4), 299–304.

Schaafsma, A., V. Limay-Rios, T. Baute, J. Smith, and Y. Xue (2015). "Neonicotinoid Insecticide Residues in Surface Water and Soil Associated with Commercial Maize (Corn) Fields in Southwestern Ontario". *PLOS ONE* 10 (2), e0118139.

Schmitz, J., P. Stahlschmidt, and C. A. Brühl (2015). "Assessing the Risk of Herbicides to Terrestrial Non-Target Plants Using Higher-Tier Studies". *Human and Ecological Risk Assessment: An International Journal* 21 (8), 2137–2154.

Schmolke, A., B. Kearns, and B. O'Neill (2018). "Plant guttation water as a potential route for pesticide exposure in honey bees: a review of recent literature". *Apidologie* 49 (5), 637–646.

Scoble, M. J. (1995). *The Lepidoptera. Form, Function and Diversity.* Oxford, New York: Oxford University Press.

Semple, K. T., A. W. J. Morriss, and G. I. Paton (2003). "Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis". *European Journal of Soil Science* 54 (4), 809–818.

Sgolastra, F., S. Hinarejos, T. L. Pitts-Singer, N. K. Boyle, T. Joseph, J. Lūckmann, N. E. Raine, R. Singh, N. M. Williams, and J. Bosch (2019). "Pesticide Exposure Assessment Paradigm for Solitary Bees". *Environmental Entomology* 48 (1), 22–35.

Sponsler, D. B., C. M. Grozinger, C. Hitaj, M. Rundlöf, C. Botías, A. Code, E. V. Lonsdorf, A. P. Melathopoulos, D. J. Smith, S. Suryanarayanan, W. E. Thogmartin, N. M. Williams, M. Zhang, and M. R. Douglas (2019). "Pesticides and pollinators: A socioecological synthesis". *Science of The Total Environment* 662, 1012–1027.

Stanley, D. A., Garratt, Michael P. D., J. B. Wickens, V. J. Wickens, S. G. Potts, and N. E. Raine (2015). "Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees". *Nature* 528, 548–550.

Stanley, D. A. and N. E. Raine (2016). "Chronic exposure to a neonicotinoid pesticide alters the interactions between bumblebees and wild plants". *Functional Ecology* 30 (7), 1132–1139.

Steen, R. (2016). "Diel activity, frequency and visit duration of pollinators in focal plants: in situ automatic camera monitoring and data processing". *Methods in Ecology and Evolution* 8 (2), 203–213.

Stehle, S. and R. Schulz (2015). "Agricultural insecticides threaten surface waters at the global scale". *Proceedings of the National Academy of Sciences* 112 (18), 5750–5755.

Sterk, G., B. Peters, Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (Bombus terrestris)". *Ecotoxicology* 25 (9), 1666–1678.

Stoate, C., A. Báldi, P. Beja, N. Boatman, I. Herzon, A. van Doorn, G. de Snoo, L. Rakosy, and C. Ramwell (2009). "Ecological impacts of early 21st century agricultural change in Europe – A review". *Journal of Environmental Management* 91 (1), 22–46.

Stoate, C., N. Boatman, R. Borralho, C. Carvalho, G. Snoo, and P. Eden (2001). "Ecological impacts of arable intensification in Europe". *Journal of Environmental Management* 63 (4), 337–365.

Stoner, K. A. (2016). "Current Pesticide Risk Assessment Protocols Do Not Adequately Address Differences between Honey Bees (Apis mellifera) and Bumble Bees (Bombus spp.)" *Frontiers in Environmental Science* 4.

Storkey, J. and D. B. Westbury (2007). "Managing arable weeds for biodiversity". *Pest Management Science* 63 (6), 517–523.

Sur, R. and A. Stork (2003). "Uptake, translocation and metabolism of imidacloprid in plants". *Bulletin of Insectology* 56, 35–40.

Thompson, H. (2016). "Extrapolation of acute toxicity across bee species: Extrapolation of acute toxicity across bee species". *Integrated Environmental Assessment and Management* 12 (4), 622–626.

Thompson, H. M. (2001). "Assessing the exposure and toxicity of pesticides to bumblebees (Bombus sp.)" *Apidologie* 32 (4), 305–321.

Thompson, H. M. and T. Pamminger (2019). "Perspective: Are honeybees suitable surrogates for use in pesticide risk assessment for non-*Apis* bees?" *Pest Management Science*.

Topping, C. J., P. S. Craig, F. de Jong, M. Klein, R. Laskowski, B. Manachini, S. Pieper, R. Smith, J. P. Sousa, F. Streissl, K. Swarowsky, A. Tiktak, and T. van der Linden (2015). "Towards a landscape scale management of pesticides: ERA using changes in modelled occupancy and abundance to assess long-term population impacts of pesticides". *The Science of the total environment* 537, 159–169.

Topping, C. J., T. S. Hansen, T. S. Jensen, J. U. Jepsen, F. Nikolajsen, and P. Odderskær (2003). "ALMaSS, an agent-based model for animals in temperate European landscapes". *Ecological Modelling* 167 (1-2), 65–82.

Topping, C. J., L. J. Kjaer, U. Hommen, T. T. Høye, T. G. Preuss, R. M. Sibly, and P. van Vliet (2014). "Recovery based on plot experiments is a poor predictor of landscape-level population impacts of agricultural pesticides: Agent-based simulation of population recovery". *Environmental Toxicology and Chemistry* 33 (7), 1499–1507.

Tosi, S., C. Costa, U. Vesco, G. Quaglia, and G. Guido (2018). "A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides". *Science of The Total Environment* 615, 208–218.

Tscharntke, T., R. Bommarco, Y. Clough, T. O. Crist, D. Kleijn, T. A. Rand, J. M. Tylianakis, S. van Nouhuys, and S. Vidal (2007). "Conservation biological control and enemy diversity on a landscape scale". *Biological Control* 43 (3), 294–309.

Tscharntke, T., Y. Clough, T. C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer, and A. Whitbread (2012). "Global food security, biodiversity conservation and the future of agricultural intensification". *Biological Conservation* 151 (1), 53–59.

Tschumi, M., M. Albrecht, J. Collatz, V. Dubsky, M. H. Entling, A. J. Najar-Rodriguez, and K. Jacot (2016). "Tailored flower strips promote natural enemy biodiversity and pest control in potato crops". *Journal of Applied Ecology* 53 (4), 1169–1176.

Tschumi, M., M. Albrecht, M. H. Entling, and K. Jacot (2015). "High effectiveness of tailored flower strips in reducing pests and crop plant damage". *Proceedings. Biological sciences / The Royal Society* 282 (1814), 20151369.

United Nations (1992). *Convention on Biological Diversity*.

United Nations (2016). *Cancun Declaration on Mainstreaming the Conservation and Sustainable Use of Biodiversity for Well-Being*. Cancun (Mexico).

Van den Brink, P. J., A. C. Alexander, M. Desrosiers, W. Goedkoop, P. L. Goethals, M. Liess, and S. D. Dyer (2011). "Traits-based approaches in bioassessment and ecological risk assessment: Strengths, weaknesses, opportunities and threats". *Integrated Environmental Assessment and Management* 7 (2), 198–208.

vanEngelsdorp, D., J. Hayes Jr., R. M. Underwood, and J. Pettis (2008). "A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008". *PloS one* 3 (12), e4071.

Vidau, C., M. Diogon, J. Aufauvre, R. Fontbonne, B. Viguès, J.-L. Brunet, C. Texier, D. G. Biron, N. Blot, H. El Alaoui, L. P. Belzunces, and F. Delbac (2011). "Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by Nosema ceranae". *PLoS ONE* 6 (6), e21550.

Vray, S., O. Rollin, P. Rasmont, M. Dufrêne, D. Michez, and N. Dendoncker (2019). "A century of local changes in bumblebee communities and landscape composition in Belgium". *Journal of Insect Conservation*.

Walker, C. H., R. Sibly, and D. B. Peakall (2016). *Principles of ecotoxicology*. CRC press.

Wardhaugh, C. W. (2015). "How many species of arthropods visit flowers?" *Arthropod-Plant Interactions* 9 (6), 547–565.

Westrich, P., U. Frommer, K. Mandery, H. Riemann, H. Ruhnke, C. Saure, and J. Voith (2011). "Rote Liste und Gesamtartenliste der Bienen (Hymenoptera, Apidae) Deutschlands". *Naturschutz und Biologische Vielfalt* 70 (3), 373–416.

Westrich, P. (1990). *Die Wildbienen Baden-Württembergs: [im Rahmen des Artenschutzprogrammes Baden Württemberg]*. 2., verb. Aufl. Stuttgart: Ulmer.

Wezel, A., M. Casagrande, F. Celette, J.-F. Vian, A. Ferrer, and J. Peigné (2014). "Agroecological practices for sustainable agriculture. A review". *Agronomy for Sustainable Development* 34 (1), 1–20.

Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson (2012). "Neonicotinoid pesticide reduces bumble bee colony growth and queen production". *Science* 336 (6079), 351–352.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts (2010). "Ecological and life-history traits predict bee species responses to environmental disturbances". *Biological Conservation* 143 (10), 2280–2291.

Winfree, R., I. Bartomeus, and D. P. Cariveau (2011). "Native Pollinators in Anthropogenic Habitats". *Annual Review of Ecology, Evolution, and Systematics* 42 (1), 1–22.

Wirtz, I. P., M. Hauer-Jákli, D. Schenke, E. Ladewig, B. Märländer, U. Heimbach, and J. Pistorius (2018). "Investigations on neonicotinoids in guttation fluid of seed treated sugar beet: Frequency, residue levels and discussion of the potential risk to honey bees". *Crop Protection* 105, 28–34.

Wood, T. J. and D. Goulson (2017). "The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013". *Environmental Science and Pollution Research*.

Woodcock, B. A., N. J. B. Isaac, J. M. Bullock, D. B. Roy, D. G. Garthwaite, A. Crowe, and R. F. Pywell (2016). "Impacts of neonicotinoid use on long-term population changes in wild bees in England". *Nature Communications* 7, 12459.

Zurbuchen, A., L. Landert, J. Klaiber, A. Müller, S. Hein, and S. Dorn (2010). "Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances". *Biological Conservation* 143 (3), 669–676.

APPENDIX

A THE IMPACT OF PESTICIDES ON FLOWER-VISITING INSECTS: A REVIEW WITH REGARD TO EUROPEAN RISK ASSESSMENT

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A

A.1 ABSTRACT

Flower-visiting insects (FVIs) are an ecologically diverse group of mobile, flying species that should be protected from pesticide effects according to European policy. However, there is an ongoing decline of FVI species which is partly caused by agricultural pesticide applications. Therefore, the risk assessment framework needs to be improved. We synthesized the peer-reviewed literature on FVI groups, their ecology, habitat, exposure to pesticides, and subsequent effects. The results show that FVIs are far more diverse than previously thought. Their habitat, the entire agricultural landscape, is potentially contaminated with pesticides through multiple pathways. Pesticide exposure of FVIs at environmental realistic levels can cause population-relevant adverse effects. This knowledge was used to critically evaluate the European regulatory framework of exposure and effect assessment. The current risk assessment should be amended to incorporate specific ecological properties of FVIs, i.e. traits. We present data-driven tools to improve the future risk assessment by making use of trait information. There are major knowledge gaps concerning the general investigation of other groups than bees, collection of comprehensive data on FVI groups and their ecology, linking habitat to FVI exposure, and study of previously neglected complex population effects. This is necessary to improve our understanding of FVIs and facilitate the development of a more protective FVI risk assessment.

A.2 INTRODUCTION

The evidence that flower-visiting insects (FVIs) are in decline is continuously growing (Goulson et al., 2015; Hallmann et al., 2017; IPBES, 2016; Ollerton, 2017; Potts et al., 2015; Powney et al., 2019; Vray et al., 2019). This is apparent in losses of domestic honey bee (*Apis mellifera*) hives in the many EU countries and the USA and a simultaneous decline in wild bee diversity and butterfly, moth, and syrphid fly populations (Goulson et al., 2015; Potts et al., 2015; Powney et al., 2019; vanEngelsdorp et al., 2008; Vray et al., 2019). Hallmann et al. (2017) showed a substantial long-term decline in flying insect biomass in nature reserves, which included many flower visitors such as butterflies, bees, flies, and beetles. This general decrease in species and abundances is caused by multiple, mostly anthropogenic, factors, one of which is exposure to pesticides (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). Other discussed causes include habitat loss and fragmentation, resource diversity decrease, climate change, parasites and pathogens, invasive species, and environmental pollution (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017).

FVIs provide a vital ecosystem service, pollination, that propels human food production and maintains flowering plant biodiversity (Klein et al., 2007; Ollerton et al., 2011). However, FVI protection is not only relevant for the protection of commercial yield and native flora. Since FVIs make up about 30% of all arthropod species worldwide, they are a major part of faunal biodiversity (Wardhaugh, 2015).

Biodiversity should be protected according to the Convention on Biological Diversity (United Nations, 1992). In 2016, the United Nations specifically called for pollinator conservation in agriculture in their Cancun Declaration (United Nations, 2016). This resulted in the formation of the continuously growing Coalition of the Willing on Pollinators that commits to protecting pollinators and their habitat from harmful anthropogenic impact (Coalition of the Willing on Pollinators, 2016). In Europe, Regulation (EC) 1107/2009 is in place, concerning the regulatory risk assessment framework to prevent unacceptable negative impact of agricultural pesticide use on biodiversity (European Commission, 2009). Therefore, FVI protection from significant adverse pesticide effects is required by European law. Since pesticides contribute to the ongoing FVI decline, it is possible that regulatory measures are insufficient to provide protection from pesticides (Goulson et al., 2015; IPBES, 2016; Ollerton, 2017).

In this review, we synthesized the available scientific literature and regulatory documents to examine the impact of pesticides on FVIs. We further discuss the suitability of European risk assessment to prevent adverse consequences of pesticide use. Species decline has mostly been noted in bees because of an economic interest in preserving viable populations of these important pollinators (IPBES, 2016; Klein et al., 2007). However, there are many other FVI taxa that are exposed to pesticides in the agricultural landscape which may lead to negative effects on their populations (Godfray et al., 2014; Godfray et al., 2015; IPBES, 2016; Potts et al., 2015). Consequently, we identified the relevant FVI groups by visitation frequency and abundance in crops and the surrounding area and described their ecology and habitat. We used this knowledge to characterize preferential pesticide exposure pathways and summarized quantitative exposure of relevant habitat compartments from residue studies. We collated effects studies to assess the impact of environmentally realistic pesticide doses on FVIs. This enabled us to critically discuss the suitability of the regulatory effect and exposure assessment. We further propose data-driven tools that improve FVI risk assessment. Finally, we show major knowledge gaps that need to be closed to increase our understanding of FVIs and develop a sufficiently protective regulatory framework.

A.3 REVIEW METHODOLOGY

We searched the peer-reviewed English-language literature published until 2018 using Google Scholar. Keywords included the following terms and their combinations: "Pollinator", "flower visiting insect", "bee", "butterfly", "moth", "fly", "beetle", "habitat", "trait", "pesticide", "insecticide", "risk assessment", "exposure", "residue", "effect", and "toxicity". We also considered Researchgate suggestions (researchgate.net) and results of a continuous Sparrho search (sparrho.com) which used the keywords "pollinator", "pesticide", and "bee". Some papers were brought to our attention through recommendations from scientific colleagues and we obtained additional papers from literature references. Semi-field and field effect studies were only included if they investigated the impact of environmentally realistic pesticide exposure levels. Finally, we screened EU regulatory documents to gain detailed knowledge about European risk assessment for bees and non-target arthropods (NTAs).

A.4 FVI GROUPS

FVIs in the context of this article are defined as insect species that directly interact with flowers in at least the flying adult life stage, in accordance with Wardhaugh (2015). Most so-called pollinators have actually only been determined as FVIs since the usual visual observations on flowers are not suitable to prove pollen deposition on the stigma, i.e. pollination. FVIs are an ecologically complex aggregation, which includes species with very different life strategies, e.g. herbivores, predators, and parasites (Ollerton, 2017). To assess the impact of pesticides on FVIs it is important to identify the relevant groups that frequently visit flowers and are abundant in the agricultural landscape. In the past, the scientific literature sparsely identified FVI groups aside from bees. Lepidopterans (moths and butterflies), and flies (mainly hover flies) are also acknowledged as important taxa. Beetles and wasps are mentioned as flower visitors of minor importance (Winfree et al., 2011). In recent years it was hypothesized that there are considerably more FVI species than previously assumed. The suspected FVI groups span from bees over moths and butterflies, beetles, wasps, and ants to flies but also include less prominent groups such as thrips, true bugs, springtails, termites and cockroaches (Ollerton, 2017; Wardhaugh, 2015). However, these classifications were only based on estimates and needed support from field research.

Several studies found that FVI communities in the agricultural landscape are indeed as diverse as theoretically suggested. Wildflower plantings were visited by many insect taxa aside from bees, lepidopterans, and hover flies in the central German agricultural landscape (Grass et al., 2016). In fact, non-bee/non-hover fly insects made up half of the visiting individual visits and 75% of FVI species (Fig. A.1). Non-hover fly dipterans were by far the largest portion of visiting species. In contrast, butterflies only made up a small share of FVI abundance, whereas the number of flower visits by beetles and non-hover fly dipterans individuals was comparable to honey bees (Grass et al., 2016). A large-scale meta-analysis also found that non-hover fly dipterans are at least as relevant as hover flies since they made up the majority of dipteran flower visits (Orford et al., 2015). Such a distribution was also found in a common plant of the agricultural landscape that is widely distributed in Europe. Common buttercup *Ranunculus acris* was mostly visited by fly species as shown in a large-scale, long-term project (Biodiversity Exploratories; Fig. A.2). Beetles were detected in similar species and individual numbers as bees (Fischer et al., 2010; Nico Blüthgen, personal communication, 2012).

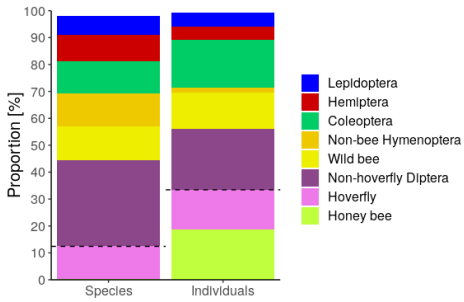

Figure A.1.: Wildflower planting flower visits in central Germany. The dashed line shows the cumulative fraction of honey bee and hover fly flower visits. Figure adapted from Grass et al. (2016).

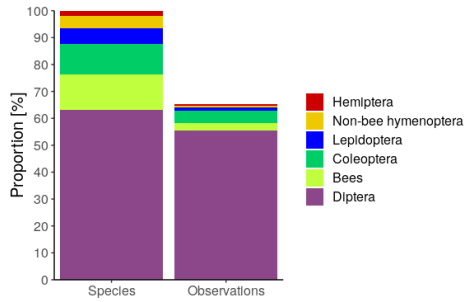

Figure A.2.: *Ranunculus acris* visits on Biodiversity Exploratories sites. (Nico Blüthgen, personal communication, 2012).

The diversity of FVI communities has been underestimated not only for native flora but also for crops. An extensive meta-analysis summarized the results of 39 field studies that investigated flower-visits in several crop systems from five continents (Rader et al., 2015). Overall, non-bee species accounted for 38% of flower visits. The visits by non-bees of oilseed rape as a typical European mass-flowering crop were quite variable (between 5-80%) and varied even within countries (5-60%).
FVI communities are far more diverse than it was acknowledged in the past. There is a general trend that visit rates vary greatly between cropping systems, native habitats, and geographic locations (Grass et al., 2016; Orford et al., 2015; Rader et al., 2015). The available literature identifies the relevant European FVI groups in crops and their semi-natural surroundings as bees, flies (non-syrphids and syrphids), lepidopterans (moths and butterflies), and beetles. However, there is only limited information to evaluate all groups. Therefore, it is currently not possible to assess the relevance of all other suspected groups, e.g. non-bee hymenopterans and hemipterans. After the identification of relevant FVI groups it is necessary to examine their ecology and their habitat to assess their potential pesticide exposure.

A.5 FVI ECOLOGY, HABITAT, AND EXPOSURE PATHWAYS

A.5.1 *Ecology*

Other than visiting flowers in at least their adult stage, FVIs differ substantially in their ecology (Ollerton, 2017). A comprehensive review of FVI ecology is beyond the scope of this paper. We, therefore, concentrate on bee species since they are extensively studied in the ecotoxicological context and cover many of the general FVI traits. Specific additional properties of other groups are still mentioned.

All bee species are obligate florivores in larval and adult life stages. This distinguishes them from all other FVI taxa where only a subset of species are flower visitors and mostly adults are florivores. Adult bees feed predominantly on nectar whereas larvae feed mostly on pollen (Michener, 2007). Other FVI groups such as moths and butterflies, and beetles also have herbivore life stages (Ebert, 1994; Koch and Freude, 1992; Scoble, 1995). Aside from the well-known domesticated western honey bee *Apis mellifera* there are a multitude of ecologically variable wild bee species in Europe. Some species are eusocial, i.e. live in colonies or aggregations, but most species are solitary. Additionally, there are many parasitic species that exploit their bee host to feed and tend to their offspring (Michener, 2007; Westrich, 1990). Bees have several nesting strategies. Most species burrow into the soil to build their nest but others also excavate deadwood, occupy preexisting cavities in soil or deadwood, or construct nests from collected material (Michener, 2007). Other FVI groups also contain soil-dwelling larval stages, e.g. flies and beetles (Frouz, 1999; Koch and Freude, 1992). There are food generalists (polylectic) and specialists (oligolectic) that in some cases forage on just one specific plant (Michener, 2007; Westrich, 1990). The active flight period and length of flight differs between bee species. Many species start mating and foraging flights in spring while others do not begin their adult phase before summer and continue until autumn (Westrich, 1990). Most species have only one brood per year (univoltine) whereas some lay eggs throughout the year (multivoltine). Voltinism varies with geography and climate (Michener, 2007). Daily activity usually peaks at midday but also in the morning and evening (Steen, 2016; Thompson, 2001). Bee species vary greatly in their foraging range, i.e. the distance they can cover to search for food resources, which ranges from hundreds of meters to ten or more kilometers (Zurbuchen et al., 2010).

A.5.2 *Habitat*

Due to their ecological profile, FVI species need a set of compartments inside a habitat to fulfill basic needs: Food, water, shelter, mating space, and nesting grounds (Table A.1). The agricultural landscape generally comprizes viable habitats which can be categorized as crop plantings and non-target areas, e.g. managed flower strips and field edge structures (Hahn et al., 2015; Marshall and Moonen, 2002; Tschumi et al., 2015). These areas differ in many aspects such as structure, plant species inventory, spacial and temporal food resource availability, natural enemies, or anthropogenic stress (Hahn et al., 2015; Marshall and Moonen, 2002; Tschumi et al., 2015). Therefore, habitat quality varies significantly which theoretically enables us to assess habitat attractiveness for FVIs.

Table A.1.: Habitat compartments that are used by FVIs.

Compartment	Life stages	Function
Airspace	Adults	Food search (foraging), mate search, nest search
Flowers	Adults and florivore larvae	Food collection (foraging), shelter, mating, nesting, nest material collection
Stem/leaves	Adults and herbivore larvae	Food collection (foraging), shelter, mating, nesting, nest material collection
Soil	Adults and soil-dwelling larvae	Nesting, shelter
Water sources	Adults	Water collection / consumption

There are numerous crops that have been classified as bee-attractive (Supporting Information Table A.S1; EFSA, 2013). However, it is currently not possible to quantitatively evaluate the suitability of a certain crop as a FVI food source. Most studies were only performed with honey bees and focus on major sources of pollen/nectar in their diet rather than the food spectrum (EFSA, 2013). Mass-flowering crops such as oilseed rape *Brassica napus* and sunflower *Helianthus annuus* are used as food sources by wild and managed bees. Their over-abundant supply of floral resources will be used to some degree even if they are not the preferred food plant of a FVI species (Coudrain et al., 2015; Holzschuh et al., 2013; Requier et al., 2015). Even virtually non-attractive crops plantings such as corn or cabbage might be FVI habitats if there is undergrowth of crop-associated wild plants, e.g. cornflower or poppy species (Balmer et al., 2014; Manandhar and Wright, 2016; Storkey and Westbury, 2007). Furthermore, crops can still provide habitat functions for FVIs even if they are not flowering, e.g. nesting grounds or temporary refuge.

Aside from crops, there are non-target areas that are used as habitat by FVIs. Field edge structures are semi-natural habitats in intensely managed agricultural areas. They provide multiple habitat functions for FVI species, e.g. refugia, feeding and breeding grounds, and migration corridors for FVI species (Denisow and Wrzesień, 2015; Marshall and Moonen, 2002; Marshall et al., 2006). Flower strips are sown with seed mixtures for insect conservation with emphasis on sustaining pollinator populations. They ensure crop pollination and also favor predacious beneficials to support biological pest control (Feltham et al., 2015; Haaland et al., 2011; Tschumi et al., 2015). These non-target areas, however, have also not been adequately studied to discuss their habitat suitability in more detail.

In absence of sufficient information and to exercise the precautionary principle, we assume in this review that the entire agricultural landscape is FVI habitat. Therefore, FVIs may potentially be exposed to pesticides while interacting with habitat compartments of crop and non-target areas.

A.5.3 *Exposure pathways*

Pesticides are transported into FVI habitats by direct application to crops (primary processes) or unintentional redirection of a fraction of the applied pesticide amount into adjacent areas (secondary processes; Fig. A.3). Primary processes include spray and solid application, e.g. seed treatment or granules (Nuyttens et al., 2013; Walker et al., 2016). Stem application and irrigation methods play a minor role in Europe (Düker and Kubiak, 2015; Miorini et al., 2017). Secondary processes are spray drift, field-edge overspray, dust dispersion, and run-off. As a result of this pesticide input into crops and non-target areas, all FVI habitat compartments are potentially contaminated (Sgolastra et al., 2019).

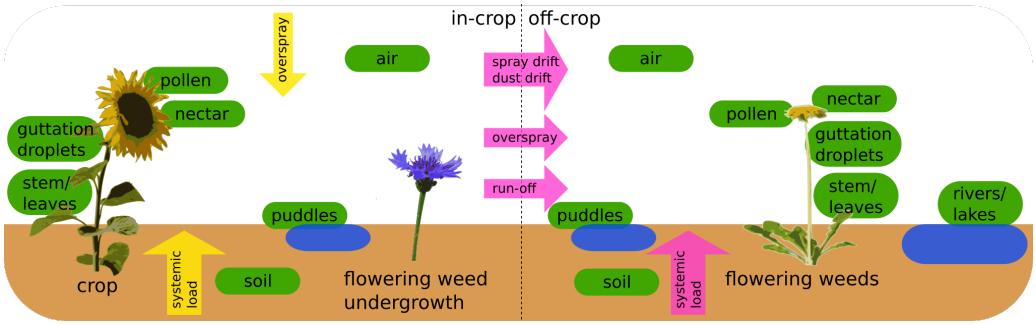

Figure A.3.: Exposure pathways from application to habitat compartments in- and off-crop habitats. Yellow up-/downwards arrows indicate primary and pink side-/upwards arrows secondary transport processes.

Exposure of airspace, pollen and nectar, stem/leaves, soil, and water sources (rivers/lakes, puddles, guttation water) can subsequently lead to FVI exposure (Fig. A.3). Pesticide applications on less attractive crops can still cause FVI exposure if there is flowering weed undergrowth, e.g. cornflower or poppy species in cereal fields, or by transport into attractive off-crop areas (Botías et al., 2015; Simon-Delso et al., 2017). The identification of potentially contaminated habitat compartments does not allow for an estimation of FVI pesticide exposure. It is therefore necessary to quantify exposure of habitat compartments and link it to FVI contamination to identify important pathways.

A.6 EXPOSURE TO PESTICIDES

A.6.1 *Individuals*

Investigations of pesticide residues levels in FVI individuals are required to assess pesticide exposure. Unfortunately, these data are only available for bees at the moment. The predominant part of bee exposure studies in recent years investigated the chemical class of neonicotinoids. Furthermore, the vast majority of these studies is concerned with honey bee exposure (Blacquière et al., 2012; Bonmatin et al., 2015; Godfray et al.,

2014; Godfray et al., 2015; Wood and Goulson, 2017). Hence, such research is overrepresented compared to other pesticide classes or bee species in the following sections.
Bees are exposed to a plethora of pesticides. Brood and adult bee samples from North American honey bee colonies contained 46 pesticides of different pesticide classes and their metabolites (Mullin et al., 2010). A French study found residues of 19 compounds in honey bee colony samples (Chauzat et al., 2011). All major pesticide classes are detected in honey bees, i.e. insecticides, fungicides, and herbicides, according to comprehensive list compiled by EFSA PPR Panel (2012). A more recent study investigated pesticide residues in different bumble bee species and found at least one insecticide or fungicide in over half of the analysed individuals (Botías et al., 2017). The majority of these individuals was exposed to multiple compounds.

A.6.2 *Nectar and pollen*

Nectar and pollen are major carriers of pesticide loads for FVIs. The aforementioned North American and French studies found residues of 98 and 19 pesticides and metabolites in collected pollen, respectively (Chauzat et al., 2011; Mullin et al., 2010). A more recent Italian study registered 18 different insecticides and fungicides in pollen over a three year long sampling period (Tosi et al., 2018). The range of maximum neonicotinoid residues in pollen and nectar was determined as 10^0-10^1 ng/g and 10^0 ng/g, respectively (Godfray et al., 2014; Goulson, 2013; Wood and Goulson, 2017). Residue levels fluctuate between crops by an order of magnitude but pollen doses are consistently higher than nectar doses (Gierer et al., 2019; Wood and Goulson, 2017). Several parameters such as dose and mode of treatment, physicochemical properties of the pesticide, crop type, season, location, soil type, weather, and sampling time of day influence pesticide doses in both matrices (Gierer et al., 2019; Wood and Goulson, 2017). Pesticide load in bee-collected pollen and nectar is often similar to residues in crops (Rundlöf et al., 2015; Wood and Goulson, 2017). However, there are also studies that found much lower contamination (Cutler and Scott-Dupree, 2014; Rolke et al., 2016). Since bees collect pollen and nectar from a wide variety of plants, the dietary spectrum partly determines their contamination. The highest levels of residues are found when a large proportion of crop pollen is collected (Botías et al., 2015; Cutler and Scott-Dupree, 2014; David et al., 2016; Pohorecka et al., 2013; Rundlöf et al., 2015). Non-cultivated plants adjacent to crops are often also contaminated with pesticides in greatly variable doses that can reach comparable levels (Botías et al., 2015; Mogren and Lundgren, 2016; Wood and Goulson, 2017). In general, high doses in nectar and pollen temporally coincide with the bloom of mass-flowering crops such as oilseed rape (Wood and Goulson, 2017). However, chronic exposure of species with a long active flight period, such as honey bees or bumble bees, might be driven by wildflower foraging. One study found that 97% of total neonicotinoid residues in pollen in June and August were actually derived from wildflowers (Botías et al., 2015).

A.6.3 *Soil*

The majority of European bee species (60-70%) nest in soil either by actively burrowing nests or using existing cavities (Westrich, 1990). Therefore, pesticide exposure by soil contact may be an important, yet underestimated pathway (Gradish et al., 2019; Sgolastra et al., 2019). Soil exposure may also be relevant for soil-dwelling life stages of other FVI groups such as fly and beetle larvae (Frouz, 1999; Koch and Freude, 1992). Systemic pesticides are usually applied directly to the soil to be taken up by crops. Only a fraction of the applied pesticide load enters the plant body whereas the major part remains in the soil (Alford and Krupke, 2017; Sur and Stork, 2003). Agricultural soils are therefore often contaminated with multiple pesticides (Hvězdová et al., 2018). Measurable neonicotinoid residues in various crop soils range from 10^{-1}-10^{1} ng/g (Botías et al., 2015; Heimbach et al., 2016; Jones et al., 2014; Wood and Goulson, 2017). To assess pesticide exposure it is not only important to know (peak) concentrations but also the persistence in the soil matrix. Half-lives of neonicotinoid insecticides range from several days to years (Goulson, 2013). Values over one year suggest possible accumulation or continuing exposure from applications in previous years. Both cases have been demonstrated for neonicotinoids by chemical analysis of crop soils (Bonmatin et al., 2005; Goulson, 2013; Jones et al., 2014).

A.6.4 *Stem/leaves*

Systemic pesticides are designed to be taken up by crops from the soil. Depending on the crop, 1.6-20% of the applied amount of neonicotinoids are absorbed into the plant body (Alford and Krupke, 2017; Sur and Stork, 2003). Several studies have also found neonicotinoid residues in wild plant stem or leaves from field margins in levels of 10^{0}-10^{1} ng/g (Botías et al., 2016; Mogren et al., 2016; Pecenka and Lundgren, 2015). FVI exposure by stem or leaf material may not be restricted to herbivore life stages. Since they use the plant body as refuge or collect parts of it as nesting material, e.g. leaf cutter bees (*Megachile* ssp.), FVI adults might also be exposed to pesticide residues by contact (Sgolastra et al., 2019).

A.6.5 *Water sources*

FVIs can potentially take up pesticides from different water sources. Ephemeral puddles on farmland have been shown to contain maximum neonicotinoid concentrations of 10^{1} ng/mL that may represent a risk towards bees (Samson-Robert et al., 2014; Schaafsma et al., 2015). Another potential water source for FVIs are guttation droplets that are exuded by some plant species at moist conditions. Concentrations of systemic neonicotinoids in crop guttation fluid vary greatly (Reetz et al., 2016; Tapparo et al., 2011; Wirtz et al., 2018). Maximum concentration have been measured at 10^{5} ng/mL (God-

fray et al., 2014; Schmolke et al., 2018). Exposure at toxicologically relevant doses is only expected in crops treated with systemic pesticides, since spray treatments lead to doses that are lower by three orders of magnitude (Bonmatin et al., 2015). Additionally, there is first evidence that seed treatment of crops can lead to contamination of guttation fluid in weeds that grow in proximity (Mörtl et al., 2019). Field-adjacent rivers and lakes are heavily contaminated with pesticides at levels that often present a risk for aquatic invertebrates (Morrissey et al., 2015; Stehle and Schulz, 2015). It was stated that exposure through surface waters might also be toxicologically relevant for bee species (Sánchez-Bayo et al., 2016).

A.6.6 *Linking habitat to individual exposure*

FVI habitats in crops and non-target areas are exposed to pesticides. However, it is generally difficult to connect the exposure of these habitats to the contamination of FVI individuals. For nectar, pollen, and stem/leaf material, this would require to break down and quantify FVI food intake. Bee adults usually procure their energy from carbohydrate-rich nectar, whereas larvae feed on pollen provision/pollen bread, a mixture of mostly protein-rich pollen and minor nectar content (Westrich, 1990). Since polylectic bee species forage on a wide variety of plant species (Coudrain et al., 2015; Sickel et al., 2015), their larval pesticide uptake is highly dependent on the proportion of contaminated nectar and pollen in their diet. Data on FVI food spectrum and corresponding pesticide exposure are scarce. There are some quantitative estimates of adult and larval bee food consumption but it is not clear how this would translate into an individual bee pesticide load (EFSA, 2013). Food intake varies greatly between bee species which makes it impossible to generalize single species estimates (Müller et al., 2006). There is insufficient information to connect stem or leaf exposure to FVI contamination, too (Sgolastra et al., 2019).

Linking soil to FVI exposure is even more difficult. Pesticides can be sorbed to the soil and become bound residues with decreased bioavailability and degradation rates. This occurs especially in hydrophobic chemicals (Gevao et al., 2000; Semple et al., 2003). Water soluble compounds such as neonicotinoids might not be so prone to sorption and therefore retain their bioavailability to a greater extent. There is currently no approach to estimate FVI exposure after soil contact.

The details of FVI water uptake are nearly unknown. There are estimates of the daily water intake of the honey bee and one wasp species (EFSA, 2013). Still, the majority of FVI species are not covered and it is unclear which water sources are used to what degree. In the case of guttation, it has been stated that this phenomenon rarely occurs in most crops, especially in high enough concentrations to be of toxicological relevance (Schmolke et al., 2018; Wirtz et al., 2018). This may also be true for exposure via surface waters and puddles. There is currently no clear link of pesticide residues in the available water sources and pesticide uptake of FVIs (Wood and Goulson, 2017).

Since FVIs are exposed to pesticides in their habitat, subsequent effects need to be assessed to evaluate the consequences for FVI populations and communities.

A.7 PESTICIDE EFFECTS

A.7.1 *General considerations*

To determine the risk of pesticide applications for FVIs it is necessary to investigate their sensitivity towards those chemicals. Only detailed information for a representative amount of species enables an assessment of the entire group. Since the honey bee is a test organism in European pesticide risk assessment there are extensive acute toxicity data of all registered pesticides for this species. However, other bee species' sensitivity towards pesticides is practically unknown and may differ substantially.
The European Commission restricted the use of the neonicotinoids imidacloprid, clothianidin, and thiamethoxam in 2013 because of high acute risks for bees. Since then, several complex semi-field and field studies have been carried out to investigate neonicotinoid effects on honey bees, non-*Apis*, and wild bee species at environmentally realistic exposure levels. Unfortunately, there is still nearly no information on pesticide effects towards all other non-bee FVI groups. Several colony-level honey bee studies found limited to negligible effects after neonicotinoid exposure (Cutler et al., 2014; Dively et al., 2015; Pilling et al., 2013; Rundlöf et al., 2015). Honey bee effects from those studies are hardly translatable to all other European bee species because of the substantial ecological differences, mainly social structure and sheer individual numbers in a population (Stoner, 2016; Wood and Goulson, 2017). Therefore, honey bee field effects will not be elaborated on in this report. See the following review articles for further information on honey bee field effects: Blacquière et al. (2012), Godfray et al. (2014), Godfray et al. (2015), Goulson (2013), Pisa et al. (2015), and Pisa et al. (2017).

A.7.2 *(Semi-)Field studies*

Reproduction
There have been several (semi-)field studies that investigated non-*Apis* bee reproduction and colony growth effects in similar experimental setups, mostly with *B. terrestris*. Bumble bee colonies were either exposed by feeding them contaminated nectar but letting them forage without restriction or setting them up next to farmland that was applied with pesticides. In synthesis, neonicotinoid exposure lead to reductions in worker, male, and queen offspring (colony growth), reduced individual growth, and skewed sex ratio in most studies (Cutler and Scott-Dupree, 2014; Ellis et al., 2017; Gels et al., 2002; Main et al., 2018; Moffat et al., 2016; Moffat et al., 2015; Rundlöf et al., 2015; Whitehorn et al., 2012). Impaired reproduction is not only caused by neonicotinoids but also by of the application of new substance classes such as sulfoximine insecticides (Siviter et al., 2018). There is only one study that examined field effects on solitary bees and recorded a total reduction in brood cell construction by *Osmia bicornis* next to clothianidin-treated oilseed rape (Rundlöf et al., 2015). However, there are also a few studies that found no adverse effects on bumble bees and solitary bees in field settings (Peters et al., 2016; Ruddle et al., 2018; Sterk et al., 2016). Discrepant outcomes between these and the

majority of studies most likely result from different exposure levels. In comparison to Rundlöf et al. (2015), those three studies used a very similar setup where they put up bumble bee colonies and solitary bee trap nests next to seed-treated oilseed rape. However, Sterk et al. (2016) and Peters et al. (2016) used winter variety oilseed rape where Rundlöf et al. (2015) used the spring variety. This resulted in a nearly tenfold difference in maximum pollen residues, which is a highly probable cause for the contrasting effects.

Foraging
There is a general pattern that the number of bee trips to flowers increases but foraging efficiency decreases after pesticide exposure. Pesticide effects on bumble bee foraging were investigated in (semi-)field studies similar to the reproduction experiments above. However, bees were exposed by pesticide-spiked sugar water in all studies. Several experiments detected an increased length of trips or a reduced number of successful trips (Feltham et al., 2014; Gill and Raine, 2014; Gill et al., 2012; Stanley et al., 2015; Stanley and Raine, 2016). A single study found only minor changes in foraging activity and pollen collection (Arce et al., 2016).

Immune system
Neonicotinoid exposure has been linked to increased disease and parasite susceptibility in honey bees in (semi-)field experiments (e.g. Alburaki et al., 2015; Dively et al., 2015; Pettis et al., 2012; Vidau et al., 2011. Such effects were not studied in wild bees. Since they have a very similar nervous and immune system to honey bees, it is possible that neonicotinoids also make wild bees also more prone to disease and parasites (Wood and Goulson, 2017). Fungicide effects on immune functions may also be relevant. Pettis et al. (2013) investigated the impact of collected crop pollen on *Nosema ceranae* prevalence in honey bees and found a correlation of infestations and pollen fungicide load.

A.7.3 *Neglected effects*

Source-sink effects
There are ecologically more complex effects that result from intra- and interspecific interactions which have been barely considered by scientific research, so far. These effects are most relevant on the population and community level. They are not exclusive to FVIs but especially relevant for this group (EFSA, 2015).
FVIs can easily move between multiple in-field and off-field habitats within a landscape. Spatial movement has therefore to be considered when investigating pesticide effects on FVI populations. Migration from semi-natural off-field habitats to pesticide-treated in-field areas could possibly result in source-sink dynamics: Individuals from a sustaining habitat migrate to a non-sustaining habitat and subsidize the sink population but also deplete the source population (Topping et al., 2015). This process can be mistaken for in-field recovery when the off-field surroundings are not considered. It has been shown in modeling studies that landscape-scale effects of pesticides cannot

be sufficiently estimated using small-scale data (Topping et al., 2015; Topping et al., 2014). Migratory population dynamics in time and space are difficult to detect using field experiments due to limited duration and restricted spatial scale. Landscape-scale modeling approaches represent promising methods to assess source-sink effects of pesticides (Topping et al., 2015).

Indirect effects

Aside from direct effects, pesticides can also impact FVIs indirectly through trophic interactions. Habitat quality may be adversely affected by reduction or modification of food and nesting resources (Relyea and Hoverman, 2006; Rohr et al., 2006). One of the main causes of FVI decline is decreased diversity and abundance of flower and nesting resources. This is caused by habitat destruction through agricultural land use practices, such as pesticide use (Forister et al., 2016; Goulson et al., 2015; IPBES, 2016; Ollerton, 2017). Scheper et al. (2014) combined pollen load data from entomological museum collections with population trends of wild bees. Decline of preferred food plant species was identified as one of two main factors associated with bee species declines. Therefore, herbicide applications might reduce FVI food plant supply and consequently lead to adverse population effects. Unfortunately, there is no information available to evaluate the relevance of indirect pesticide effect towards FVIs.

Ecosystem services (Pollination/biodiversity)

In contrast to protection goals that were defined by authorities (European Commission, 2009; United Nations, 1992) there is little to no research regarding the effects of pesticide applications on FVI ecosystem services, such as pollination or biodiversity. First evidence of a direct pesticide pollination effect in a field setting was found in a semi-field cage experiment (Stanley et al., 2015). *Bombus terrestris* females were exposed to thiamethoxam and allowed to forage on apple trees which subsequently reduced apple seed production. However, this is not a pollination effect in the economic sense since the number of seeds does not influence apple market value.

It is difficult to directly detect FVI diversity or population effects in field experiments, since it would take years and extensive sampling campaigns to collect the necessary data. A meta-analysis related bee species distribution monitoring data over an 18 year period in the UK to neonicotinoid use in oilseed rape (Woodcock et al., 2016). Population persistence was negatively affected in, both, bee species that forage on oilseed and those that usually do not. However, the effect was three times stronger in oilseed rape foragers. Therefore, neonicotinoid use in a mass-flowering crop possibly caused bee species decline. However, this result of pesticide effects on FVI diversity is only correlative and cannot be connected directly to pesticide use.

After collating information on the relevant groups, their ecology and habitat, exposure to pesticides, and subsequent effects, we will critically evaluate the European exposure and effect assessment for its suitability concerning FVIs.

A.8 REGULATORY DEFICITS AND DEVELOPMENT

A.8.1 *European risk assessment*

The European pesticide risk assessment is a proactive administrative measure that should ensure the protection of non-target species as outlined in Regulation (EC) 1107/2009 (European Commission, 2009). FVIs are currently covered with risk assessment schemes for bees (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b) and non-target arthropods (NTAs; Candolfi et al., 2001) within the framework of the terrestrial ecotoxicology guidance document (SANCO, 2002).
However, ongoing FVI declines that are partly caused by pesticides suggest the possibility that the current risk assessment is not sufficiently protective (Godfray et al., 2015; Goulson et al., 2015; IPBES, 2016; Potts et al., 2015). The European Food Safety Authority (EFSA) identified major shortcomings in FVI risk assessment and suggested improvements for the bee and NTA guidance documents (EFSA, 2015; EFSA PPR Panel, 2012). Consequently, they drafted a new bee guidance document which should improve the risk assessment process (EFSA, 2013). This process of revising old guidance and devising a new framework is far from finished. The revised bee guidance document has yet to be ratified and a NTA guidance document has not yet been developed. Therefore, scientific input is needed to facilitate the regulatory development.

A.8.2 *Bee risk assessment*

In the current regulatory framework, the impact of pesticides on bee species is assessed in a separate scheme in contrast to all other FVIs (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b). Exposure and effect assessment is generally carried out as follows.
Potential exposure of bees is estimated for in-field scenarios (Table A.2). At the first tier, contact contamination of individuals is evaluated by using application rates of pesticide products. Furthermore, oral exposure is considered by using data from plant residue and metabolism studies. Higher tier exposure assessment includes pesticide residue studies of relevant matrices such as dead bees, nectar, pollen, wax, or honey (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b). First tier risk assessment requires effect testing for acute contact and oral mortality in honey bees. In the case of systemic pesticide brood feeding tests can be necessary. In higher tier testing several more realistic honey bee test systems can be used to refine the evaluation process if further information is required, e.g. chronic oral tests, semi-field studies using tunnel tents, or field tests (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b).
In a revision of the current guidance, EFSA identified major deficits with regard the ecology of FVIs (EFSA, 2013; EFSA PPR Panel, 2012). The current exposure assessment does not include off-field areas, which are also FVI habitat and should therefore be considered (Table A.2). Furthermore, FVI contamination by dust from solid application as well as exposure by water sources are not incorporated. It was criticized that the entire

spectrum of bee species is not well-represented since the honey bee is used as the only surrogate. Other bee species' sensitivity towards pesticides is usually unknown (Arena and Sgolastra, 2014; Uhl et al., 2016). Since relative susceptibility varies for different pesticides, it is difficult to extrapolate acute toxicity data from the honey bee to wild bees (Biddinger et al., 2013; Uhl et al., 2016). Wild bee species also have different ecological properties than the honey bee which leads to contrasting results in complex higher tier tests (Arena and Sgolastra, 2014; Rundlöf et al., 2015; Stoner, 2016). EFSA further stated that current semi-field and field designs generally allow for too much data variance and do not provide enough statistical power (OEEP/EPPO, 2010a; OEEP/EPPO, 2010b).

As a reaction to the deficits in current bee risk assessment, EFSA drafted the new bee guidance document which includes substantial improvements (EFSA, 2013). Exposure assessment incorporates additional pesticide uptake pathways such as dust from seed treatment, guttation water, puddles, and surface water (Table A.2). Aside from in-field exposure, off-field exposure is also incorporated via deposition factors for spray, granular, and seed treatment application. Residue studies should also include plant material or bees foraging on the treated crop as well as bees returning to the hive in higher tier exposure assessment (EFSA, 2013). Two additional test species were selected because of their different acute sensitivity and ecological differences that are relevant for higher tier testing; a bumble bee (*Bombus terrestris*) and a solitary bee (*Osmia bicornis/cornuta*). In first tier effect assessment, chronic oral and larval toxicity tests were added. EFSA (2013) further called for modified study designs in higher tier effect assessment to decrease data variance and enhance statistical power. This includes larger tunnel/field size, higher number of replicates and colonies per site, greater distance between sites, the use of sister queens in colonies, and prolonged study duration.

In spite of the extensive regulatory changes that EFSA (2013) proposed, there still remain deficits and open questions that arise from their recommendations (Table A.2). The suggested exposure assessment does not include soil as a contamination source although it is acknowledged to be relevant (Gradish et al., 2019; Sgolastra et al., 2019). They discuss honey dew and extrafloral nectar as potential exposure sources but do not provide information to justify their importance for FVI exposure. Regarding effect assessment, there is reasonable doubt that the proposed additional test species will decrease uncertainty. Notwithstanding the limited available database, it can be concluded that both species are usually less sensitive than the honey bee in acute toxicity tests (Uhl et al., 2016). It may still be reasonable to use these species for higher tier testing where ecological differences influence toxicity to a greater extent (Cutler and Scott-Dupree, 2014; Rundlöf et al., 2015). However, there are currently no established chronic or larval laboratory, semi-field, or field test protocols for both species. Furthermore, the ambitious study design improvements might be difficult to implement.

There are several specific issues that are generally not included in European risk assessment and are also not considered by EFSA (2013). Neglected effects include landscape-scale source-sink effects, indirect effect trough trophic interactions, and ecosystem service effects (pollination, biodiversity). These effects have not been studied well but are very relevant for the environmental safety evaluation of pesticide products. In addition,

there is no assessment of effects after exposure to multiple pesticides, e.g. tank mixtures or sequential applications.

A.8.3 *Non-target arthropod risk assessment (non-bee FVIs)*

All non-bee FVI groups are covered within the current NTA risk assessment framework (Candolfi et al., 2001). NTA exposure assessment is performed separately for in-field overspray and off-field spray drift scenarios which include calculations of maximum residue levels (Table A.2). The NTA effect evaluation is performed with several predatory or parasitic arthropods (e.g. *Aphidius rhopalosiphi*, *Typhlodromus pyri*). In first tier evaluation, acute and chronic mortality laboratory tests are conducted whereas higher tier testing includes extended laboratory and aged pesticide residue studies, semi-field, and field experiments to study more subtle pesticide impact under more realistic conditions, i.e. lethal and sublethal effects. Four additional beneficial test species are proposed for products with special mode of action or higher tier assessment which are derived from integrated pest management (*Orius laevigatus*, *Chrysoperla carnea*, *Coccinella septempunctata*, *Aleochara bilineata*).

Similar to bee risk assessment the current NTA scheme needs to be adjusted to allow for a protective evaluation of pesticide impact on non-bee FVIs (Table A.2). As discussed by EFSA (2015), exposure caused by dust drift after sowing of pesticide-treated seeds needs to be assessed. Furthermore, there is no oral toxicity testing in the first tier assessment, which would be relevant for FVIs that consume nectar, pollen or stem/leaf material. Moreover, non-bee FVIs are not specifically accounted for by surrogate organisms.

To alleviate shortcomings in the current guidance, EFSA (2015) published a scientific opinion on NTA risk assessment which is the precursor of an upcoming new NTA guidance document. They revised the exposure evaluation to include estimates of pesticide uptake through food (nectar, pollen, stem/leaf material) and dust as well as the contamination of soil surfaces (Table A.2). Furthermore, one explicit FVI species (lepidopteran larvae) has been proposed as an additional test species for effect assessment. They proposed a landscape-scale risk assessment for mobile species such as FVIs which is a major change of previous proceedings. This should ensure that in-field effects do not lead to unacceptable reductions in off-field populations (EFSA, 2015). Previously neglected issues such as indirect effects, source-sink dynamics, and ecosystem service effects are also discussed. They further mention that sequential and simultaneous use of different pesticides should be included into risk assessment.

However, EFSA (2015) did not address all deficits of the current framework and raised open questions with their recommendations for a future NTA guidance (Table A.2). Exposure assessment of guttation water is not included as well as in-soil residue evaluation. Due to the multitude of different life strategies and ecological niches of non-bee FVIs, it remains unclear if one test species will sufficiently represent this group, especially in higher tier effect assessment. The NTA scientific opinion is overall lacking in concrete protocols for effect and exposure assessment. A new NTA guidance document is supposed to follow up with more tangible recommendations.

Upcoming FVI guidance should make use of data-driven approaches to pesticide impact assessment. These regulatory tools allow for large-scale evaluations of FVI populations by incorporating ecological information.

Table A.2.: Relevant guidelines, regulatory deficits and development. + indicates an addition to the existing guidance.

	Regulatory documents	FVI test organisms	Effect assessment	Exposure assessment	Exposure scenarios	Deficits
Bees	Current guidance document (OEEP/EPPO, 2010b; OEEP/EPPO, 2010a)	*A. mellifera*	First tier: Acute contact/oral, brood feeding Higher tier: chronic oral, semi-field, field	Spray, nectar, pollen	In-field	Test organism not representative, insufficient exposure assessment
	New guidance document (EFSA, 2013)	+ *O. bicornis*, *B. terrestris*	First tier: + Chronic oral, chronic larvae Higher tier: + improved quality in semi-field, field protocols	+ Dust, guttation water, surface waters, puddles	+ Off-field	No soil exposure assessment, uncertainty about suitability of additional test species, missing or unvalidated semi-field and field test protocols, ecologically complex issues not incorporated
Non-bee FVIs	Current guidance document (Candolfi et al., 2001)	None	First tier: Acute/chronic contact Higher tier: Extended lab, aged residue, semi-field, field	Spray/residue	In-field, off-field	No FVI species, insufficient exposure assessment
	New scientific opinion (EFSA, 2015)	+ 1 lepidopteran species	Higher tier: + Landscape-scale assessment	+ Nectar, pollen, plant material, dust	No change	No in-soil & guttation water exposure assessment, uncertainty if additional test species is adequate surrogate for FVIs, lacking concrete guidance

A.9 FUTURE REGULATORY TOOLS

A.9.1 *Trait-based analysis*

Our knowledge of the species we want to protect and their environment can enable us to develop a risk assessment that is better suited for specific groups such as FVIs. It has been indicated that it is rather difficult to identify representative surrogate species for this diverse group (Heard et al., 2017; Uhl et al., 2016). Therefore, alternative approaches have to be considered that facilitate the assessment of pesticide impact on FVI communities. Ecological traits (i.e. species-specific properties) determine the breadth of the ecological niche and therefore the susceptibility of FVI populations to environmental factors. The narrower the niche, the higher the sensitivity to external stressors (de Palma et al., 2015; Forrest et al., 2015; Hofmann et al., 2019; Williams et al., 2010). Therefore, it is possible to allocate FVI species to ecologically similar categories and assess their populations' vulnerability to stressors such as pesticides (Fig. A.4; Brittain and Potts, 2011; Hofmann et al., 2019; Sponsler et al., 2019). Several traits have been identified to be relevant for population vulnerability in bee species, e.g. mobility, sociality, nesting, lecty, flight season/duration, and voltinism (Table A.S2 in Supporting Information). By combining toxicity and trait data in a modeling approach, it would be possible to make broader predictions about the consequences of pesticide use on FVI communities (Brittain and Potts, 2011; de Palma et al., 2015; Forrest et al., 2015; Williams et al., 2010).

Trait-based approaches for risk assessment have already been proposed with emphasis on the aquatic environment (Rubach et al., 2011; Van den Brink et al., 2011). The underlying concept can be easily translated to FVIs and their specific properties. A comprehensive trait database for European bees is already available for bee species vulnerability classification (Roberts et al., 2016). Vulnerability models would need to be validated with extensive monitoring data. Unfortunately, for all other FVI groups there is significantly less information about the ecological parameters that influence vulnerability and there are no applicable databases available.

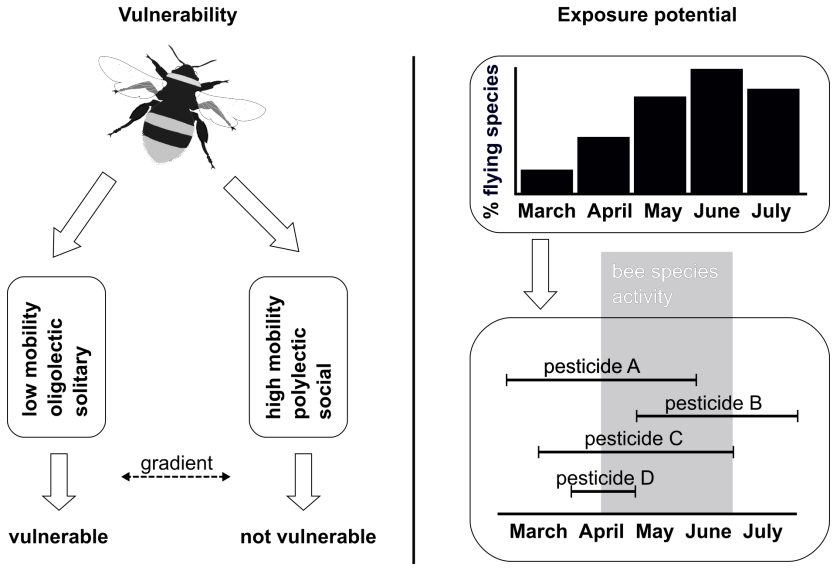

Figure A.4.: Potential applications of trait data to estimate FVI vulnerability to stressors and likelihood of pesticide exposure.

Similar to the effect assessment, exposure evaluation of FVI species could also be improved by analyzing trait data. The influx of pesticides into FVI habitats is not necessarily resulting in exposure of FVI species. However, ecological trait information can be used to assess uptake probability and identify relevant exposure pathways (Brittain and Potts, 2011; de Palma et al., 2015; Sgolastra et al., 2019; Sponsler et al., 2019). A combination of trait data with pesticide application information and residue levels in habitat matrices could enable a quantitative estimation of FVI contamination through specific pathways. There are a number of traits that influence exposure potential. These ecological properties include flight activity throughout the year, daily flight activity, food plant preference (lecty), nesting (location and construction), sociality, and mobility for bees (Brittain and Potts, 2011; de Palma et al., 2015; Sgolastra et al., 2019; Thompson, 2001). Application dates in field cultures and pesticides persistence can be combined with the active flight period of bee species to assess the proportion of species that are potentially exposed to a specific substance (Sponsler et al., 2019). A trait-based exposure analysis could also be performed for European bee species using the aforementioned database (Roberts et al., 2016). A linkage of habitat to FVI exposure is currently not possible for many relevant matrices such as soil, stem or leaf material, and non-nectar fluids. If the existing information gaps are closed, it may be possible to devise a holistic general framework that connects trait-based effect and exposure assessment as it was proposed for aquatic organisms (Rubach et al., 2011; Van den Brink et al., 2011).

A.9.2 *Landscape-scale modeling*

Since FVIs are mobile species, knowledge about their spatio-temporal population dynamics is required for a protective assessment of pesticide impact. This was recognized by EFSA (2015) in their NTA scientific opinion, where they argued that a landscape-scale risk assessment should be developed. A feasible approach is employing a model system that predicts the effects of pesticide applications on populations within the agricultural landscape (Rortais et al., 2017). The animal, landscape, and man simulation system (ALMaSS) is one possible framework that could be used to evaluate pesticide impact on predefined key species (Topping et al., 2003). It can be used to implement agent-based animals population models within a comprehensive and dynamic landscape simulation. This allows for a realistic simulation of pesticide use patterns on a spatio-temporal scale. Animal behavior parameters are modeled to predict exposure and effects at the individual level which translates into population impact. The ALMaSS suite of models can already be applied to several arthropod, bird, and mammalian species. However, such a complex system requires detailed knowledge of the investigated landscape (land use and management) as well as extensive information about the ecology of model species (Topping et al., 2003). Landscape-scale models also need to be accompanied by FVI monitoring to validate their predictions for the use in risk assessment. There are other approaches such as the BEEHAVE model that might be easier to implement at the cost of reduced explanatory power at the landscape level (Becher et al., 2014; Rortais et al., 2017). This model was designed to simulate pesticide risk to honey bee colonies. An adapted version has been developed to provide the same functionality for bumble bees (Becher et al., 2018). It is unclear if other FVIs groups than bees can be integrated into this framework.

Both presented regulatory approaches are suitable to improve future pesticide risk assessment. The main limiting factor for their application is FVI ecological data availability.

A.10 RESEARCH RECOMMENDATIONS

In this review, we identified the relevant FVI groups in the agricultural landscape as bees, flies (non-syrphids and syrphids), lepidopterans (moths and butterflies), beetles, and wasps (Grass et al., 2016; Orford et al., 2015; Rader et al., 2015). There is only very limited information available to evaluate all possible groups such as non-bee Hymenoptera and Hemiptera. Proportions of species and individuals of the respective groups vary in different crop systems (Rader et al., 2015) and semi-natural habitats (Grass et al., 2016; Orford et al., 2015). FVIs are flying, mobile species that live in the entire agricultural landscape. They use both farmland and non-target areas such as flower strips, field margins or hedgerows as habitat (e.g. Coudrain et al., 2015; Denisow and Wrzesień, 2015; Feltham et al., 2015; Holzschuh et al., 2013; Marshall and Moonen, 2002; Tschumi et al., 2015). There is insufficient information available to assess suitable

habitats of the specific FVI groups and their function in more detail. They use several compartments of these habitats to fulfill specific ecological functions such as foraging, mating, and nesting (Michener, 2007; Westrich, 1990). Pesticide applications on crops theoretically lead to contamination of FVI habitat compartments. Therefore, FVI species are potentially exposed to pesticide through multiple pathways. Analytic studies show that FVIs are contaminated with numerous pesticides (Botías et al., 2017; Chauzat et al., 2011; Mullin et al., 2010). There is also extensive evidence of pesticide residues in crops and non-target areas (Wood and Goulson, 2017). Pesticide residues have been detected in all habitat compartments which include nectar and pollen (e.g. Chauzat et al., 2011; Mullin et al., 2010; Tosi et al., 2018), soil (Hvězdová et al., 2018; Jones et al., 2014), stem and leaves (Botías et al., 2016; Mogren and Lundgren, 2016), and water sources (Samson-Robert et al., 2014; Schaafsma et al., 2015; Schmolke et al., 2018; Stehle and Schulz, 2015; Wirtz et al., 2018). However, it is not possible to link habitat to FVI exposure with the current knowledge base. There is a lack of information regarding the exposure of all non-bee FVI groups. FVIs are affected by many pesticides, most notably neonicotinoid insecticides, at environmental realistic doses. Bee (semi-)field studies found adverse effects on ecologically relevant parameters such as reproduction, foraging, and immune functions (e.g. Alburaki et al., 2015; Dively et al., 2015; Feltham et al., 2015; Gill et al., 2012; Rundlöf et al., 2015; Whitehorn et al., 2012). Furthermore, there are ecologically important effects on the population/community level that have been neglected so far by research such as source-sink effects, indirect effects, and effects on the ecosystem services pollination and biodiversity (EFSA, 2015).

The existing and proposed future risk assessment frameworks contain some deficits regarding the exposure and effect evaluation for FVIs. Both the current bee and NTA risk assessment (Candolfi et al., 2001; OEEP/EPPO, 2010a; OEEP/EPPO, 2010b) fail to cover the specific ecological properties of FVI species. EFSA (2013) and EFSA (2015) drafted new regulatory documents that improve the risk assessment process. However, there are still unaddressed issues and uncertainties that need to be resolved to achieve a protective risk assessment scheme for FVIs. Data-driven tools can help to improve FVI risk assessment by using ecological information. Trait data can be used determine their exposure to pesticides and the vulnerability of FVI populations to stressors (Rubach et al., 2011; Van den Brink et al., 2011). This information could be combined with toxicity, pesticide application, and residue data to assess pesticide impact on FVI communities in a connected framework. Another promising approach is landscape-scale modeling which allows for an evaluation of the pesticide exposure of FVI populations and subsequent effects, in space and time (Rortais et al., 2017; Topping et al., 2003). Both approaches need comprehensive databases that include FVI species traits, landscape composition, land use, pesticide toxicity, and residues in relevant matrices which are currently not sufficiently available.

Throughout this review, we highlighted knowledge gaps that need to be closed in order to better understand FVIs and assess the effects of pesticide applications in their habitat. Therefore, we call for general research on the following subjects:

- Identification of all relevant FVI groups aside from bee species
- Study of ecology and habitat of all FVI groups
- Implementation of extensive FVI population monitoring campaigns to determine threat level of specific groups
- Creation of a comprehensive FVI ecological trait database
- Determination of FVI habitat exposure with consideration to relevant matrices and creation of a pesticide residue database
- Linkage of habitat to FVI exposure with special regard to non-bee FVIs
- Assessment of pesticide effects with a focus on population-relevant parameters, especially for non-bee FVIs
- Investigation of neglected effects such as source-sink, indirect, and ecosystem service (pollination, biodiversity) effects
- Development and advancement of suitable trait-based approaches for a impact assessment on the landscape scale

Aside from this scientific input, there is a need for regulatory decision making processes to move away from arbitrary conservative assumptions and overcomplicated risk assessment schemes towards a more substantiated and holistic approach which incorporates large-scale evaluation methods and utilizes ecological information.

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A.12 CONFLICT OF INTEREST

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A.13 AUTHOR CONTRIBUTIONS

Conceptualisation PU CAB.
Literature review PU.
Writing - original draft PU.
Writing - review & editing PU CAB.

A.14 DATA AVAILABILITY

Aside from the Supporting Information, there are no data to be reported.

A.15 REFERENCES

Alburaki, M., S. Boutin, P.-L. Mercier, Y. Loublier, M. Chagnon, and N. Derome (2015). "Neonicotinoid-Coated Zea mays Seeds Indirectly Affect Honeybee Performance and Pathogen Susceptibility in Field Trials". *PloS one* 10 (5), e0125790.

Alford, A. and C. H. Krupke (2017). "Translocation of the neonicotinoid seed treatment clothianidin in maize". *PLOS ONE* 12 (3), e0173836.

Arce, A. N., T. I. David, E. L. Randall, A. Ramos Rodrigues, T. J. Colgan, Y. Wurm, and R. J. Gill (2016). "Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting". *Journal of Applied Ecology* 54 (4), 1199–1208.

Arena, M. and F. Sgolastra (2014). "A meta-analysis comparing the sensitivity of bees to pesticides". *Ecotoxicology* 23 (3), 324–334.

Balmer, O., C. E. Géneau, E. Belz, B. Weishaupt, G. Förderer, S. Moos, N. Ditner, I. Juric, and H. Luka (2014). "Wildflower companion plants increase pest parasitation and yield in cabbage fields: Experimental demonstration and call for caution". *Biological Control* 76, 19–27.

Becher, M. A., V. Grimm, P. Thorbek, J. Horn, P. J. Kennedy, and J. L. Osborne (2014). "BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure". *Journal of Applied Ecology* 51 (2), 470–482.

Becher, M. A., G. Twiston-Davies, T. D. Penny, D. Goulson, E. L. Rotheray, and J. L. Osborne (2018). "*Bumble* -BEEHAVE: A systems model for exploring multifactorial causes of bumblebee decline at individual, colony, population and community level". *Journal of Applied Ecology* 55.

Biddinger, D. J., J. L. Robertson, C. Mullin, J. Frazier, S. A. Ashcraft, E. G. Rajotte, N. K. Joshi, and M. Vaughn (2013). "Comparative toxicities and synergism of apple orchard pesticides to Apis mellifera (L.) and Osmia cornifrons (Radoszkowski)". *PloS one* 8 (9), e72587.

Blacquière, T., G. Smagghe, C. A. M. Gestel, and V. Mommaerts (2012). "Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment". *Ecotoxicology* 21 (4), 973–992.

Bonmatin, J.-M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, Mitchell, E A D, D. A. Noome, N. Simon-Delso, and A. Tapparo (2015). "Environmental fate and exposure; neonicotinoids and fipronil". *Environmental science and pollution research international* 22 (1), 35–67.

Bonmatin, J. M., P. A. Marchand, R. Charvet, I. Moineau, E. R. Bengsch, and M. E. Colin (2005). "Quantification of imidacloprid uptake in maize crops". *Journal of agricultural and food chemistry* 53 (13), 5336–5341.

Botías, C., A. David, E. M. Hill, and D. Goulson (2016). "Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects". *Science of The Total Environment* 566-567, 269–278.

Botías, C., A. David, E. M. Hill, and D. Goulson (2017). "Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes". *Environmental Pollution* 222, 73–82.

Botías, C., A. David, J. Horwood, A. Abdul-Sada, E. Nicholls, E. Hill, and D. Goulson (2015). "Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees". *Environmental science & technology* 49 (21), 12731–12740.

Brittain, C. and S. G. Potts (2011). "The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination". *Basic and Applied Ecology* 12 (4), 321–331.

Candolfi, M., K. Barrett, P. Campbell, R. Forster, N. Grandy, M. Huet, G. Lewis, P. Oomen, R. Schmuck, and H. Vogt (2001). *Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods: From the ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropods Regulatory Testing), a joint BART, EOOP/CoE, OECD and IOBC Workshop organized in conjunction with SETAC-Europe and EC: Held at Wageningen International Conference Centre; Wageningen, The Netherlands; 21-23 March 2000*. Pensacola FL (USA): Setac Office.

Chauzat, M.-P., A.-C. Martel, N. Cougoule, P. Porta, J. Lachaize, S. Zeggane, M. Aubert, P. Carpentier, and J.-P. Faucon (2011). "An assessment of honeybee colony matrices, Apis mellifera (Hymenoptera: Apidae) to monitor pesticide presence in continental France". *Environmental Toxicology and Chemistry* 30 (1), 103–111.

Coalition of the Willing on Pollinators (2016). *Declaration on the Coalition of the Willing on Pollinators*. Cancun (Mexico).

Coudrain, V., S. Rittiner, F. Herzog, W. Tinner, and M. H. Entling (2015). "Landscape distribution of food and nesting sites affect larval diet and nest size, but not abundance of Osmia bicornis". *Insect Science* 23 (5), 746–753.

Cutler, G. C. and C. D. Scott-Dupree (2014). "A field study examining the effects of exposure to neonicotinoid seed-treated corn on commercial bumble bee colonies". *Ecotoxicology* 23 (9), 1755–1763.

Cutler, G. C., C. D. Scott-Dupree, M. Sultan, A. D. McFarlane, and L. Brewer (2014). "A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success". *PeerJ* 2, e652.

David, A., C. Botías, A. Abdul-Sada, E. Nicholls, E. L. Rotheray, E. M. Hill, and D. Goulson (2016). "Widespread contamination of wildflower and bee-collected pollen

with complex mixtures of neonicotinoids and fungicides commonly applied to crops". *Environment international* 88, 169–178.

de Palma, A., M. Kuhlmann, S. P. Roberts, S. G. Potts, L. Börger, L. N. Hudson, I. Lysenko, T. Newbold, A. Purvis, and I. Kaplan (2015). "Ecological traits affect the sensitivity of bees to land-use pressures in European agricultural landscapes". *Journal of Applied Ecology* 52 (6), 1567–1577.

Denisow, B. and M. Wrzesień (2015). "The Importance of Field-Margin Location for Maintenance of Food Niches for Pollinators". *Journal of Apicultural Science* 59 (1), 27–37.

Dively, G. P., M. S. Embrey, A. Kamel, D. J. Hawthorne, and J. S. Pettis (2015). "Assessment of chronic sublethal effects of imidacloprid on honey bee colony health". *PLoS ONE* 10 (3), e0118748.

Düker, A. and R. Kubiak (2015). "Stem application of metalaxyl for the protection of Vitis vinifera L.('Riesling') leaves and grapes against downy mildew (Plasmopara viticola)". *VITIS-Journal of Grapevine Research* 48 (1), 43–48.

Ebert, G., ed. (1994). *Die Schmetterlinge Baden-Württembergs, Bd.3, Nachtfalter 1: Allgemeiner Teil: Benutzerhinweise, Beobachtungsmethoden bei Nachtfaltern, Aspekte zur Nahrungskonkurrenz unter Nachtfaltern, Ergebnisse. Spezieller Teil: Hepialidae, Cossidae, Zygaenidae, Limacodidae, Psychidae, Thyrididae*. Vol. 3. Die Schmetterlinge Baden-Württembergs. Stuttgart: Ulmer Verlag.

EFSA (2013). "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 11 (7), 3295.

EFSA (2015). "Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods". *EFSA Journal* 13 (2).

EFSA PPR Panel (2012). "Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 10 (5), 2668 pp.

Ellis, C., K. J. Park, P. Whitehorn, A. David, and D. Goulson (2017). "The Neonicotinoid Insecticide Thiacloprid Impacts upon Bumblebee Colony Development under Field Conditions". *Environmental Science & Technology* 51 (3), 1727–1732.

European Commission (2009). "Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009: concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC". *Official Journal of the European Union*.

Feltham, H., K. Park, and D. Goulson (2014). "Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency". *Ecotoxicology* 23 (3), 317–323.

Feltham, H., K. Park, J. Minderman, and D. Goulson (2015). "Experimental evidence that wildflower strips increase pollinator visits to crops". *Ecology and evolution* 5 (16), 3523–3530.

Fischer, M., O. Bossdorf, S. Gockel, F. Hänsel, A. Hemp, D. Hessenmöller, G. Korte, J. Nieschulze, S. Pfeiffer, D. Prati, S. Renner, I. Schöning, U. Schumacher, K. Wells, F. Buscot, E. K. Kalko, K. E. Linsenmair, E.-D. Schulze, and W. W. Weisser (2010). "Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories". *Basic and Applied Ecology* 11 (6), 473–485.

A

Forister, M. L., B. Cousens, J. G. Harrison, K. Anderson, J. H. Thorne, D. Waetjen, C. C. Nice, M. De Parsia, M. L. Hladik, R. Meese, H. van Vliet, and A. M. Shapiro (2016). "Increasing neonicotinoid use and the declining butterfly fauna of lowland California". *Biology Letters* 12 (8), 20160475.

Forrest, J. R. K., R. W. Thorp, C. Kremen, N. M. Williams, and Y. Clough (2015). "Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape". *Journal of Applied Ecology* 52 (3), 706–715.

Frouz, J. (1999). "Use of soil dwelling Diptera (Insecta, Diptera) as bioindicators: a review of ecological requirements and response to disturbance". *Agriculture, Ecosystems & Environment* 74 (1-3), 167–186.

Gels, J. A., D. W. Held, and D. A. Potter (2002). "Hazards of insecticides to the bumble bees Bombus impatiens (Hymenoptera: Apidae) foraging on flowering white clover in turf". *Journal of Economic Entomology* 95 (4), 722–728.

Gevao, B., K. Semple, and K. Jones (2000). "Bound pesticide residues in soils: a review". *Environmental Pollution* 108 (1), 3–14.

Gierer, F., S. Vaughan, M. Slater, H. M. Thompson, J. S. Elmore, and R. D. Girling (2019). "A review of the factors that influence pesticide residues in pollen and nectar: Future research requirements for optimising the estimation of pollinator exposure". *Environmental Pollution* 249, 236–247.

Gill, R. J. and N. E. Raine (2014). "Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure". *Functional Ecology* 28 (6), 1459–1471.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine (2012). "Combined pesticide exposure severely affects individual-and colony-level traits in bees". *Nature* 491 (7422), 105–108.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, G. Petrokofsky, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean (2014). "A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators". *Proceedings. Biological sciences / The Royal Society* 281 (1786), 20140558.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean (2015). "A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators". *Proceedings. Biological sciences / The Royal Society* 282 (1818), 20151821.

Goulson, D. (2013). "An overview of the environmental risks posed by neonicotinoid insecticides". *Journal of Applied Ecology* 50 (4), 977–987.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray (2015). "Bee declines driven by combined stress from parasites, pesticides, and lack of flowers". *Science* 347 (6229), 1255957.

Gradish, A. E., J. van der Steen, C. D. Scott-Dupree, A. R. Cabrera, G. C. Cutler, D. Goulson, O. Klein, D. M. Lehmann, J. Lückmann, B. O'Neill, N. E. Raine, B. Sharma, and H. Thompson (2019). "Comparison of Pesticide Exposure in Honey Bees (Hymenoptera: Apidae) and Bumble Bees (Hymenoptera: Apidae): Implications for Risk Assessments". *Environmental Entomology* 48 (1), 12–21.

Grass, I., J. Albrecht, F. Jauker, T. Diekötter, D. Warzecha, V. Wolters, and N. Farwig (2016). "Much more than bees—Wildflower plantings support highly diverse flower-visitor communities from complex to structurally simple agricultural landscapes". *Agriculture, Ecosystems & Environment* 225, 45–53.

Haaland, C., R. E. Naisbit, and L.-F. Bersier (2011). "Sown wildflower strips for insect conservation: a review". *Insect Conservation and Diversity* 4 (1), 60–80.

Hahn, M., T. Schmidt, and C. A. Brühl (2015). "Pesticides and non-target terrestrial invertebrates". In: *Protection of biodiversity in the risk assessment and risk management of pesticides with a focus on arthropods, soil organisms, and amphibians. Report of the Research and Development Project Nr 3709 65 421.* Ed. by C. A. Brühl, Alscher A., Berger G., Bethwell C, Graef F., Hahn M., Schmidt T., and Weber B. Vol. 76. Texte. Federal Environment Agency (Germany), 244.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. de Kroon (2017). "More than 75 percent decline over 27 years in total flying insect biomass in protected areas". *PLOS ONE* 12 (10), e0185809.

Heard, M. S., J. Baas, J.-. L. Dorne, E. Lahive, A. G. Robinson, A. Rortais, D. J. Spurgeon, C. Svendsen, and H. Hesketh (2017). "Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species?" *Science of The Total Environment* 578, 357–365.

Heimbach, F., A. Russ, M. Schimmer, and K. Born (2016). "Large-scale monitoring of effects of clothianidin dressed oilseed rape seeds on pollinating insects in Northern Germany: implementation of the monitoring project and its representativeness". *Ecotoxicology* 25 (9), 1630–1647.

Hofmann, M. M., C. M. Zohner, and S. S. Renner (2019). "Narrow habitat breadth and late-summer emergence increases extinction vulnerability in Central European bees". *Proceedings of the Royal Society B: Biological Sciences* 286 (1898), 20190316.

Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter (2013). "Mass-flowering crops enhance wild bee abundance". *Oecologia* 172 (2), 477–484.

Hvězdová, M., P. Kosubová, M. Košíková, K. E. Scherr, Z. Šimek, L. Brodský, M. Šudoma, L. Škulcová, M. Sáňka, M. Svobodová, L. Krkošková, J. Vašíčková, N. Neuwirthová, L. Bielská, and J. Hofman (2018). "Currently and recently used pesticides in Central European arable soils". *Science of The Total Environment* 613-614, 361–370.

IPBES (2016). *The assessment report on pollinators, pollination and food production of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*.

Jones, A., P. Harrington, and G. Turnbull (2014). "Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years". *Pest management science* 70 (12), 1780–1784.

Klein, A.-M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke (2007). "Importance of pollinators in changing landscapes for world crops". *Proceedings of the Royal Society B: Biological Sciences* 274 (1608), 303–313.

Koch, K. and H. Freude (1992). *Die Käfer Mitteleuropas: Ökologie*. Vol. 3. Goecke & Evers.

Main, A. R., E. B. Webb, K. W. Goyne, and D. Mengel (2018). "Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis". *Ecological Applications* 28 (5), 1232–1244.

Manandhar, R. and M. G. Wright (2016). "Effects of Interplanting Flowering Plants on the Biological Control of Corn Earworm (Lepidoptera: Noctuidae) and Thrips (Thysanoptera: Thripidae) in Sweet Corn". *Journal of Economic Entomology* 109 (1), 113–119.

Marshall, E. J. and A. C. Moonen (2002). "Field margins in northern Europe: their functions and interactions with agriculture". *Agriculture, Ecosystems & Environment* 89 (1-2), 5–21.

Marshall, E., T. M. West, and D. Kleijn (2006). "Impacts of an agri-environment field margin prescription on the flora and fauna of arable farmland in different landscapes". *Agriculture, Ecosystems & Environment* 113 (1-4), 36–44.

Michener, C. D. (2007). *The bees of the world*. 2. ed. Baltimore: Johns Hopkins Univ. Press.

Miorini, T. J., C. G. Raetano, and S. E. Everhart (2017). "Control of white mold of dry bean and residual activity of fungicides applied by chemigation". *Crop Protection* 94, 192–202.

Moffat, C., S. T. Buckland, A. J. Samson, R. McArthur, V. Chamosa Pino, K. A. Bollan, J. T.-J. Huang, and C. N. Connolly (2016). "Neonicotinoids target distinct nicotinic acetylcholine receptors and neurons, leading to differential risks to bumblebees". *Scientific Reports* 6, 24764.

Moffat, C., J. G. Pacheco, S. Sharp, A. J. Samson, K. A. Bollan, J. Huang, S. T. Buckland, and C. N. Connolly (2015). "Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (Bombus terrestris)". *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 29 (5), 2112–2119.

Mogren, C. L. and J. G. Lundgren (2016). "Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status". *Scientific Reports* 6, 29608.

Mogren, C. L., T. A. Rand, S. W. Fausti, and J. G. Lundgren (2016). "The Effects of Crop Intensification on the Diversity of Native Pollinator Communities". *Environmental Entomology* 45 (4), 865–872.

Morrissey, C. A., P. Mineau, J. H. Devries, F. Sanchez-Bayo, M. Liess, M. C. Cavallaro, and K. Liber (2015). "Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review". *Environment International* 74, 291–303.

Mörtl, M., B. Darvas, Á. Vehovszky, J. Győri, and A. Székács (2019). "Contamination of the guttation liquid of two common weeds with neonicotinoids from coated maize seeds planted in close proximity". *Science of The Total Environment* 649, 1137–1143.

Müller, A., S. Diener, S. Schnyder, K. Stutz, C. Sedivy, and S. Dorn (2006). "Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships". *Biological Conservation* 130 (4), 604–615.

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp, and J. S. Pettis (2010). "High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health". *PloS one* 5 (3), e9754.

Nuyttens, D., W. Devarrewaere, P. Verboven, and D. Foqué (2013). "Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review". *Pest Management Science* 69 (5), 564–575.

OEEP/EPPO (2010a). "Efficacy evaluation of plant protection products. Side-effects on honeybees". *Bulletin OEPP/EPPO Bulletin* 40, 313–319.

OEEP/EPPO (2010b). "Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees". *Bulletin OEPP/EPPO Bulletin* 40, 323–331.

Ollerton, J. (2017). "Pollinator Diversity: Distribution, Ecological Function, and Conservation". *Annual Review of Ecology, Evolution, and Systematics* 48, 353–76.

Ollerton, J., R. Winfree, and S. Tarrant (2011). "How many flowering plants are pollinated by animals?" *Oikos* 120 (3), 321–326.

Orford, K. A., I. P. Vaughan, and J. Memmott (2015). "The forgotten flies: the importance of non-syrphid Diptera as pollinators". *Proceedings of the Royal Society B: Biological Sciences* 282 (20142934).

Pecenka, J. R. and J. G. Lundgren (2015). "Non-target effects of clothianidin on monarch butterflies". *The Science of Nature* 102 (3-4).

Peters, B., Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (Osmia bicornis)". *Ecotoxicology* 25 (9), 1679–1690.

Pettis, J. S., E. M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose, and D. vanEngelsdorp (2013). "Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae". *PLoS ONE* 8 (7), e70182.

Pettis, J. S., D. vanEngelsdorp, J. Johnson, and G. Dively (2012). "Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema". *Naturwissenschaften* 99 (2), 153–158.

Pilling, E., P. Campbell, M. Coulson, N. Ruddle, and I. Tornier (2013). "A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam". *PLoS ONE* 8 (10), e77193.

Pisa, L. W., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, M. McField, C. A. Morrissey, D. A. Noome, J. Settele, N. Simon-Delso, J. D. Stark, Van der Sluijs, J P, H. van Dyck, M. Wiemers, and D. Goulson (2015). "Effects of neonicotinoids and fipronil on non-target invertebrates". *Environmental science and pollution research international* 22 (1), 68–102.

Pisa, L., D. Goulson, E.-C. Yang, D. Gibbons, F. Sánchez-Bayo, E. Mitchell, A. Aebi, J. van der Sluijs, C. J. K. MacQuarrie, C. Giorio, E. Y. Long, M. McField, M. Bijleveld van Lexmond, and J.-M. Bonmatin (2017). "An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems". *Environmental Science and Pollution Research*.

Pohorecka, K., P. Skubida, P. Semkiw, A. Miszczak, D. Teper, P. Sikorski, K. Zagibajło, M. Skubida, D. Zdańska, and A. Bober (2013). "Effects of exposure of honey bee colonies to neonicotinoid seed–treated maize crops". *Journal of Apicultural Science* 57 (2).

Potts, S. G., K. Biesmeijer, R. Bommarco, T. Breeze, L. Carvalheiro, M. Franzén, J. P. González-Varo, A. Holzschuh, D. Kleijn, A.-M. Klein, B. Kunin, T. Lecocq, O. Lundin, D. Michez, P. Neumann, A. Nieto, L. Penev, P. Rasmont, O. Ratamäki, V. Riedinger, Roberts, S. P. M., M. Rundlöf, J. Scheper, P. Sørensen, I. Steffan-Dewenter, Stoev, P., Vilà, M., and O. Schweiger (2015). *Status and trends of European pollinators. Key findings of the STEP project*. Ed. by Pensoft Publishers. 74 pp. Sofia.

Powney, G. D., C. Carvell, M. Edwards, R. K. A. Morris, H. E. Roy, B. A. Woodcock, and N. J. B. Isaac (2019). "Widespread losses of pollinating insects in Britain". *Nature Communications* 10 (1).

Rader, R., I. Bartomeus, L. A. Garibaldi, Garratt, Michael P. D., B. G. Howlett, R. Winfree, S. A. Cunningham, M. M. Mayfield, A. D. Arthur, G. K. S. Andersson, R. Bommarco, C. Brittain, L. G. Carvalheiro, N. P. Chacoff, M. H. Entling, B. Foully, B. M. Freitas, B. Gemmill-Herren, J. Ghazoul, S. R. Griffin, C. L. Gross, L. Herbertsson, F. Herzog, J. Hipólito, S. Jaggar, F. Jauker, A.-M. Klein, D. Kleijn, S. Krishnan, C. Q. Lemos, S. A. M. Lindström, Y. Mandelik, V. M. Monteiro, W. Nelson, L. Nilsson, D. E. Pattemore, N. de O. Pereira, G. Pisanty, S. G. Potts, M. Reemer, M. Rundlöf, C. S. Sheffield, J. Scheper, C. Schüepp, H. G. Smith, D. A. Stanley, J. C. Stout, H. Szentgyörgyi, H. Taki, C. H. Vergara, B. F. Viana, and M. Woyciechowski (2015). "Non-bee insects are important contributors to global crop pollination". *Proceedings of the National Academy of Sciences* 113 (1), 146–151.

Reetz, J. E., W. Schulz, W. Seitz, M. Spiteller, S. Zühlke, W. Armbruster, and K. Wallner (2016). "Uptake of Neonicotinoid Insecticides by Water-Foraging Honey Bees (Hymenoptera: Apidae) Through Guttation Fluid of Winter Oilseed Rape". *Journal of Economic Entomology* 109 (1), 31–40.

Relyea, R. and J. Hoverman (2006). "Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems". *Ecology Letters* 9 (10), 1157–1171.

Requier, F., J.-F. Odoux, T. Tamic, N. Moreau, M. Henry, A. Decourtye, and V. Bretagnolle (2015). "Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds". *Ecological Applications* 25 (4), 881–890.

Roberts, S. P. M., S. G. Potts, and M. Kuhlmann (2016). *Trait database of European bees*. unpublished. Excerpt provided 2016-07-14.

Rohr, J. R., J. L. Kerby, and A. Sih (2006). "Community ecology as a framework for predicting contaminant effects". *Trends in ecology & evolution* 21 (11), 606–613.

Rolke, D., M. Persigehl, B. Peters, G. Sterk, and W. Blenau (2016). "Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey". *Ecotoxicology* 25 (9), 1691–1701.

Rortais, A., G. Arnold, J.-L. Dorne, S. J. More, G. Sperandio, F. Streissl, C. Szentes, and F. Verdonck (2017). "Risk assessment of pesticides and other stressors in bees: Principles, data gaps and perspectives from the European Food Safety Authority". *Science of The Total Environment* 587-588, 524–537.

Rubach, M. N., R. Ashauer, D. B. Buchwalter, H. De Lange, M. Hamer, T. G. Preuss, K. Töpke, and S. J. Maund (2011). "Framework for traits-based assessment in ecotoxicology". *Integrated Environmental Assessment and Management* 7 (2), 172–186.

Ruddle, N., C. Elston, O. Klein, A. Hamberger, and H. Thompson (2018). "Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee *Osmia bicornis*: Thiamethoxam-treated oilseed rape and *Osmia bicornis* reproduction". *Environmental Toxicology and Chemistry* 37 (4), 1071–1083.

Rundlöf, M., Andersson, Georg K S, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone, and H. G. Smith (2015). "Seed coating with a neonicotinoid insecticide negatively affects wild bees". *Nature* 521, 77–80.

Samson-Robert, O., G. Labrie, M. Chagnon, and V. Fournier (2014). "Neonicotinoid-Contaminated Puddles of Water Represent a Risk of Intoxication for Honey Bees". *PLoS ONE* 9 (12), e108443.

Sánchez-Bayo, F., K. Goka, and D. Hayasaka (2016). "Contamination of the Aquatic Environment with Neonicotinoids and its Implication for Ecosystems". *Frontiers in Environmental Science* 4 (71).

SANCO (2002). *Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC*. European Commission Health & Consumer Protection Directorate-General and Directorate E-Food Safety, plant health animal health and welfare and international questions and E1 - Plant health.

Schaafsma, A., V. Limay-Rios, T. Baute, J. Smith, and Y. Xue (2015). "Neonicotinoid Insecticide Residues in Surface Water and Soil Associated with Commercial Maize (Corn) Fields in Southwestern Ontario". *PLOS ONE* 10 (2), e0118139.

Scheper, J., M. Reemer, R. van Kats, W. A. Ozinga, G. T. J. van der Linden, J. H. J. Schaminée, H. Siepel, and D. Kleijn (2014). "Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in The Netherlands". *Proceedings of the National Academy of Sciences* 111 (49), 17552–17557.

Schmolke, A., B. Kearns, and B. O'Neill (2018). "Plant guttation water as a potential route for pesticide exposure in honey bees: a review of recent literature". *Apidologie* 49 (5), 637–646.

Scoble, M. J. (1995). *The Lepidoptera. Form, Function and Diversity*. Oxford, New York: Oxford University Press.

Semple, K. T., A. W. J. Morriss, and G. I. Paton (2003). "Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis". *European Journal of Soil Science* 54 (4), 809–818.

Sgolastra, F., S. Hinarejos, T. L. Pitts-Singer, N. K. Boyle, T. Joseph, J. Lūckmann, N. E. Raine, R. Singh, N. M. Williams, and J. Bosch (2019). "Pesticide Exposure Assessment Paradigm for Solitary Bees". *Environmental Entomology* 48 (1), 22–35.

Sickel, W., M. J. Ankenbrand, G. Grimmer, A. Holzschuh, S. Härtel, J. Lanzen, I. Steffan-Dewenter, and A. Keller (2015). "Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach". *BMC ecology* 15, 20.

Simon-Delso, N., G. San Martin, E. Bruneau, C. Delcourt, and L. Hautier (2017). "The challenges of predicting pesticide exposure of honey bees at landscape level". *Scientific Reports* 7 (1).

Siviter, H., M. J. F. Brown, and E. Leadbeater (2018). "Sulfoxaflor exposure reduces bumblebee reproductive success". *Nature* 561 (7721), 109–112.

Sponsler, D. B., C. M. Grozinger, C. Hitaj, M. Rundlöf, C. Botías, A. Code, E. V. Lonsdorf, A. P. Melathopoulos, D. J. Smith, S. Suryanarayanan, W. E. Thogmartin, N. M. Williams, M. Zhang, and M. R. Douglas (2019). "Pesticides and pollinators: A socioecological synthesis". *Science of The Total Environment* 662, 1012–1027.

Stanley, D. A., Garratt, Michael P. D., J. B. Wickens, V. J. Wickens, S. G. Potts, and N. E. Raine (2015). "Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees". *Nature* 528, 548–550.

Stanley, D. A. and N. E. Raine (2016). "Chronic exposure to a neonicotinoid pesticide alters the interactions between bumblebees and wild plants". *Functional Ecology* 30 (7), 1132–1139.

Steen, R. (2016). "Diel activity, frequency and visit duration of pollinators in focal plants: in situ automatic camera monitoring and data processing". *Methods in Ecology and Evolution* 8 (2), 203–213.

Stehle, S. and R. Schulz (2015). "Agricultural insecticides threaten surface waters at the global scale". *Proceedings of the National Academy of Sciences* 112 (18), 5750–5755.

Sterk, G., B. Peters, Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (Bombus terrestris)". *Ecotoxicology* 25 (9), 1666–1678.

Stoner, K. A. (2016). "Current Pesticide Risk Assessment Protocols Do Not Adequately Address Differences between Honey Bees (Apis mellifera) and Bumble Bees (Bombus spp.)" *Frontiers in Environmental Science* 4.

Storkey, J. and D. B. Westbury (2007). "Managing arable weeds for biodiversity". *Pest Management Science* 63 (6), 517–523.

Sur, R. and A. Stork (2003). "Uptake, translocation and metabolism of imidacloprid in plants". *Bulletin of Insectology* 56, 35–40.

Tapparo, A., C. Giorio, M. Marzaro, D. Marton, L. Soldà, and V. Girolami (2011). "Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds". *Journal of Environmental Monitoring* 13 (6), 1564.

Thompson, H. M. (2001). "Assessing the exposure and toxicity of pesticides to bumblebees (Bombus sp.)" *Apidologie* 32 (4), 305–321.

Topping, C. J., P. S. Craig, F. de Jong, M. Klein, R. Laskowski, B. Manachini, S. Pieper, R. Smith, J. P. Sousa, F. Streissl, K. Swarowsky, A. Tiktak, and T. van der Linden (2015). "Towards a landscape scale management of pesticides: ERA using changes in modelled occupancy and abundance to assess long-term population impacts of pesticides". *The Science of the total environment* 537, 159–169.

Topping, C. J., T. S. Hansen, T. S. Jensen, J. U. Jepsen, F. Nikolajsen, and P. Odderskær (2003). "ALMaSS, an agent-based model for animals in temperate European landscapes". *Ecological Modelling* 167 (1-2), 65–82.

Topping, C. J., L. J. Kjaer, U. Hommen, T. T. Høye, T. G. Preuss, R. M. Sibly, and P. van Vliet (2014). "Recovery based on plot experiments is a poor predictor of landscape-level population impacts of agricultural pesticides: Agent-based simulation of population recovery". *Environmental Toxicology and Chemistry* 33 (7), 1499–1507.

Tosi, S., C. Costa, U. Vesco, G. Quaglia, and G. Guido (2018). "A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides". *Science of The Total Environment* 615, 208–218.

Tschumi, M., M. Albrecht, M. H. Entling, and K. Jacot (2015). "High effectiveness of tailored flower strips in reducing pests and crop plant damage". *Proceedings. Biological sciences / The Royal Society* 282 (1814), 20151369.

Uhl, P., L. A. Franke, C. Rehberg, C. Wollmann, P. Stahlschmidt, L. Jeker, and C. A. Brühl (2016). "Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment". *Scientific Reports* 6, 34439.

United Nations (1992). *Convention on Biological Diversity*.

United Nations (2016). *Cancun Declaration on Mainstreaming the Conservation and Sustainable Use of Biodiversity for Well-Being*. Cancun (Mexico).

Van den Brink, P. J., A. C. Alexander, M. Desrosiers, W. Goedkoop, P. L. Goethals, M. Liess, and S. D. Dyer (2011). "Traits-based approaches in bioassessment and eco-

logical risk assessment: Strengths, weaknesses, opportunities and threats". *Integrated Environmental Assessment and Management* 7 (2), 198–208.

vanEngelsdorp, D., J. Hayes Jr., R. M. Underwood, and J. Pettis (2008). "A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008". *PloS one* 3 (12), e4071.

Vidau, C., M. Diogon, J. Aufauvre, R. Fontbonne, B. Viguès, J.-L. Brunet, C. Texier, D. G. Biron, N. Blot, H. El Alaoui, L. P. Belzunces, and F. Delbac (2011). "Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by Nosema ceranae". *PLoS ONE* 6 (6), e21550.

Vray, S., O. Rollin, P. Rasmont, M. Dufrêne, D. Michez, and N. Dendoncker (2019). "A century of local changes in bumblebee communities and landscape composition in Belgium". *Journal of Insect Conservation*.

Walker, C. H., R. Sibly, and D. B. Peakall (2016). *Principles of ecotoxicology*. CRC press.

Wardhaugh, C. W. (2015). "How many species of arthropods visit flowers?" *Arthropod-Plant Interactions* 9 (6), 547–565.

Westrich, P. (1990). *Die Wildbienen Baden-Württembergs: [im Rahmen des Artenschutzprogrammes Baden Württemberg]*. 2., verb. Aufl. Stuttgart: Ulmer.

Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson (2012). "Neonicotinoid pesticide reduces bumble bee colony growth and queen production". *Science* 336 (6079), 351–352.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts (2010). "Ecological and life-history traits predict bee species responses to environmental disturbances". *Biological Conservation* 143 (10), 2280–2291.

Winfree, R., I. Bartomeus, and D. P. Cariveau (2011). "Native Pollinators in Anthropogenic Habitats". *Annual Review of Ecology, Evolution, and Systematics* 42 (1), 1–22.

Wirtz, I. P., M. Hauer-Jákli, D. Schenke, E. Ladewig, B. Märländer, U. Heimbach, and J. Pistorius (2018). "Investigations on neonicotinoids in guttation fluid of seed treated sugar beet: Frequency, residue levels and discussion of the potential risk to honey bees". *Crop Protection* 105, 28–34.

Wood, T. J. and D. Goulson (2017). "The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013". *Environmental Science and Pollution Research*.

Woodcock, B. A., N. J. B. Isaac, J. M. Bullock, D. B. Roy, D. G. Garthwaite, A. Crowe, and R. F. Pywell (2016). "Impacts of neonicotinoid use on long-term population changes in wild bees in England". *Nature Communications* 7, 12459.

Zurbuchen, A., L. Landert, J. Klaiber, A. Müller, S. Hein, and S. Dorn (2010). "Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances". *Biological Conservation* 143 (3), 669–676.

A.S1 SUPPORTING INFORMATION

Table A.S1.: Bee-attractive European crops. Adapted from (EFSA, 2013).

Cultivation system	Crop	Comment
Arable crops	Alfalfa, asparagus, beans (*Phaseolus* ssp.), blueberries, broad beans/horse beans (*Vicia faba*), buckwheat, castorbeans, chick peas, chillies and peppers, clover, cow peas, cranberries, cucumber and gherkin, currants, eggplant, gooseberries, peanuts, hemp, legumes for silage (e.g. *Lotus corniculatus*, *Lespedeza* spp., *Pueraria lobata*, *Sesbania* spp., *Onobrychis sativa*, *Hedysarum coronarium*), lentils, lineseed, lupins, maize, melon, mustard, okra-peas, peppermint, poppy, potatoes, pumpkins, squash and gourds, pyrethrum (*Chrysanthemum cinerariifolium*), oilseed rape, raspberries (and similar berries), safflower, cotton, serradella/birdsfoot (*Ornithopus sativus*), sesame, soybeans, spices (e.g. *Laurus nobilis*, *Anethum graveolens*, *Trigonella foenum-graecum*, *Crocus sativus*, *Thymus vulgaris*, *Curcuma longa*), strawberries, sugar beet, sunflower, tomatoes, vetches (*Vicia sativa*), viper's grass (*Scorzona hispanica*), watermelons	
	Anise, badian fennel, corian, artichokes, cabbage and other brassica, carrots, cauliflower and broccoli, chicory, garlic, leeks and other alliaceous vegetables (e.g. *Allium porrum*, *A. schoenoprasum*), onions, tobacco, turnips	Harvested before flowering
	Barley, oats, rice, rye, rye grass for forage and silage (e.g. *Lolium multiflorum*, *L. perenne*), sorghum, triticale, wheat	Can attract FVIs via guttation water
Orchard	Almonds, apples, apricots, avocados, bananas, carobs, cherries, chestnuts, coffee, dates, elder, figs, grapefruit, hazelnuts, kiwi fruit, lemons and limes, olives, oranges, peaches and nectarines, pears, persimmons, pistachios, plums and sloes, quinces, tangerine, mandarine and clementine, walnuts	
Vineyard	Grapes	

Table A.S2.: Ecological traits of European bee species and their implications for population susceptibility to environmental stress.

Trait	Explanation
Mobility	The foraging distance is correlated with bee size and determines how far bees can fly to collect food and nest building resources (Greenleaf et al., 2007; Michener, 2007). Small species with low mobility have been shown to be vulnerable to intensive agriculture. Bigger, more mobile species are most likely more resistant since they can use more diverse foraging grounds in case of disturbance (de Palma et al., 2015).
Sociality	Social bee species colonies have higher foraging and reproductive capacity. This should allow them to better compensate against stressors compared to solitary bees (de Palma et al., 2015). However, due to the sheer amount of resources needed for a colony, these species might forage on a wider variety of plants which would increase chances of (multiple) pesticide exposure (Brittain and Potts, 2011). Larvae of parasitic species assume the social strategy of the host and are therefore affected by stress in a similar way.
Nesting	Different strategies such as aboveground vs. belowground nesting or active nest excavation vs. renting may result in different vulnerabilities in bee populations. However, evidence is inconclusive which strategies are more robust (de Palma et al., 2015; Williams et al., 2010). Furthermore, pesticide exposure from different matrices might be dependent on the environmental compartments that bees nest in.
Lecty	Dietary specialists (oligolectic species) react negatively to environmental stress (e.g. agricultural intensification, habitat loss) due to their limitation on few or just one food plant. Generalist, polylectic species can switch to alternative food plants (de Palma et al., 2015; Forrest et al., 2015; Williams et al., 2010).
Flight season/duration	A short flight season corresponds with high sensitivity to stress events since the variety of plants that resources can be collected from and the time to do so is restricted. Species with longer flight seasons have more time to forage on additional plants (de Palma et al., 2015; Forrest et al., 2015).
Voltinism	Univoltine species might be vulnerable to changes in their environment in the time of reproduction whereas multivoltine species may be able to compensate due to two or more brood cycles within a year (Brittain and Potts, 2011). This has not been established.

A.S1.1 *References*

Brittain, C. and S. G. Potts (2011). "The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination". *Basic and Applied Ecology* 12 (4), 321–331.

de Palma, A., M. Kuhlmann, S. P. Roberts, S. G. Potts, L. Börger, L. N. Hudson, I. Lysenko, T. Newbold, A. Purvis, and I. Kaplan (2015). "Ecological traits affect the sensitivity of bees to land-use pressures in European agricultural landscapes". *Journal of Applied Ecology* 52 (6), 1567–1577.

EFSA (2013). "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 11 (7), 3295.

Forrest, J. R. K., R. W. Thorp, C. Kremen, N. M. Williams, and Y. Clough (2015). "Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape". *Journal of Applied Ecology* 52 (3), 706–715.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen (2007). "Bee foraging ranges and their relationship to body size". *Oecologia* 153 (3), 589–596.

Michener, C. D. (2007). *The bees of the world*. 2. ed. Baltimore: Johns Hopkins Univ. Press.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts (2010). "Ecological and life-history traits predict bee species responses to environmental disturbances". *Biological Conservation* 143 (10), 2280–2291.

A

B INTERSPECIFIC SENSITIVITY OF BEES TOWARDS DIMETHOATE AND IMPLICATIONS FOR ENVIRONMENTAL RISK ASSESSMENT

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B

B.1 ABSTRACT

Wild and domesticated bee species are exposed to a variety of pesticides which may drive pollinator decline. Due to wild bee sensitivity data shortage, it is unclear if the honey bee *Apis mellifera* is a suitable surrogate species in the current EU risk assessment scheme. Furthermore, the underlying causes for sensitivity differences in bees are not established. We assessed the acute toxicity (median lethal dose, LD50) of dimethoate towards multiple bee species, generated a species sensitivity distribution and derived a hazardous dose (HD5). Furthermore, we performed a regression analysis with body weight and dimethoate toxicity. HD5 lower 95% confidence limit was equal to honey bee mean LD50 when applying a safety factor of 10. Body weight proved to be a predictor of interspecific bee sensitivity but did not explain the pattern completely. Using acute toxicity values from honey bees and a safety factor of 10 seems to cover the interspecific sensitivity range of bees in the case of dimethoate. Acute endpoints of proposed additional test species, the buff-tailed bumblebee *Bombus terrestris* and the red mason bee *Osmia bicornis*, do not improve the risk assessment for the entire group. However, this might not apply to other insecticides such as neonicotinoids.

B.2 INTRODUCTION

Agricultural crops and wild plants are mostly pollinated by insects and bees play a major role. Wild and domesticated bee species are affected by multiple environmental factors (Goulson et al., 2015). Since the last century the USA and Europe have experienced substantial losses of domesticated honey bee (*Apis mellifera*) colonies and simultaneous decline in wild bee diversity (Goulson et al., 2015; on the Status of Pollinators in North America, 2007; Potts et al., 2015; Potts et al., 2010; vanEngelsdorp et al., 2008). In Germany 52% of wild bee species are included in the Red List (Westrich et al., 2008).

Decline of pollinator species might be related to pesticide use in agricultural landscapes amongst other factors such as parasites and habitat loss (Goulson et al., 2015). Honey bees have received some attention in terms of their sensitivity towards pesticides (Desneux et al., 2007; vanEngelsdorp and Meixner, 2010) and are included in the regulatory risk assessment framework of the placement of pesticides on the market (Regulation (EC) 1107/2009). It was recently suggested that toxicity towards wild bees could be extrapolated from honey bee data. In a meta-analysis, Arena and Sgolastra (2014) found that in most cases wild bee species are less sensitive to common insecticides than honey bees when comparing LD50 values obtained from acute toxicity studies. This was consistent for five out of six tested insecticide classes, whereas wild bees displayed equal to higher sensitivity to neonicotinoids (median factor 1.06). Since relative susceptibility patterns vary for different insecticides, it is difficult to extrapolate acute toxicity data of a specific insecticide from the honey bee to a specific wild bee species using the current data (Biddinger et al., 2013; Helson et al., 1994). Moreover, recent field studies on oilseed rape revealed that deducing responses from honey

bee populations to wild bees may not be adequate in realistic exposure scenarios either (Cutler et al., 2014; Rundlöf et al., 2015). Interspecific susceptibility patterns towards insecticides seem to be substance-specific at least at generic level (Helson et al., 1994; Scott-Dupree et al., 2009). Indicators for different sensitivities of bee species towards insecticides are not clearly established. Body weight and size are often stated to be predictive traits but there are other possible factors such as metabolism and cuticular physiology. Since only few wild bee species have been subject to ecotoxicological studies, reliable evidence of the relationship between sensitivity and such traits remains to be provided (Arena and Sgolastra, 2014).

Currently, the honey bee is the only pollinator species that is required to be evaluated in the EU pesticide risk assessment scheme (SANCO, 2002). However, wild bee species such as bumble bees and solitary bees differ substantially from the honey bee in their ecological properties, e.g. sociality, life cycle, behaviour, which might affect their population responses. Pesticide effects on solitary bee populations and to an extent even bumble bee colonies might be more pronounced than on honey bees since effects on individuals cannot be buffered by sheer numbers as in the hive of a superorganism (Arena and Sgolastra, 2014; Rundlöf et al., 2015). Participants of a Society of Environmental Toxicology and Chemistry (SETAC) 2011 workshop in Pensacola (USA) pleaded for evaluating pesticide effects (lethal and sublethal) towards non-*Apis* species in laboratory, semi-field and field studies (Fischer and Moriarty, 2011). The European Food Safety Authority EFSA (2013) also identified a lack of information on the sensitivity of bumble bees and solitary bees. They proposed to include the buff-tailed bumblebee *Bombus terrestris* and the red mason bee *Osmia bicornis* into EU pesticide risk assessment.

In the current lower tier testing scheme, pesticides are categorised as having a low risk towards bees through contact exposure when the quotient of application rate and contact LD50 of the surrogate species, the honey bee is lower than 50 (OEEP/EPPO, 2010). EFSA (2013) proposed an additional assessment factor of 10 to account for interspecific differences in bee sensitivity. They referred to Arena and Sgolastra (2014) who found a factor of 10 to be protective in 95% of all cases in a meta-analysis of multiple insecticides, comparing endpoints of the honey bee and 19 wild bee species, 9 of which are tropical.

The species sensitivity distribution (SSD) is one approach to infer from laboratory test results on the effects that a pesticide has on bee species communities in the agricultural landscape. The underlying idea of the SSD is that interspecific sensitivity follows a statistical distribution. By fitting a suitable distribution to the data the dose at which 5% of species in a community are affected by a pesticide (HD5) can be derived (Posthuma et al., 2002). To ensure a proper level of safety, i.e. reduce uncertainty, it was recommended to use the lower 95% confidence limit of the HD5 (lower limit HD5) (Maltby et al., 2005; Newman et al., 2000). To establish a SSD ecologically representative and comparable toxicity data are needed, as well as an appropriate statistical analysis method (Newman et al., 2000; Wheeler et al., 2002).

In order to adequately assess the risk pesticides pose to bees a comprehensive database is needed. Sensitivity data for European bee species are scarce, covering only a few species that are bred for pollination services so far. The aim of the present study was

to measure sensitivity of multiple bee species towards one insecticide to study interspecific sensitivity variability in bee species. We chose species that occur in the European agricultural landscape. These species may forage on crops and are therefore potentially exposed to insecticides in the field. We chose dimethoate as it is used as toxic reference in honey bee acute toxicity studies. Our first goal was to collect sufficient data from dose-response experiments to generate a SSD and deduce the effect of dimethoate on wild bee species. Subsequently, we compared the lower limit HD5 to the honey bee contact LD50 divided by 10 as proposed by EFSA (2013). This enabled us to ascertain if the honey bee is a suitable surrogate organism for all bee species. Furthermore, we assessed if this safety factor covers the sensitivity range of wild bee species. Secondly, the sensitivity and weight data of multiple bee species was evaluated to deduce if body weight is a predictor of bee sensitivity.

B.3 METHODS

B.3.1 *Insecticide*

We used a formulation of dimethoate (Perfekthion®, BASF, 40% a.i. (w/w)). It is an organophosphate insecticide which acts on the nervous system by inhibiting acetylcholinesterase and is highly toxic to honey bees (University of Hertfordshire, 2013).

B.3.2 *Provision of test species*

Five different bee species were used: the buff-tailed bumble bee (workers) *Bombus terrestris* (Linneaus), the red mason bee (♀ & ♂) *Osmia bicornis* (Linneaus), the sweat bee (♀) *Lasioglossum malachurum* (Kirby), the mining bee (♀) *Andrena flavipes* (Panzer) and the ivy bee (♀) *Colletes hederae* Schmidt & Westrich. Medium-sized *B. terrestris* colonies (60-80 workers) were obtained from a commercial breeder (Biofa AG, Rudolf-Diesel-Str. 2, 72525 Münsingen, Germany). *O. bicornis* were ordered as cocoons (WAB-Mauerbienenzucht, Sonnentauweg 47, 78467 Konstanz, Germany). Since males and females of *O. bicornis* were available, we also tested males of this species to infer on sex-specific sensitivity. All other species were caught at feeding grounds or nesting sites in the agricultural landscape around Landau, Germany with permission of regional authorities. Collected bees were examined to be viable and morphospecies were confirmed by visual inspection. All bee species were kept in an environmental chamber under experimental conditions, i.e. same environmental conditions, test cages, food etc., until the experiment was started. All species that were caught were collected on the day before test start so that the bees could acclimatise to experimental conditions. *O. bicornis* cocoons were put in the environmental chamber under test conditions for bees to eclose. It took around 3 days for enough males to emerge and around 5 for females. *B. terrestris* workers were collected from the colonies the day before test start. Further information on wild bee collection and identification can be found in the Supplementary Information.

B.3.3 *Experimental procedure*

Acute, contact toxicity tests were performed with all test species. All tests were conducted according to the ringtest protocol for solitary bee acute contact toxicity developed by the International Commission for Plant-Pollinator Relationships (ICPPR) with minor modifications in some tests that are noted below (Roessink, 2014). Before the experiment, bees were fed *ad libitum* with sucrose solution 50% (w/w) through plastic syringes. Bees were transferred to test cages (1 L plastic boxes sealed with a perforated lid) the day before application to acclimatize overnight. In the case of *B. terrestris* and *O. bicornis* 30 bees per treatment were set up (10 per cage, n = 3). The remaining species could not be collected in such large quantities in the agricultural landscape. Consequently, the number of bees per cage had to be reduced in these tests. Fifteen *L. malachurum* females per treatment were tested (5 per cage, n = 3). For *A. flavipes* and *C. hederae* the number of bees per treatment was 9 (3 per cage, n = 3). Environmental conditions were set to 8:16h day/night rhythm (light intensity < 10 lux at day), 60% humidity and 21°C. Temperature for *B. terrestris* and *L. malachurum* was increased to 25°C to better accommodate them following recommendations by EFSA (2013). Bees were anaesthetised for the transfer to the test cages and for the application. All species were chilled at 4°C and put in a petri dish on ice for the application, whereas bumble bees were anaesthetised with CO_2 since chilling did not calm them down to allow safe handling. Moribund bees were rejected and replaced by healthy bees prior to the test start. Wet and dry weight were determined for all bee species: Anaesthetised *B. terrestris* and *O. bicornis* specimens were weighed before treatment application. Individuals of all other species were weighed after the experiment to avoid loss of bees due to excessive handling since the number of specimens was already limited. We tested six treatments per bee species: a control of deionised water containing 0.5% (v/v) wetting agent (Tween® 80; Carl Roth GmbH + Co. KG) and five dimethoate treatments. Dimethoate doses of 1.25, 2.5, 5, 10 and 20 µg a.i./bee were chosen for *B. terrestris*. *O. bicornis* specimens were applied with 0.625, 1.25, 2.5, 5 and 10 µg a.i./bee. For individuals of the remaining species we used 0.0896, 0.224, 0.56, 1.4 and 3.5 µg a.i./bee. Dimethoate solutions were prepared by diluting the respective concentration in deionised water containing 0.5% wetting agent (Tween® 80). Bees were applied with 1 µL or 5 µL in case of *B. terrestris* on the dorsal side of the thorax between the neck and wing base using a Hamilton micro syringe (Hamilton Bonaduz AG). A paper tissue was inserted into test cages after treatment solution was fully absorbed (10 to 15 min) to provide a hiding place. Bumble bees had to be anaesthetised once more for that procedure. Following the application bees were returned to the environmental chamber and fed 50% sucrose solution *ad libitum*. After 48 h mortality was assessed. For *O. bicornis* ♀ 3 separate test runs were performed. In all 8 experiments control mortality was ≤10% except for *B. terrestris* (13%) and *A. flavipes* (22%). A subsample of 28 bees of all species were dried afterwards at 60°C for 48 h and weighed again. Furthermore, samples of treatment solutions were chemically analysed to verify actual treatment doses for all *B. terrestris* and *O. bicornis* ♀ experiments (see Supplementary Information).

B.3.4 *Statistical analysis*

Median lethal dose values (LD50) were calculated for all tested species by fitting a dose-response model to the data. Models were chosen by visual data inspection and using Akaike information criterion (AIC). Control mortality was corrected for by using Abbott's formula (Newman, 2012). Where multiple LD50 values were available a geometric mean LD50 was computed. Interspecific differences in sensitivity were analysed by performing hypothesis tests using the confidence interval (CI) overlap method (Bonferroni-adjusted) described in Wheeler et al. (2006). A species sensitivity distribution (SSD) was fitted to 48h LD50 values of all examined species (Posthuma et al., 2002). From that distribution we derived the 5% hazardous dose (HD5) and calculated its parametric bootstrap 95% confidence intervals (CIs, 5000 iterations) to obtain the lower limit HD5. To check for a dependency of bee sensitivity and weight we fitted a linear model using 48h LD50 values as response and fresh or dry weight as predictor variable. LD50 literature values of comparable studies for *A. mellifera*, *O. lignaria* and *O. cornifrons* were included in dose-response modelling and regression analysis (Supplementary Table B.S7). Furthermore, we calculated fresh and dry weight-normalised LD50 to facilitate comparability of our results with other studies. Dimethoate effects on two of the smallest German bee species (*Hylaeus gredleri* ♂, *Nomioides minutissimus* ♀, personal communication, Matthias Kitt, ecological consultant, Raiffeisenstraße 39, 76872 Minfeld, GERMANY) were estimated using the weight-sensitivity regression model. These were compared to the calculated HD50. Dry weights were obtained from pinned specimens. All statistical analyses were conducted with R 3.1.2 (R Core Team, 2014). We used the "drc" package (Ritz and Streibig, 2005) for dose-response modelling and "fitdistrplus" (Delignette-Muller et al., 2014) for fitting the SSD.

B.4 RESULTS

B.4.1 *Species sensitivity distribution*

We studied the effect of dimethoate on 5 European bee species that are abundant in the agricultural landscape. All species are categorised under "least concern" in the Red List (Westrich et al., 2008). Dimethoate sensitivity varied substantially between bee species in the following decreasing order (note that some species occur twice since there is no statistically significant difference of their LD50 to values of two other species that are different): *L. malachurum=A. flavipes>A. flavipes=C. hederae=O. bicornis* ♂*>O. bicornis* ♀*=B. terrestris* (Table B.1, Supplementary Table B.S3, Fig. B.S4). However, when examining LD50 values at per fresh weight basis the order changes to: *C. hederae=A. flavipes=L. malachurum>A. flavipes=L. malachurum=B. terrestris>O. bicornis* ♂*=O. bicornis* ♀ (Table B.1, Supplementary Table B.S4, Fig. B.S5). Calculated per dry weight, sensitivity order changes again: *C. hederae=A. flavipes>A. flavipes=L. malachurum>B. terrestris=O. bicornis* ♂*>O. bicornis* ♀ (Table B.1, Supplementary Table B.S5, Fig. B.S6). *O. bicornis* ♀ was always among the most resistant species whereas *A. flavipes* was always among the

most sensitive. *O. bicornis* ♀ were less sensitive than *O. bicornis* ♂ (Supplementary Table B.S3).

HD5 was calculated to be 0.08 µg a.i./bee and the lower limit HD5 0.02 µg a.i./bee (Fig. B.1, Supplementary Table B.S6). The lower limit HD5 is equal to the mean 48h LD50 for *A. mellifera* calculated from literature data (0.18; Supplementary Table B.S7) divided by a safety factor of 10.

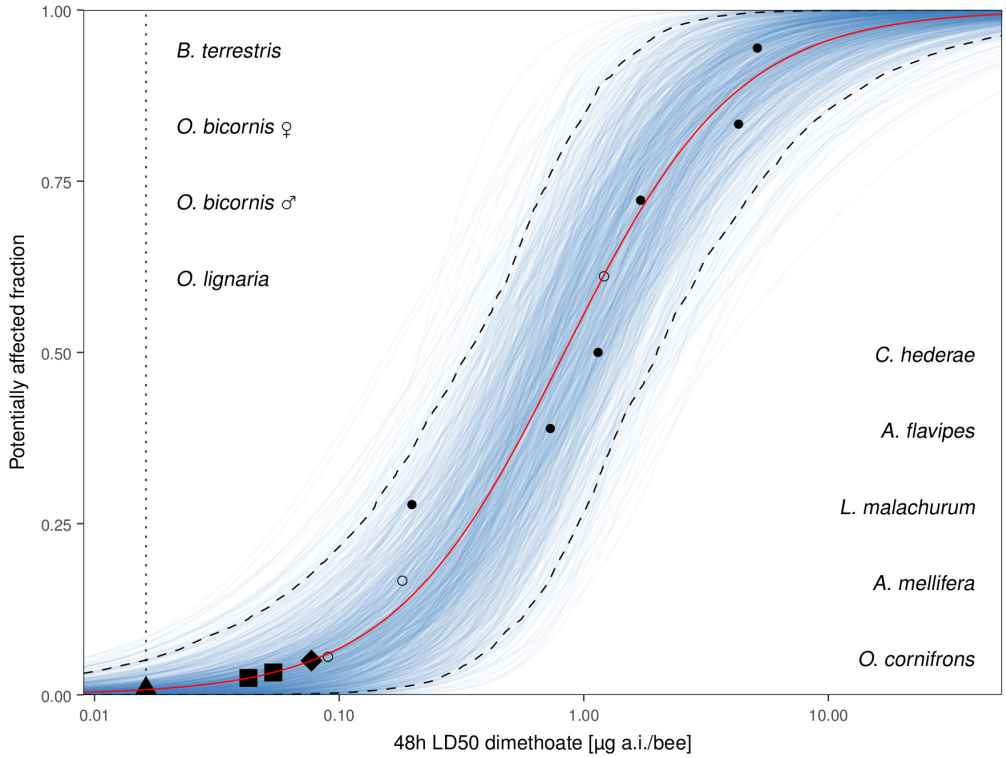

Figure B.1.: Species sensitivity distribution of dimethoate calculated from multiple bee species' sensitivity (red line). ● & ○ denote 48h LD50 values of bee species (○ are literature values). Species names are aligned by sensitivity in ascending order from bottom to top on the same y-axis coordinate as their respective ●/○. Dashed lines enclose parametric bootstrap 95% CI (1000 iterations). Blue, transparent lines display all parametric bootstrap samples. ◆ marks the HD5 value, ▲ the lower limit HD5 and ■ the extrapolated LD50 values of *Hylaeus gredleri* ♂ and *Nomioides minutissimus* ♀. The proposed regulatory threshold of honey bee LD50/10 is indicated by the dotted line. LD50 values for *A. mellifera*, *O. cornifrons* and *O. lignaria* were taken from other studies (Supplementary Table B.S7).

Table B.1.: Dimethoate sensitivity of studied bee species.

Species	Mean fresh weight	Mean dry weight	LD50	95% CI	LD50 Fresh weight-normalised	95% CI	LD50 Dry weight-normalised	95% CI
	[mg]	[mg]	[µg a.i./bee]		[µg a.i./g bee]		[µg a.i./g bee]	
Lasioglossum malachurum	11.0	3.7	0.20	0.16 – 0.24	18.08	14.70 – 21.46	53.40	43.42 – 63.37
Andrena flavipes	47.3	21.6	0.73	0.07 – 1.39	15.44	1.57 – 29.31	33.78	3.44 – 64.11
Colletes hederae	105.5	43.4	1.14	0.72 – 1.57	10.84	6.83 – 14.85	26.35	16.61 – 36.09
Osmia bicornis ♂	37.7	17.6	1.71	1.37 – 2.04	45.27	36.31 – 54.22	96.90	77.73 – 116.07
Osmia bicornis ♀	93.6	30.4	4.29	3.72 – 4.91	45.89	39.80 – 52.47	141.46	122.68 – 161.73
Bombus terrestris	205.0	55.8	5.13	4.10 – 6.15	25.00	20.00 – 30.00	91.87	73.49 – 110.26
Nomioides minutissimus ♀*	NA	0.8	0.04	NA – NA	NA	NA – NA	NA	NA – NA
Hylaeus gredleri ♂*	NA	1.0	0.05	NA – NA	NA	NA – NA	NA	NA – NA

*Toxicity values are extrapolated using the computed weight-sensitivity relationship.

B.4.2 *Weight-sensitivity regression*

The studied bee species cover a wide weight range (Supplementary Table B.S2, Figs B.S2, B.S3). Workers from the heaviest species, *B. terrestris* (205 mg), were on average 19 times heavier than females from the lightest species, *L. malachurum* (11mg; Wilcoxon rank sum test, $p<0.001$). Body weight did influence wild bee species' dimethoate sensitivity. We found a linear relationship of 48h LD50 and weight (fresh and dry) when analysing the collected wild bee toxicity data (Fig. B.2). This relationship is best described by a power function (exponential function of the general form $f(x) = c \cdot x^p$; Table B.2). However, incorporating literature values of *A. mellifera*, *O. lignaria* and *O. cornifrons* (Supplementary Table B.S7) into the model resulted in considerable decline in model fit. We extrapolated the 48h LD50 values of two small German bee species (*Hylaeus gredleri* ♂ and *Nomioides minutissimus* ♀) to be 0.05 and 0.04 µg a.i./bee, respectively. These LD50 values are situated between the HD5 and the lower limit HD5 (Table B.1, Fig. B.1).

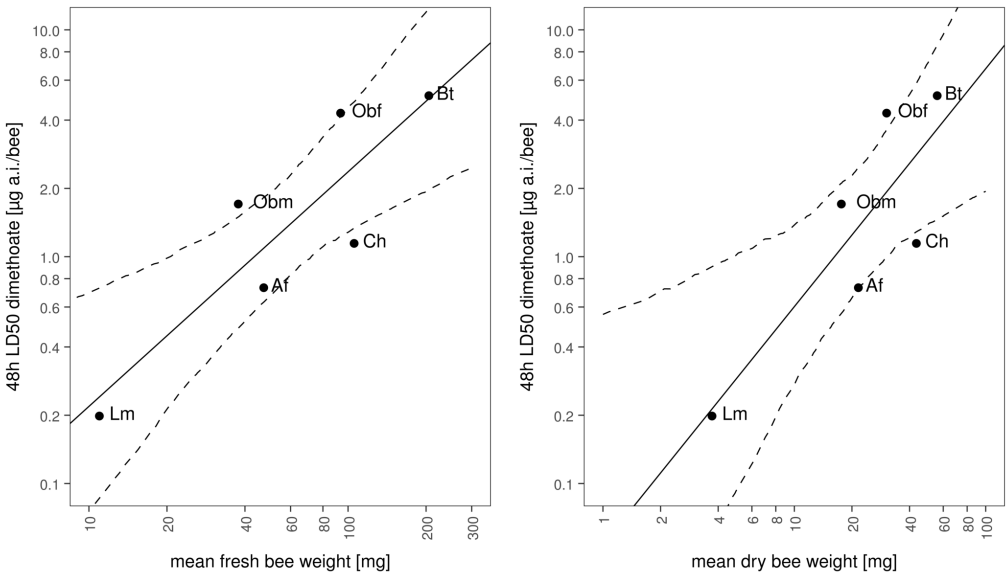

Figure B.2.: Relationship between bee weight (fresh and dry) and sensitivity towards dimethoate. Dots mark weight and sensitivity of the following species: Lm - *Lasioglossum malachurum*, Af - *Andrena flavipes*, Ch - *Colletes hederae*, Obm - *Osmia bicornis* ♂, Obf - *Osmia bicornis* ♀, Bt - *Bombus terrestris*. Both axes on logarithmic scale. Dashed lines enclose parametric bootstrap 95% CI (1000 iterations).

Table B.2.: Summary of different models to predict LD50 values from bee weight. Models vary in predictor and inclusion or omission of literature values. The explanatory variable "x" of this model is fresh or dry weight [mg] whereas the response variable "y" is the 48h LD50 of dimethoate [µg a.i./bee]. Parameter "a" is the slope of the function and "b" its intercept with the y-axis. Model function can be alternatively expressed as $y = 10^b \cdot x^a$.

Model	Predictor	Literature values	R^2	Parameter	Estimate	SE	p
$log_{10}(y) = a \cdot log_{10}(x) + b$	fresh weight	yes	0.34	a	0.8087	0.4623	0.131
				b	-1.4550	0.8579	0.141
		no	0.76	a	1.0339	0.2879	0.022
				b	-1.6938	0.5216	0.031
	dry weight	yes	0.37	a	1.1068	0.5512	0.085
				b	-1.5846	0.7591	0.075
		no	0.70	a	1.0490	0.3399	0.037
				b	-1.2693	0.4723	0.055

B

B.5 DISCUSSION

Suitability of *A. mellifera* as the sole surrogate species in acute toxicity testing was questioned by EFSA (2013). To reduce uncertainty additional bee species could be incorporated in pesticide risk assessment. The OECD honey bee guideline for acute contact toxicity testing requires the use of young adult worker bees of similar age (OECD, 1998). It is not exactly stated how old bees should be which may lead to variation in age across research facilities. Since cuticular resistance and detoxification capacity develop with age but not before eclosion in honey bees (Falcón et al., 2014; Słowińska et al., 2015; Smirle and Winston, 1988) different susceptibilities might be obtained from honey bee tests. Young solitary bees may even be relatively less susceptible due to a fully matured cuticle and already elevated antioxidant enzyme levels before eclosion (Dmochowska-Ślęzak et al., 2015; Elias-Neto et al., 2014). Consequently, the honey bee may be a sufficient surrogate organism in some cases at least in lower tier testing with contact exposure. In any case bee age should be exactly defined in lower tier testing guidelines to reduce variability of generated LD50 values.

For reasons of reproducibility and costs of laboratory studies the SSD approach can be an acceptable compromise to higher tier testing. It produces ecologically relevant results which might be used as additional data, or an alternative to the complex and cost-intensive semi-field or field studies (Maltby et al., 2005). However, the significance of SSD results for more complex systems has only been studied in aquatic experiments. There is a need to verify if this holds true for terrestrial settings. One conceptual shortcoming of the HD5 as a toxic endpoint is that it deems the most sensitive species expendable. However, those species might share the same ecological niche. In our case sensitive species are likely to be small species when considering the weight-sensitivity relationship (Fig. B.2). When extrapolating toxicity of two of the smallest bee species in Germany with our weight-sensitivity regression model LD50 values were still higher than the lower limit HD5. Therefore, we cannot confirm that small, sensitive bee species are put at risk by using the HD5 in risk assessment.

In our study the safety factor of 10 recommended by EFSA (2013) seems to cover the acute sensitivity range of wild bee species. We modeled dimethoate sensitivity of multiple bee species and found that the lower limit HD5 is equal to the mean 48h LD50 value of honey bees divided by this safety factor (Fig. B.1). Therefore, testing the honey bee and employing a safety factor of 10 seems to be adequate for lower tier risk assessment of dimethoate. However, bee species acute toxicity data we inferred from are still limited. Dimethoate is a well-studied insecticide that the honey bee is rather sensitive to (Arena and Sgolastra, 2014). For neonicotinoids, however, Arena and Sgolastra (2014) reported several studies where other bee species were at least as susceptible as the honey bee. Therefore, a safety factor of 10 might not encompass interspecific sensitivity in the case of those insecticides. There still is reasonable doubt that the honey bee is a feasible surrogate for all bee species since relative sensitivities of bee species vary with each pesticide (Arena and Sgolastra, 2014; Biddinger et al., 2013). The additional testing of a bumble bee and a solitary species was proposed by EFSA (2013) to reduce uncertainty. We argue that test species should be chosen according to their sensitivity and ecological relevance. The two species (*B. terrestris*, *O. bicornis*) recommended by EFSA (2013) were the least sensitive towards the toxic reference dimethoate in our experiments (LD50s 28.5 and 23.8 times higher than honey bee). Moreover, *B. terrestris* was also generally less sensitive than the honey bee in the studies surveyed by Arena and Sgolastra (2014) and Sanchez-Bayo and Goka (2014). Both species are commercially bred for pollination services in agricultural systems where pesticides are frequently used (*O. bicornis* in e.g. apple orchards, *B. terrestris* in greenhouses). Therefore, they can be procured in high numbers for testing and can be handled quite well in the laboratory. However, it is unclear which additional information is to be gained from testing rather pesticide-resistant species. To substantially reduce uncertainty in lower tier risk assessment sensitive species should be studied. To achieve that goal a comprehensive database of interspecific sensitivity of bees is needed. Furthermore, differences in responses of bee species to pesticides should also be considered in higher tier testing. Pesticide impact on bee species in the field is governed by ecological differences as shown by Rundlöf et al. (2015). We propose that bee risk assessment should rather focus more on testing multiple species in realistic settings than in the laboratory. Several traits are assumed to determine interspecific sensitivity differences in bees, mainly body size and weight. However, data on bee species sensitivity is scarce which hinders reliable inference on predictive factors (Arena and Sgolastra, 2014). We evaluated sensitivity and weight data of multiple bee species to deduce if body weight is a predictor of bee sensitivity. Comparing 48h LD50 values of five European bee species we found that dimethoate toxicity increases with decreasing bee species weight (Table B.2, Fig. B.2). Incorporating literature values considerably decreased model fit. The reason might be laboratory-specific differences in bee health status, e.g. pathogen or virus levels, as well as varying sensitivity of honey bee strains from different parts of the world (Rinkevich et al., 2015). Furthermore, body weight and sensitivity data could only be procured from separate studies. Besides the traits summarized by Arena and Sgolastra (2014) there are additional ecological factors that may substantially affect bee sensitivity towards pesticides. Amongst other things uptake, metabolism and excre-

tion of topically applied pesticide solutions define their toxic impact. The generally accepted uptake mechanism is that pesticides are diluted in both layers of the cuticle and subsequently distributed in the hemolymph to reach the central nervous system (Winteringham, 1969). Cuticular maturation may have an effect on pesticide uptake since permeability decreases during this hardening and darkening process. Cuticular hydrocarbon profiles differ between honey bee pupae, newly-emerged workers and adult foragers (Falcón et al., 2014). Unlike in honey bees, solitary bee cuticle is fully developed at eclosion (Elias-Neto et al., 2014). Interspecific differences in cuticular composition may be an additional factor but there are no studies on that subject. Once a pesticide has entered the insect body, its actual toxic effect on the insect depends on the organism's capacity to metabolize and subsequently excrete it. Such detoxification processes are controlled by enzyme activity. Common European bee species such as the *B. terrestris*, the solitary bee *Megachile rotundata* and the honey bee *A. mellifera* were reported to show similar levels of genes that are associated with detoxification processes (Xu et al., 2013). Nevertheless, there are interspecific differences in the buildup of these enzyme levels during bee development. In adult honey bees the detoxification capacity is quite low at eclosion and increases as they become foragers (Słowińska et al., 2015; Smirle and Winston, 1988). In the solitary bee *O. bicornis*, however, antioxidant enzyme levels are already building up before eclosion (Dmochowska-Ślęzak et al., 2015). Our data suggest that body weight is a governing factor of bee sensitivity towards dimethoate but it remains unclear if this holds true for all pesticides in general. Further research on interspecific sensitivity of bees is needed.

In this study we computed a SSD from dimethoate acute toxicity data of wild bee species. The derived lower limit HD5 was equivalent to the honey bee LD50 value divided by a safety factor of 10. This value also encompasses two of the smallest wild bee species which LD50 values were calculated from a weight-sensitivity relationship. For dimethoate no further information is gained by conducting acute laboratory tests with the two wild bee species *B. terrestris* and *O. bicornis* as suggested by EFSA (2013). We recommend to investigate wild bee toxicity for other insecticide groups and reconsider the proposed acute testing scheme. Adding wild bee species to environmental risk assessment for pesticides seems to be important when considering field-relevant effects where differences in sociality and behaviour affect sensitivity, but not so when testing on an organism level in a laboratory.

B.6 ACKNOWLEDGEMENTS

We are thankful to Matthias Kitt for providing information on where to collect wild bees and helping with species determination. Furthermore, we thank Ivo Roessink for chemical analysis of test solutions.

B.7 AUTHOR CONTRIBUTIONS STATEMENT

C.A.B, L.J. & P.S. conceived the manuscript. L.A.F., C.R., P.S. & P.U. performed the laboratory experiments. P.U. & L.A.F. analysed the data. P.U., L.A.F. & C.W. wrote the manuscript with editorial advice from C.A.B, P.S. & L.J. All authors approved the final manuscript.

B.8 ADDITIONAL INFORMATION

Data on *O. bicornis* and *B. terrestris* was generated as part of an International Commission for Plant-Pollinator Relationships (ICPPR) ringtest. The authors declare no competing financial interests.

B.9 REFERENCES

Arena, M. and F. Sgolastra (2014). "A meta-analysis comparing the sensitivity of bees to pesticides". *Ecotoxicology* 23 (3), 324–334.

Biddinger, D. J., J. L. Robertson, C. Mullin, J. Frazier, S. A. Ashcraft, E. G. Rajotte, N. K. Joshi, and M. Vaughn (2013). "Comparative toxicities and synergism of apple orchard pesticides to Apis mellifera (L.) and Osmia cornifrons (Radoszkowski)". *PloS one* 8 (9), e72587.

Cutler, G. C., C. D. Scott-Dupree, M. Sultan, A. D. McFarlane, and L. Brewer (2014). "A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success". *PeerJ* 2, e652.

Delignette-Muller, M. L., R. Pouillot, J.-B. Denis, and C. Dutang (2014). *fitdistrplus: help to fit of a parametric distribution to non-censored or censored data*. Version 1.0-2. URL: http://CRAN.R-project.org/package=fitdistrplus/.

Desneux, N., A. Decourtye, and J. Delpuech (2007). "The sublethal effects of pesticides on beneficial arthropods". *Annu. Rev. Entomol* 52, 81–106.

Dmochowska-Ślęzak, K., K. Giejdasz, M. Fliszkiewicz, and K. Żółtowska (2015). "Variations in antioxidant defense during the development of the solitary bee Osmia bicornis". *Apidologie* 46 (4), 432–444.

EFSA (2013). "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 11 (7), 266 pp.

Elias-Neto, M., A. L. O. Nascimento, A. M. Bonetti, F. S. Nascimento, S. Mateus, C. A. Garófalo, and M. M. G. Bitondi (2014). "Heterochrony of cuticular differentiation in eusocial corbiculate bees". *Apidologie* 45 (4), 397–408.

Falcón, T., M. J. Ferreira-Caliman, F. M. Franco Nunes, É. D. Tanaka, F. S. do Nascimento, and M. M. Gentile Bitondi (2014). "Exoskeleton formation in Apis mellifera: Cuticular hydrocarbons profiles and expression of desaturase and elongase genes during pupal and adult development". *Insect Biochemistry and Molecular Biology* 50, 68–81.

Fischer, D. and T. Moriarty (2011). *Pesticide risk assessment for pollinators: Summary of a SETAC Pellston Workshop*. Pensacola FL (USA).

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray (2015). "Bee declines driven by combined stress from parasites, pesticides, and lack of flowers". *Science (New York, N.Y.)* 347 (6229), 1255957.

Helson, B. V., K. N. Barber, and P. D. Kingsbury (1994). "Laboratory toxicology of six forestry insecticides to four species of bee (Hymenoptera Apoidea)". *Archives of environmental contamination and toxicology* 27 (1), 107–114.

Maltby, L., N. Blake, T. C. Brock, and Van den Brink, Paul J. (2005). "Insecticide species sensitivity distributions: Importance of test species selection and relevance to aquatic ecosystems". *Environmental Toxicology and Chemistry* 24 (2), 379.

Newman, M. C. (2012). *Quantitative ecotoxicology*. CRC Press.

Newman, M. C., D. R. Ownby, L. C. A. Mezin, D. C. Powell, T. R. L. Christensen, S. B. Lerberg, and B.-.-.-A. Anderson (2000). "Applying species–sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient numbers of species". *Environmental toxicology and chemistry* 19 (2), 508–515.

OECD (1998). *Test No. 214: Honeybees, Acute Contact Toxicity Test*.

OEEP/EPPO (2010). "Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees". *Bulletin OEPP/EPPO Bulletin* 40, 323–331.

On the Status of Pollinators in North America, C. (2007). *Status of pollinators in North America*. Washington, D.C.: National Academy of Sciences.

Posthuma, L., G. W. Suter II, and T. P. Traas (2002). *Species sensitivity distributions in ecotoxicology*. CRC Press.

Potts, S. G., K. Biesmeijer, R. Bommarco, T. Breeze, L. Carvalheiro, M. Franzén, J. P. González-Varo, A. Holzschuh, D. Kleijn, A.-M. Klein, B. Kunin, T. Lecocq, O. Lundin, D. Michez, P. Neumann, A. Nieto, L. Penev, P. Rasmont, O. Ratamäki, V. Riedinger, Roberts, S. P. M., M. Rundlöf, J. Scheper, P. Sørensen, I. Steffan-Dewenter, Stoev, P., Vilà, M., and O. Schweiger (2015). *Status and trends of European pollinators. Key findings of the STEP project.* Ed. by Pensoft Publishers. 72 pp. Sofia.

Potts, S. G., S. Roberts, R. Dean, G. Marris, M. Brown, R. Jones, P. Neumann, and J. Settele (2010). "Declines of managed honey bees and beekeepers in Europe". *Journal of Apicultural Research* 49 (1), 15.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. Version 3.1.2 (2014-10-31). Vienna (Austria). URL: http://www.R-project.org/.

Rinkevich, F. D., J. W. Margotta, J. M. Pittman, R. G. Danka, M. R. Tarver, J. A. Ottea, and K. B. Healy (2015). "Genetics, Synergists, and Age Affect Insecticide Sensitivity of the Honey Bee, Apis mellifera". *PLoS ONE* 10 (10), e0139841.

Ritz, C. and J. C. Streibig (2005). "Bioassay analysis using R". *Journal of Statistical Software* 12 (5), 1–22. URL: http://CRAN.R-project.org/package=drc/.

Roessink, I., ed. (2014). *Ringtest Solitary bee acute contact toxicity*. ICPPR workgroup non-Apis bees.

Rundlöf, M., Andersson, Georg K S, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone, and H. G. Smith (2015). "Seed coating with a neonicotinoid insecticide negatively affects wild bees". *Nature*.

Sanchez-Bayo, F. and K. Goka (2014). "Pesticide residues and bees–a risk assessment". *PLOS ONE* 9 (4), e94482.

SANCO (2002). *Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC*. European Commission Health & Consumer Protection Directorate-General and Directorate E-Food Safety, plant health animal health and welfare and international questions and E1 - Plant health.

Scott-Dupree, C. D., L. Conroy, and Harris, C. R. (2009). "Impact of currently used or potentially useful insecticides for canola agroecosystems on Bombus impatiens (Hymenoptera Apidae), Megachile rotundata (Hymentoptera: Megachilidae), and Osmia lignaria (Hymenoptera: Megachilidae)". *Journal of Economic Entomology* 102 (1), 177–182.

Słowińska, M., J. Nynca, J. Wilde, B. Bąk, M. Siuda, and A. Ciereszko (2015). "Total antioxidant capacity of honeybee haemolymph in relation to age and exposure to pesticide, and comparison to antioxidant capacity of seminal plasma: Apidologie". *Apidologie*, 1–10.

Smirle, M. J. and M. L. Winston (1988). "Detoxifying enzyme activity in worker honey bees: An adaptation for foraging in contaminated ecosystems". *Canadian Journal of Zoology* 66 (9), 1938–1942.

University of Hertfordshire (2013). *The Pesticide Properties DataBase (PPDB): developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006-2013*. URL: http://sitem.herts.ac.uk/aeru/footprint/en/index.htm/.

vanEngelsdorp, D., J. Hayes Jr., R. M. Underwood, and J. Pettis (2008). "A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008". *PloS one* 3 (12), e4071 EP -.

vanEngelsdorp, D. and M. D. Meixner (2010). "A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them". *Journal of invertebrate pathology* 103 Suppl 1, S80–95.

Westrich, P., U. Frommer, K. Mandery, H. Riemann, H. Ruhnke, C. Saure, and J. Voith (2008). "Rote Liste der Bienen Deutschlands (Hymenoptera, Apidae)(4. Fassung, Dezember 2007)". *Eucera* 1 (3), 33–87.

Wheeler, M. W., R. M. Park, and A. J. Bailer (2006). "Comparing median lethal concentration values using confidence interval overlap or ratio tests". *Environmental toxicology and chemistry* 25 (5), 1441–1444.

Wheeler, E. P. Grist, K. M. Leung, D. Morritt, and M. Crane (2002). "Species sensitivity distributions: data and model choice". *Marine Pollution Bulletin* 45 (1), 192–202.

Winteringham, F. P. (1969). "Mechanisms of selective insecticidal action". *Annual review of entomology* 14 (1), 409–442.

Xu, J., J. P. Strange, D. L. Welker, and R. R. James (2013). "Detoxification and stress response genes expressed in a western North American bumble bee, Bombus huntii (Hymenoptera: Apidae)". *BMC genomics* 14, 874.

B.S1 SUPPORTING INFORMATION

B.S1.1 *Collection and identification of wild bees*

Wild bees bee were caught at feeding grounds or nesting sites. Three species were found in sufficient numbers: *L. malachurum*, *A. flavipes* and *C. hederae* (Fig. B.S1). All of them are nesting in aggregations and are therefore quite abundant at their nesting sites (Bischoff et al., 2005; Westrich, 1990). Bees were either collected from flowers with small plastic cups or caught with a hand net between morning and midday. Afterwards, bees were directly anaesthetised by cooling and transferred to group cages where they were fed 50% sugar solution ad libitum. *L. malachurum* collected in a vineyard (8°5′19″E / 49°7′47″N), *A. flavipes* on a wildflower meadow (8°11′32″E / 49°8′57″N) and *C. hederae* at a small loess wall behind a vineyard (8°9′53″E / 49°13′28″N). After the termination of the experiments the surviving wild bees were killed by freezing and species were accurately determined. Precise identification was only possible with dead bees under a binocular. Studied specimen proved to be of the desired species except for two individuals that were actually *L. pauxillum* and *L. calceatum* instead of *L. malachurum*.

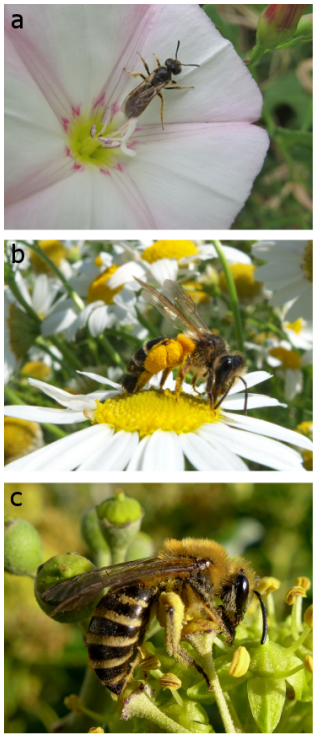

Figure B.S1.: Captured bee species. *L. malachurum* ♀ in a field bindweed flower (a), *A. flavipes* ♀ on feverfew flower (b), *C. hederae* ♀ collecting pollen on ivy (c). Photo credit: Carsten A. Brühl.

B.S1.2 *Dose verification of treatment solutions*

Samples of the highest and lowest concentrated treatment solutions were taken in all *B. terrestris* and *O. bicornis* ♀ test runs and stored in a freezer at -21°C until shipping. They were shipped to Ivo Roessink (Alterra, Wageningen UR, 6700AA Wageningen, The Netherlands) for quantitative HPLC analysis of their dimethoate content (Table B.S1). Only test runs were the measured dimethoate concentration was within ± 5% of the nominal concentration were deemed valid.

Table B.S1.: Results of analytical dose verification.

Species	Test run	Dimethoate concentration [µg/µL]		Deviation [%]
		Nominal	Measured	
B. terrestris	1	0.2500	0.2543	+1.74
		4.0000	4.1770	+4.43
O. bicornis ♀	1	0.6250	0.6249	-0.16
		10.0000	10.2706	+2.71
	2	0.6250	0.5964	-4.58
		10.0000	9.8831	-1.17
	3	0.6250	0.6223	-0.43
		10.0000	10.2286	+2.29

Further information

Preparation of Calibration Solutions
The standard stock solution with a concentration of 250 mg/L DIMETHOATE was prepared in methanol using reference material with a purity of 98.5% (Dr. Ehrenstorfer). Stock solution was stored in the freezer at temperature lower than -10°C.

External calibration standards with concentrations between 2.5 and 500 ng/mL were freshly prepared prior analysis by diluting the stock solution with acetonitrile/Milli-Q water (20v/80v) directly in GC vials, using a dilutor Hamilton 600. Duplo samples of about 3.0 mL were taken from the dosage solutions by mean of a glass pipette and transferred into a 4-mL brown vial, containing 1.0 mL acetonitrile. After homogenizing using a vortex one of the duplo's was stored in the refrigerator at 4°C (range 2 to 8°C) and the other one, was diluted prior to analysis with acetonitrile/MilliQ-water (20v/80v) and analyzed directly (without extraction or concentration) by means of LC-MSMS. The dilution has been done also directly in GC vials, using a dilutor Hamilton 600. Injected samples were quantified by dimethoate peak area using the calibration curve constructed from calibration standards included in the same sample sequence. The concentrations of the samples never exceeded the highest standard of the calibration curve. The curve fit was linear and forced through origin (x-axis zero; y-axis zero).

LC-MS/MS-Conditions for dimethoate

Instrument

Autosampler: Agilent G1329A
Pump: Agilent G1312A (binary pump)
Detector: Agilent G63110A QQQ
Source: Agilent G1948 Electrospray
Column thermostat: Agilent G1316A

Separation

Eluent A: MilliQ-water (Advantage A10) + 0.1% formic acid
Eluent B: Acetonitril + 0.1% formic acid

Gradient:	Time	%B
	0.0	60
	2.0	60
	3.0	80
	6.0	80
	7.0	60
	8.0	60

Injection Volume: 50 µL
Flow Rate: 0.7 mL/min
Column: Agilent Zorbax Eclipse XDB C18 (4.6 mm x 150 mm, 5 micron)
Column temperature: 40°C

Detection

Ionization Mode: Positive
Heater Gas Temperature: 350 °C
Spray Voltage: 3000 V
Nebulizer pressure: 50 psi
Nitrogen flow: 10 L/min
Scan Mode: Multiple reaction monitoring (MRM)

Compound	Precursor Ion	Product Ion	Fragmentation	Collision Energy
dimethoate	230	198.8	60	5
dimethoate	230	171	60	9
dimethoate	239	125	60	21

Retention time: about 2.75 min.
LOD in the injected samples: 0.03 ng/mL
LOQ in the injected samples: 0.09 ng/mL

Table B.S2.: Weight of studied bee species.

Species	Fresh weight [mg]	95% CI [mg]	Dry weight [mg]	95% CI [mg]
L. malachurum	10.97	4.28 – 17.66	3.71	1.34 – 6.09
O. bicornis ♂	37.69	7.19 – 68.19	17.61	4.58 – 30.63
A. flavipes	47.26	19.35 – 75.17	21.61	14.44 – 28.77
O. bicornis ♀	93.57	62.72 – 124.42	30.36	23.30 – 37.42
C. hederae	105.52	66.36 – 144.68	43.43	29.00 – 57.86
B. terrestris	205.01	116.84 – 293.19	55.79	29.53 – 82.04

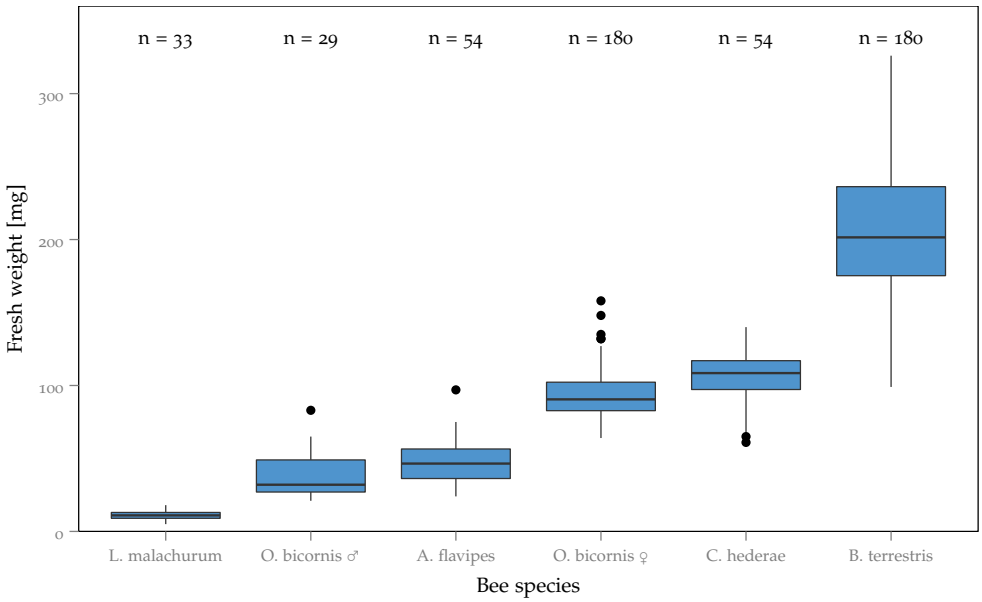

Figure B.S2.: Boxplots of fresh weight for studied bee species.

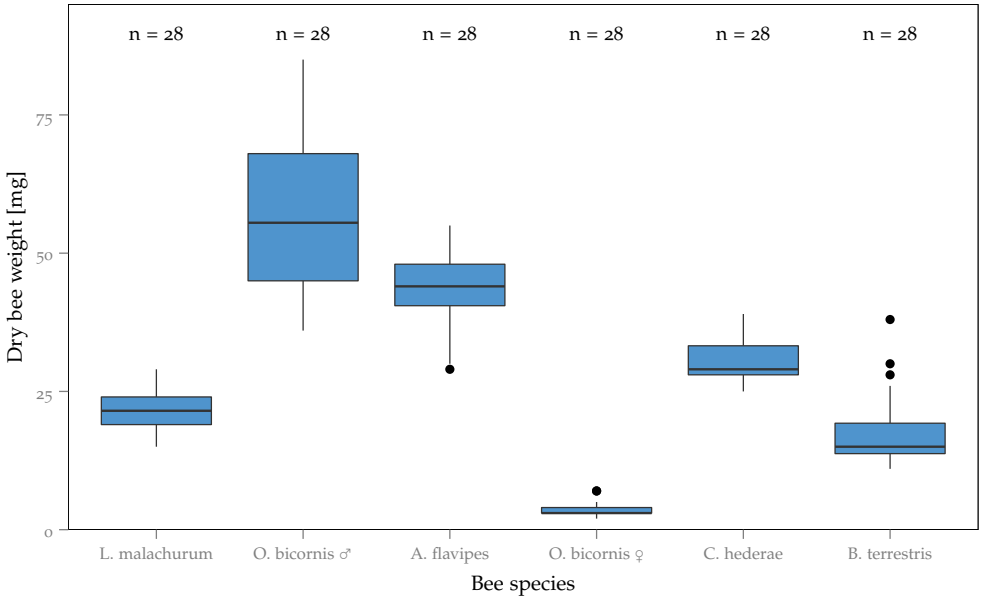

Figure B.S3.: Boxplots of dry weight for studied bee species.

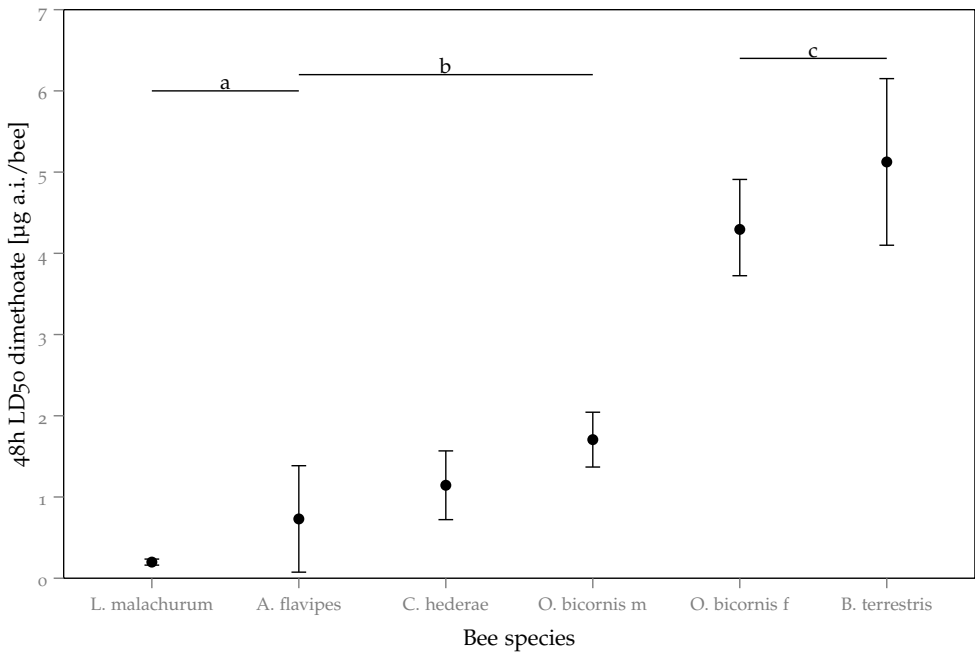

Figure B.S4.: LD50 values for the studied bee species. Means and 95% CIs. Letters indicate statistical significance ($p < 0.05$).

Table B.S3.: Comparison of 48 h dimethoate LD50 values of studied bee species.

Compared species		Mean difference	95% CI	p
Species 1	Species 2	[µg a.i./bee]	[µg a.i./bee]	
A. flavipes	*L. malachurum*	0.53	-0.38 – 1.44	0.086
C. hederae	*L. malachurum*	0.95	0.36 – 1.53	<0.001
O. bicornis ♂	*L. malachurum*	1.51	1.04 – 1.98	<0.001
O. bicornis ♀	*L. malachurum*	4.10	3.21 – 4.99	<0.001
B. terrestris	*L. malachurum*	4.93	3.51 – 6.35	<0.001
C. hederae	*A. flavipes*	0.41	-0.67 – 1.49	1
O. bicornis ♂	*A. flavipes*	0.98	-0.04 – 2.00	0.075
O. bicornis ♀	*A. flavipes*	3.56	2.30 – 4.83	<0.001
B. terrestris	*A. flavipes*	4.40	2.71 – 6.08	<0.001
O. bicornis ♂	*C. hederae*	0.56	-0.19 – 1.31	0.413
O. bicornis ♀	*C. hederae*	3.15	2.09 – 4.21	<0.001
B. terrestris	*C. hederae*	3.98	2.44 – 5.52	<0.001
O. bicornis ♀	*O. bicornis* ♂	2.59	1.59 – 3.59	<0.001
B. terrestris	*O. bicornis* ♂	3.42	1.92 – 4.91	<0.001
B. terrestris	*O. bicornis* ♀	0.83	-0.84 – 2.51	1

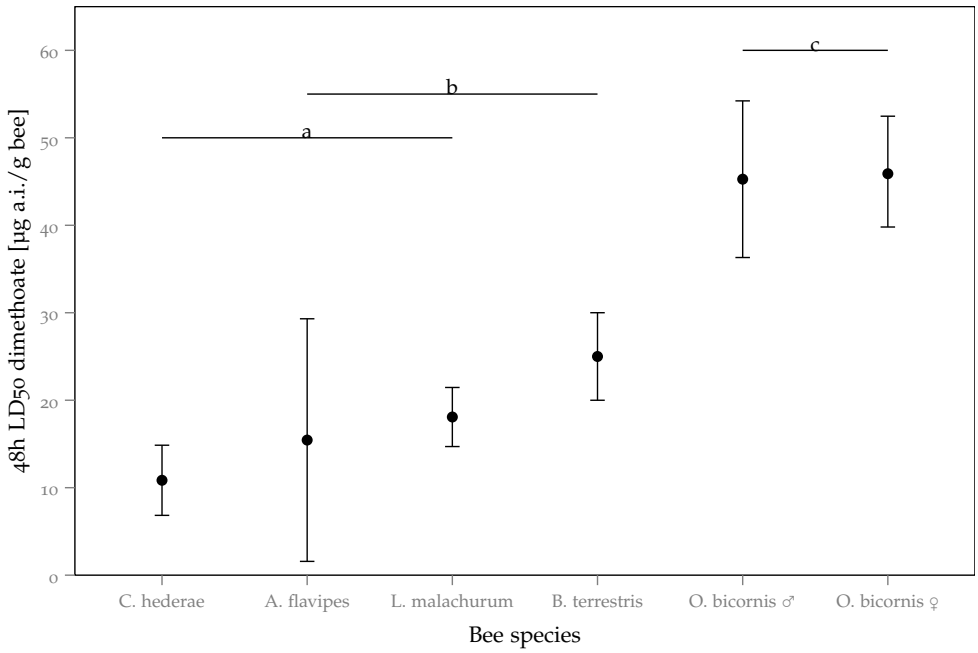

Figure B.S5.: Fresh weight-normalised LD50 values for the studied bee species. Means and 95% CIs. Letters indicate statistical significance ($p < 0.05$).

Table B.S4.: Comparison of fresh weight-normalised 48 h dimethoate LD50 values of studied bee species.

Compared species		Mean difference	95% CI	p
Species 1	Species 2	[µg a.i./g bee]	[µg a.i./g bee]	
A. flavipes	*L. malachurum*	-2.64	-24.01 – 18.74	1
C. hederae	*L. malachurum*	-7.24	-15.09 – 0.62	0.103
O. bicornis ♂	*L. malachurum*	27.19	12.86 – 41.52	<0.001
O. bicornis ♀	*L. malachurum*	27.81	17.06 – 38.56	<0.001
B. terrestris	*L. malachurum*	6.92	-2.12 – 15.96	0.367
C. hederae	*A. flavipes*	-4.60	-26.22 – 17.02	8.152
O. bicornis ♂	*A. flavipes*	29.82	5.10 – 54.55	0.006
O. bicornis ♀	*A. flavipes*	30.45	7.62 – 53.28	0.002
B. terrestris	*A. flavipes*	9.56	-12.52 – 31.64	1
O. bicornis ♂	*C. hederae*	34.42	19.73 – 49.12	<0.001
O. bicornis ♀	*C. hederae*	35.05	23.82 – 46.28	<0.001
B. terrestris	*C. hederae*	14.16	4.55 – 23.76	<0.001
O. bicornis ♀	*O. bicornis* ♂	0.63	-15.80 – 17.05	1
B. terrestris	*O. bicornis* ♂	-20.27	-35.63 – -4.91	0.002
B. terrestris	*O. bicornis* ♀	-20.89	-32.98 – -8.81	<0.001

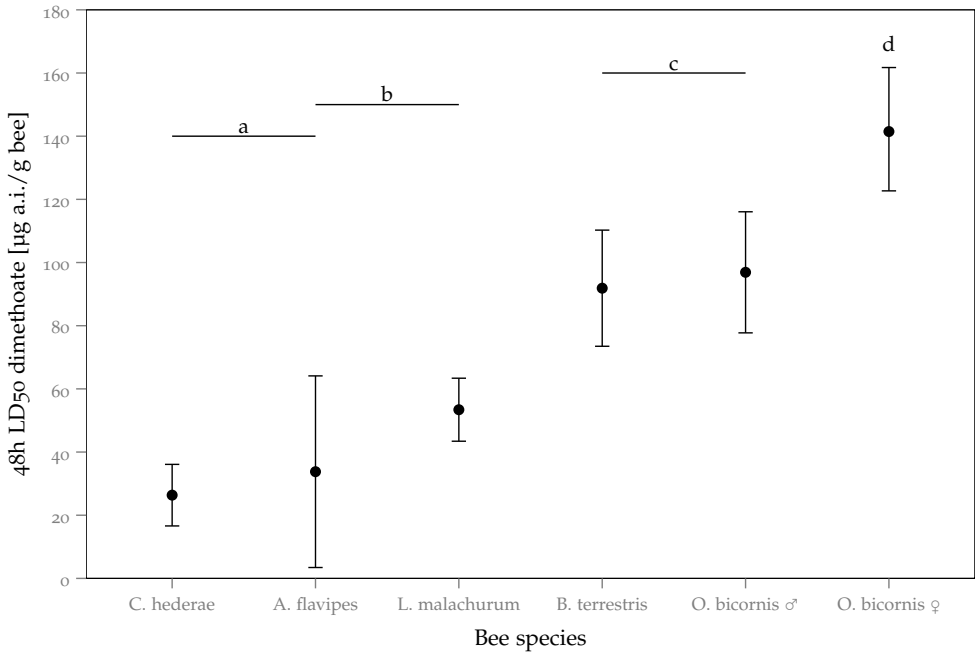

Figure B.S6.: Dry weight-normalised LD50 values for the studied bee species. Means and 95% CIs. Letters indicate statistical significance ($p < 0.05$).

Table B.S5.: Comparison of dry weight-normalised 48 h dimethoate LD50 values of studied bee species.

Compared species		Mean difference	95% CI	p
Species 1	Species 2	[µg a.i./g bee]	[µg a.i./g bee]	
A. flavipes	*L. malachurum*	-19.62	-67.44 – 28.20	1
C. hederae	*L. malachurum*	-27.05	-47.93 – -6.17	0.002
O. bicornis ♂	*L. malachurum*	43.50	11.15 – 75.86	0.001
O. bicornis ♀	*L. malachurum*	88.07	55.23 – 120.90	<0.001
B. terrestris	*L. malachurum*	38.48	7.15 – 69.80	0.005
C. hederae	*A. flavipes*	-7.43	-55.14 – 40.28	1
O. bicornis ♂	*A. flavipes*	63.12	9.39 – 116.86	0.009
O. bicornis ♀	*A. flavipes*	107.69	53.66 – 161.71	<0.001
B. terrestris	*A. flavipes*	58.10	4.98 – 111.21	0.021
O. bicornis ♂	*C. hederae*	70.55	38.35 – 102.75	<0.001
O. bicornis ♀	*C. hederae*	115.11	82.44 – 147.79	<0.001
B. terrestris	*C. hederae*	65.52	34.36 – 96.68	<0.001
O. bicornis ♀	*O. bicornis* ♂	44.56	3.59 – 85.54	0.022
B. terrestris	*O. bicornis* ♂	-5.03	-44.80 – 34.75	1
B. terrestris	*O. bicornis* ♀	-49.59	-89.75 – -9.43	0.005

Table B.S6.: Dimethoate SSD model information.

Model type	Model parameters			
	Scale	SE	Location	SE
log-logistic	0.3514	0.0951	-0.0787	0.2090

Table B.S7.: Contact dimethoate 48h LD50 values for bee species from literature.

Species	LD50 [µg a.i./bee]	Geometric mean LD50 [µg a.i./bee]	Source
A. mellifera	0.16	0.18	Ladurner et al. (2005)
A. mellifera	0.31		Biddinger et al. (2013)
A. mellifera	0.12		Stevenson (1968)
O. cornifrons	0.09	0.09	Biddinger et al. (2013)
O. lignaria	1.21	1.21	Ladurner et al. (2005)

B.S1.3 *References*

Biddinger, D. J., J. L. Robertson, C. Mullin, J. Frazier, S. A. Ashcraft, E. G. Rajotte, N. K. Joshi, and M. Vaughn (2013). "Comparative toxicities and synergism of apple orchard pesticides to Apis mellifera (L.) and Osmia cornifrons (Radoszkowski)". *PloS one* 8 (9), e72587.

Bischoff, I., E. Eckelt, and M. Kuhlmann (2005). "On the biology of the ivy-bee Colletes hederae Schmidt & Westrich, 1993 (Hymenoptera, Apidae)". *BONNER ZOOLOGISCHE BEITRAGE* 53 (1/2), 27.

Ladurner, E., J. Bosch, W. P. Kemp, and S. Maini (2005). "Assessing delayed and acute toxicity of five formulated fungicides to Osmia lignaria Say and Apis mellifera". *Apidologie* 36 (3), 449.

Stevenson, J. H. (1968). "Laboratory studies on the acute contact and oral toxicities of insecticides to honeybees". *Annals of Applied Biology* 61 (3), 467–472.

Westrich, P. (1990). *Die Wildbienen Baden-Württembergs: [im Rahmen des Artenschutzprogrammes Baden Württemberg]*. 2., verb. Aufl. Stuttgart: Ulmer.

B

C | IS *OSMIA BICORNIS* AN ADEQUATE REGULATORY SURROGATE? COMPARING ITS ACUTE CONTACT SENSITIVITY TO *APIS MELLIFERA*

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C

C.1 ABSTRACT

Bees provide essential ecosystem services and help maintain floral biodiversity. However, there is an ongoing decline of wild and domesticated bee species. Since agricultural pesticide use is a key driver of this process, there is a need for a protective risk assessment. To achieve a more protective registration process, two bee species, *Osmia bicornis*/*Osmia cornuta* and *Bombus terrestris*, were proposed by the European Food Safety Authority as additional test surrogates to the honey bee *Apis mellifera*. We investigated the acute toxicity (median lethal dose, LD50) of multiple commercial insecticide formulations towards the red mason bee (*O. bicornis*) and compared these values to honey bee regulatory endpoints. In two thirds of all cases, *O. bicornis* was less sensitive than the honey bee. By applying an assessment factor of 10 on the honey bee endpoint, a protective level was achieved for 87% (13 out 15) of all evaluated products. Our results show that *O. bicornis* is rarely an adequate additional surrogate species for lower tier risk assessment since it is less sensitive than the honey bee for the majority of investigated products. Given the currently limited database on bee species sensitivity, the honey bee seems sufficiently protective in acute scenarios as long as a reasonable assessment factor is applied. However, additional surrogate species can still be relevant for ecologically meaningful higher tier studies.

C.2 INTRODUCTION

Bees are important pollinators of wild and cultivated flora, which makes them essential providers of ecosystem services and maintainers of floral biodiversity (Klein et al., 2007; Ollerton et al., 2011). Aside from the honey bee, *Apis mellifera*, there are other managed bees along with a broad spectrum of wild bee species that contribute substantially to plant pollination (Rader et al., 2015). However, there is an ongoing trend of wild bee species decreasing in abundance and diversity all over the world (Sánchez-Bayo and Wyckhuys, 2019). Furthermore, honey bee hive numbers are also substantially decreasing in North America and many European countries (Ollerton, 2017). Among various environmental factors, e.g. habitat loss and fragmentation, parasites, agricultural pesticide use has been identified as one of the key drivers of bee decline (Goulson et al., 2015). The ecological challenge of flying insect decline in general seems to have been underestimated and consequently disregarded in the past. As a recent study by Hallmann et al. (2017) shows, there has been a severe 75% decline in flying insect biomass in several German natural reserves over roughly the last three decades (Hallmann et al., 2017).

In the European agricultural landscape, bees can be exposed to a variety of pesticides that target all major pests: herbicides, fungicides, insecticides (Chauzat et al., 2011; Mullin et al., 2010). They are not only contaminated during foraging on crops but also from visitations of field-adjacent wild flowers (Botías et al., 2015). Bees can be exposed to pesticides by direct overspray as well as oral uptake of and contact with nectar and pollen while foraging (Gradish et al., 2019; Sgolastra et al., 2019). They can

also be fed contaminated pollen and nectar as larvae. Furthermore, there is potential uptake of soil residues by adults and larvae of soil-nesting species (Gradish et al., 2019; Sgolastra et al., 2019; Wood and Goulson, 2017). Moreover, consumption of non-nectar fluids such as puddle water, guttation droplets or extrafloral nectar may also lead to contamination (Bonmatin et al., 2015; Gradish et al., 2019; Sgolastra et al., 2019; van der Sluijs et al., 2015). Consequently, bee species are exposed to pesticides through various environmental matrices throughout their lifespan (Gradish et al., 2019; Sgolastra et al., 2019).

To prevent adverse impacts of pesticide applications on bee populations, toxic effects of these substances on bee species need to be understood. However, the majority of toxicity testing in laboratory and field setups has been performed using the honey bee, a bred livestock species, whereas all other bee species are far less well-understood in their sensitivity (Wood and Goulson, 2017).

Furthermore, the honey bee is the only pollinator species that is tested for its reaction towards pesticides in the current risk assessment scheme according to Regulation (EC) 1107/2009 (SANCO, 2002). However, other bee species (i.e. bumble bees, solitary bees) may show quite different responses to pesticide exposure due to differences in physiology and ecology (Arena and Sgolastra, 2014). To account for these significant differences and collect information regarding the sensitivity of bumble bees and solitary bees, the European Food Safety Authority (EFSA) proposed the inclusion of additional surrogate species into EU pesticide risk assessment: The buff-tailed bumble bee, *Bombus terrestris*, and an *Osmia* species (the red mason bee, *Osmia bicornis* or the European orchard bee, *Osmia cornuta*) (EFSA, 2013). However, there has been reasonable doubt that these two species are adequate to provide additional safety in lower tier risk assessment. Uhl et al. (2016) tested five European bee species in acute contact exposure scenarios with a formulated insecticide product (PERFEKTHION®) containing dimethoate, which is often used as a toxic standard in regulatory testing. They found that *B. terrestris* and *O. bicornis* were the least sensitive species when compared to a dataset of their own results and collected literature data. Another study by Heard et al. (2017) compared the acute oral sensitivity of the honey bee towards several pesticides (active ingredients) to *B. terrestris* and *O. bicornis*. They found contrasting sensitivity ratios depending on substance since both non-*Apis* bee species were sometimes more, and sometimes less, sensitive. *Bombus terrestris* was generally less sensitive than the honey bee in acute toxicity studies that were compiled by Arena and Sgolastra (2014). They could not collect *O. bicornis*/*O. cornuta* data, but other *Osmia* species (*O. cornifrons*, *O. lignaria*) were usually also more resistant to toxicant stress than *A. mellifera*. Moreover, EFSA (2013) proposed an assessment factor of 10 to account for interspecific differences when testing only honey bees. This approach proved to be protective in 95% of cases in the meta-analysis by Arena and Sgolastra (2014). It is unclear, however, if this factor would be protective for the proposed test species due to the slim database of their sensitivity (Heard et al., 2017; Uhl et al., 2016).

There is a need to assess the suitability of the new test species that EFSA (2013) proposed. Only sensitive species will reduce uncertainty in lower tier risk assessment. However, with the current database, it is not possible to properly evaluate whether

the proposed species are adequate. Therefore, we tested one of these proposed surrogate species, *O. bicornis*, with commercial formulations of multiple common insecticides. We performed acute contact toxicity laboratory tests to derive 48h contact median lethal doses (LD50s). We wanted to assess the acute toxic potency of several insecticides from various classes on *O. bicornis*. Furthermore, our goal was to compare those toxicity endpoints to honey bee data from pesticide regulation that are used to assess their safety regarding bees. This enabled us to evaluate whether *O. bicornis* is usually more sensitive than the honey bee, which would make it a suitable additional surrogate species for lower tier risk assessment. Additionally, we examined if an assessment factor of 10 is protective when comparing honey bee to *O. bicornis* sensitivity.

C.3 MATERIALS AND METHODS

C.3.1 *Insecticides*

The majority of tested insecticides were chosen with respect to the application frequency of their commercial products in apple, grapes and winter oilseed rape (Table C.1) which represent three main cultivation types in Germany (Julius Kühn-Institut, 2018). Additionally, formulations of four insecticides that are not frequently applied were included because of the following reasons: Imidacloprid exposure has been implicated as a major factor in bee decline (Wood and Goulson, 2017). Dimethoate is often used as a toxic reference in bee ecotoxicity studies. Chlorpyrifos was chosen for inclusion as a second organophosphate insecticide in addition to dimethoate. Furthermore, flupyradifurone is a relatively new insecticide with low acute toxicity towards honey bees for which registration has been applied for use in multiple EU countries (European Commission, 2014). Insecticides were assigned to pesticide classes according to the Compendium of Pesticide Common Names (Alan Wood, 2018). Representative formulated products that contain those pesticides as active ingredients (a.i.) were chosen for testing (Table C.1). Most of these formulations are, or were, registered in Germany in recent years aside from Pyrinex® (a.i. chlorpyrifos) and Sivanto® SL 200 G (a.i. flupyradifurone). To ease readability, only active ingredient instead of formulated product names are used hereafter.

Table C.1.: Tested insecticides and their usage in German agriculture. The usage share signifies the prominence of a certain compound with regard to all pesticide applications. It is based on the standardised treatment index (STI) which is defined as the number of pesticide applications in a crop in relation to the application rate and cultivated area (Julius Kühn-Institut, 2018; Sattler et al., 2007). Data from Julius Kühn-Institut (2018).

Insecticide (a.i.)	Class	Usage share of a.i. [%] per culture (2015/2016)			Tested product
		Apple	Grapes	Winter oilseed rape	
alpha-cypermethrin	pyrethroid	/	/	16.8 / 16.1	FASTAC® SC
beta-cyfluthrin	pyrethorid	/	/	12.1 / 13.3	Bulldock®
deltamethrin	pyrethorid	/	/	3.4 /	Decis® Forte
etofenprox	pyrethroid	/	/	12.4 / 18.5	Trebon® 30 EC
lambda-cyhalothrin	pyrethroid	/	/ 3.3	19.5 / 24.6	Karate® Zeon
zeta-cypermethrin	pyrethorid	/	/	2.8 / 4.5	Fury® 10 EW
acetamiprid	neonicotinoid	5.2 / 8.4	/	2.0 /	Mospilan® SG
imidacloprid	neonicotinoid	/	/ 3.0	/	Confidor® WG 70
thiacloprid	neonicotinoid	12.5 / 10.2	/	16.1 / 6.9	Calypso®
dimethoate	organophosphate	/	/	/	PERFEKTHION®
chlorpyrifos	organophosphate	/	/	/	Pyrinex®
chlorantraniliprole	pyridylpyrazole	23.7 / 26.9	/	/	Coragen®
flupyradifurone	unclassified	/	/	/	Sivanto® SL 200 G
indoxacarb	oxadiazine	3.8 / 3.3	44.3 / 34.6	2.3 / 2.9	AVAUNT® 150 EC
pirimicarb	carbamate	19.5 / 15.0	/	/	Pirimor®
spinosad	spinosyn	/	/ 27.7	/	SpinTor®

C

C.3.2 *Experimental procedure*

The red mason bee, *Osmia bicornis* (Linneaus, 1758), was used as test species. Bees were ordered as uneclosed adults in cocoons (WAB-Mauerbienenzucht, Konstanz, Germany), received at the end of February 2017 and stored dry at 4°C until experimental preparation started.

Acute, contact toxicity of 16 insecticide formulation towards *O. bicornis* females was investigated (see Supporting Information Table C.S1 for a timeline of the experiments). To that end, a protocol for solitary bee acute contact toxicity testing from the International Commission on Plant-Pollinator Relationships (ICPPR) was followed or partly adapted (Roessink et al., 2016). This protocol is a precursor of a standardised testing guideline. Prior to the experiments, bee cocoons were taken from the refrigerator and placed in an environmental chamber at test conditions to let females hatch. Male bees were also collected after hatching to prevent mating with females and used for range finding tests. Female bees' eclosion time was usually between five to seven days. After eclosion, females were again stored at 4°C until one day before application to reduce stress until enough individuals for a test were available. At this date, they were transferred in to the environmental chamber in test cages (1 L plastic boxes sealed with a perforated lid) and fed *ad libitum* with sucrose solution 50% (w/w) through 2 mL plastic syringes to acclimatise overnight. Twenty bees were assigned to each treatment (usually 5 per

cage, n = 4). See the raw data for details on individual study setups (Uhl et al., 2018). Environmental conditions were set to 16:8h day/night cycle, 60% relative humidity and 21°C. In the summer of 2017 there was a malfunction of the environmental chamber which caused the light to stay on throughout the whole day. Two test runs were therefore conducted with constant lighting (dimethoate, indoxacarb). Since control mortality was below the quality criterium of 10% in those runs, they were evaluated as valid, nonetheless. Anaesthetisation of bees was necessary before the transfer to test cages. To achieve a calm state, bees were chilled at 4°C. During this process they were also weighed. Bees were anaesthetised a second time before treatment application which was performed in a petri dish. In cases where the ambient temperature was too high to keep bees calm after chilling, petri dishes were put on ice for additional cooling. Moribund bees were rejected and replaced with healthy bees prior to the test start.
Treatment solutions were prepared as follows: a control of deionised water containing 0.5% (v/v) wetting agent (Triton™ X-100, Sigma-Aldrich) and at least five treatment solutions of the respective insecticide. Concentrations and number of insecticide treatments were determined after conducting range finding tests with male bees before the main test. Results of these pretests were extrapolated to females using the weight difference of both sexes. Insecticide solutions were prepared by diluting the respective concentration in deionised water containing 0.5% wetting agent. In the first tests, bees were applied with 2 µL treatment solution on the dorsal side of the thorax between the neck and wing base using a Hamilton micro syringe (Hamilton Bonaduz AG). Due to easier handling, an Eppendorf Multipette® plus (Eppendorf AG) was used later for most of the tests. In three tests (chlorantraniliprole, flupyradifurone, pirimicarb), the applied volume had to be increased to 4 µL to dilute high doses. See the raw data for details (Uhl et al., 2018). After ten to 15 min the treatment solution was fully absorbed and a paper tissue was inserted into test cages to provide a hiding place. Following the application bees were returned to the environmental chamber and fed 50% sucrose solution *ad libitum*. Mortality was assessed after 24, 48, 72 and 96h. For dimethoate, a second test run was performed as part of an ICPPR ring test. Control mortality after 48h was ≤10% in all experiments except for flupyradifurone and chlorantraniliprole (both 15%). Those two cases were evaluated and are considered valid since in the ICCPR test protocol it is discussed that control mortality thresholds might be increased to 15 or 20% in the long run.

C.3.3 *Data analysis*

Median acute lethal dose values (contact 48h LD50) were calculated for all tested insecticidal products by fitting a dose-response model to the data. Raw data are available through an online repository (Uhl et al., 2018). Models were chosen by visual data inspection and using Akaike information criterion (AIC). Furthermore, it was ensured that appropriate models were used for tests with control mortality (no fixed lower limit). Where multiple LD50 values were available, a geometric mean LD50 was computed. Weight-normalised LD50 values were further calculated by dividing LD50 values by mean fresh weight of all bees in a respective test. All statistical anal-

yses were conducted with R 3.4.4 (R Core Team, 2017). We used the "drc" package (Ritz and Streibig, 2005) for dose-response modeling (version 3.0-1). Honey bee contact 48h LD50 values were gathered by screening regulatory documents (EC review, report, EFSA conclusion, rapporteur member state draft/renewal assessment reports). Furthermore, we contacted national and European authorities, manufacturers and EFSA to collect data and verify them. For a detailed account of the data collection process and various data sources please see Supporting Information Appendix C.S1.1 and Tables C.S2, C.S3. To compare *A. mellifera* and *O. bicornis* endpoints, sensitivity ratios ($R = LD50_{A.\ mellifera}$ / $LD50_{O.bicornis}$) were calculated according to Arena and Sgolastra (2014) for all tested insecticides. Honey bee endpoints were not available as weight-normalised values. Therefore, sensitivity of both species could only be compared without taking the weight of test individuals into account.

C.4 RESULTS

Sensitivity of *O. bicornis* towards all tested insecticides varied considerably (Table C.2, Supporting Information Figures C.S1-C.S17). The maximum LD50 value of pirimicarb was 3679 times higher than the minimum LD50 of imidacloprid. The median LD50 value of all pesticides was 1.21 µg a.i./bee. About 69% of substances had LD50 values below 2 µg a.i./bee whereas 38% had LD50s under 0.2 µg a.i./bee. Bee mean fresh-weight differed across all tests (range 77.7 to 112.7 mg, mean of all tests 91.6 mg). The indoxacarb test that included the heaviest bees shows a 23% deviation and the thiacloprid test with the least heavy bees a 15% deviation from mean weight. Such variations subsequently also occur in weight-normalised LD50 values.

Table C.2.: Comparison of *O. bicornis* acute contact toxicity with honey bee regulatory endpoints. Insecticides are ordered by sensitivity ratio.

Pesticide	*O. bicornis*					*A. mellifera*	
	LD50	95% CI	Fresh weight	Weight-normalised LD50	95% CI	LD50	R
	[µg a.i./bee]		[mg]	[µg a.i./g bee]		[µg a.i./bee]	
zeta-cypermethrin	0.13	0.09 – 0.17	100.8	1.31	0.93 – 1.69	0.002	<0.1
spinosad	2.06	1.61 – 2.51	80.0	25.73	20.13 – 31.33	0.05	<0.1
indoxacarb	1.26	0.90 – 1.63	112.7	11.21	7.94 – 14.48	0.08	0.1
dimethoate	1.32	1.14 – 1.49	99.9	13.20	11.44 – 14.89	0.111	0.1
pirimicarb	115.07	95.96 – 134.18	85.6	1343.61	1120.47 – 1566.74	36.1	0.3
alpha-cypermethrin	0.24	0.16 – 0.33	85.9	2.84	1.89 – 3.80	0.09	0.4
lambda-cyhalothrin	0.14	0.10 – 0.17	93.5	1.45	1.06 – 1.85	0.055	0.4
deltamethrin	0.06	0.04 – 0.07	100.1	0.57	0.43 – 0.71	0.029	0.5
chlorpyrifos	4.19	2.91 – 5.46	92.9	45.07	31.37 – 58.78	3.19	0.8
beta-cyfluthrin	0.04	0.02 – 0.05	100.4	0.35	0.20 – 0.50	0.032	0.9
flupyradifurone	10.59	6.06 – 15.11	83.0	127.52	72.96 – 182.08	17.1	1.6
acetamiprid	1.72	0.85 – 2.59	95.0	18.10	8.96 – 27.23	9.26	5.4
imidacloprid	0.03	0.03 – 0.04	94.6	0.33	0.27 – 0.39	0.245	7.8
chlorantraniliprole	5.92	4.26 – 7.57	79.0	74.91	53.94 – 95.87	>100	16.9
thiacloprid	1.16	0.74 – 1.58	77.7	14.91	9.50 – 20.31	20.8	18.0
etofenprox	0.18	0.14 – 0.22	84.9	2.09	1.63 – 2.55	NA	NA

In two thirds of all cases, *O. bicornis* was less sensitive than the honey bee (15 out of 16 insecticides could be evaluated; no regulatory honey bee data are available for etofenprox product). When dividing the respective honey bee endpoint by an assessment factor of 10, it was lower than the *O. bicornis* endpoint for 87% of all tested substances (Table C.2). The two remaining insecticides where *O. bicornis* would still be more sensitive are formulations of chlorantraniliprole and thiacloprid. When analysing sensitivity ratios by insecticide class, it was shown that for organophosphates and pyrethroids values are all below one, i.e. *O. bicornis* was less sensitive than the honey bee (Fig. C.1). In the case of the three tested neonicotinoids, *O. bicornis* was always more sensitive.

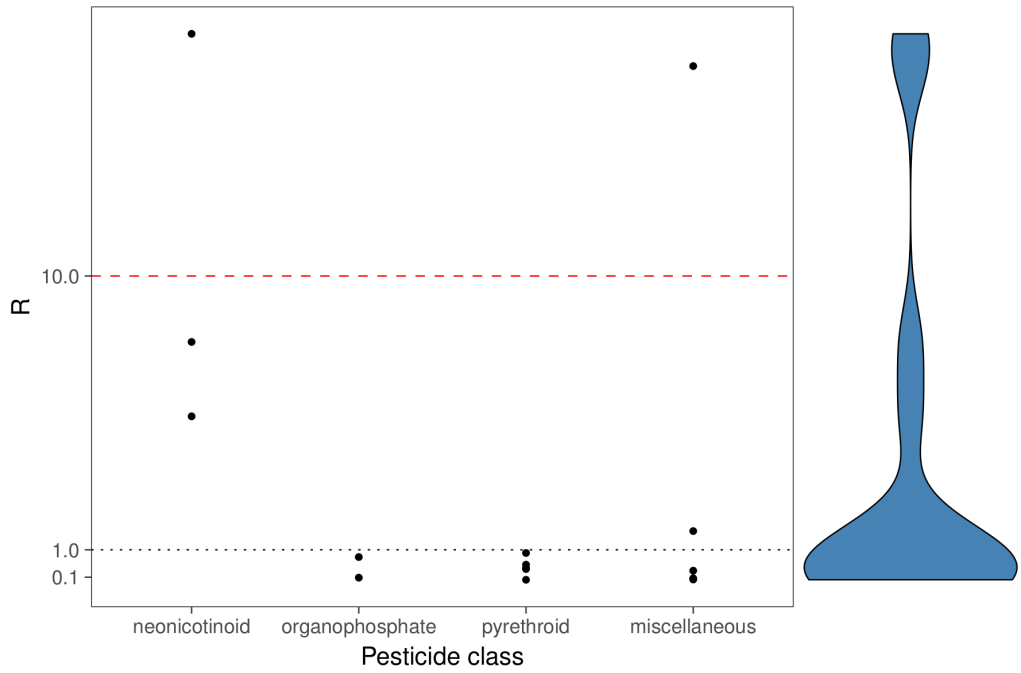

Figure C.1.: Sensitivity ratio (R) of all tested insecticides grouped by insecticide class. The dotted, grey line signifies equal sensitivity of *O. bicornis* and *A. mellifera*. The dashed, red line indicates the insecticides whose toxicity towards *O. bicornis* would be covered when dividing the honey bee endpoint by an assessment factor of 10. The violin plot on the right shows the distribution of data points.

C.5 DISCUSSION

In our study, we assessed the acute contact toxicity of several insecticides from several classes towards *O. bicornis*. Our goal was to compare these data to honey bee endpoints obtained from the pesticide registration process to infer on the suitability of *O. bicornis* as an additional regulatory surrogate species. Furthermore, we wanted to infer if applying an assessment factor of 10 on honey bee LD50 values would be protective for *O. bicornis*.

Acute sensitivity of *O. bicornis* varied substantially between pesticides, which was expected given that the available honey bee endpoints also vary considerably (Table C.2). Mean *O. bicornis* female weight also fluctuated between tests, which might have slightly affected their measured sensitivity. However, this effect was not big enough to affect the ranking of insecticides when ordered by acute toxicity. Therefore, these LD50 values are still valid for the comparison with regulatory honey bee values. Since bee individual weight is one factor that influences sensitivity towards pesticides (Uhl et al., 2016), calculating toxicity on a per weight basis leads to more precise and comparable results. Consequently, acute toxicity endpoints should generally also be reported in a weight-normalised format (see Table C.2).

To create a more protective environmental risk assessment for bees, EFSA (2013) proposed the inclusion of two additional bee species as surrogates (*B. terrestris*, *O. bicornis*/*O. cornuta*). These species should accompany the current sole test species, the honey bee. However, in acute toxicity testing, the addition of new species is only reasonable if they are generally more sensitive than the test species already in place. For two thirds of the insecticides we tested, *O. bicornis* was indeed less sensitive than the honey bee (Table C.2). This trend is in agreement with the findings of Uhl et al. (2016) who performed acute contact toxicity tests with five bee species and combined their dataset with LD50 values taken from literature. They found that two proposed test species, *O. bicornis* and *B. terrestris*, were less sensitive towards dimethoate than several bee species, including the honey bee. Heard et al. (2017) conducted acute to chronic oral tests (up to 240h) with *B. terrestris* and *O. bicornis* and five organic pesticides, cadmium and arsenic. Their results were inconclusive as to whether the proposed additional test species or the honey bee was acutely more sensitive. If only acute endpoints are considered, *O. bicornis* was more sensitive for two out of six substances that could be evaluated (48h LD50; clothianidin, tau-fluvalinate).

When evaluating this combined information, it becomes evident that *O. bicornis* (and possibly *B. terrestris*) is seldomly an adequate supplementary surrogate species for acute testing of pesticides, since its inclusion would not provide additional safety for the risk assessment process for most pesticides. There is insufficient data to evaluate *O. cornuta*. As postulated by Uhl et al. (2016), test species should be chosen according to their sensitivity in acute effect studies. However, the proposed test species were selected because they are bred for commercial pollination, can be obtained easily in large numbers and cope well under laboratory conditions. While those criteria are important for conducting laboratory experiments in general, they should not be decisive for the selection of surrogate species. The honey bee may be a better choice in acute contact toxicity tests since the not fully matured cuticle of young workers makes it more susceptible towards pesticides compared to solitary bees (Dmochowska-Ślęzak et al., 2015; Elias-Neto et al., 2014). Furthermore, there are differences in the immune response of young adults. In honey bees, the individual detoxification capacity is relatively low after hatching and increases from thereon as they age (Słowińska et al., 2015; Smirle and Winston, 1988). However, antioxidant enzyme levels already rise in *O. bicornis* adults before eclosion, which is another explanation for their lower sensitivity towards pesticides compared to honey bees at least at this life stage (Dmochowska-Ślęzak et al., 2015).

We could show for 87% of the tested insecticides that dividing the honey bee endpoint by an assessment factor of 10 is sufficient to cover *O. bicornis*' sensitivity (Fig. C.1). This assessment factor was found to be protective in 95% of all cases that were analysed in the meta-analysis of Arena and Sgolastra (2014). After testing multiple bee species with dimethoate, Uhl et al. (2016) reaffirmed this conclusion using a species sensitivity distribution (SSD) approach. Moreover, Heard et al. (2017) state that the honey bee is also an adequate surrogate species for acute oral testing as long as a reasonable assessment factor is applied. A factor of 10 would also have been protective for *O. bicornis* in their study of acute oral toxicity. However, they note that there are exceptions

for some substances, e.g. neonicotinoids. Arena and Sgolastra (2014) already mentioned that for this class, wild bee species showed equal or higher sensitivity than the honey bee. This trend is also visible in our data: *O. bicornis* was more sensitive towards all three tested neonicotinoids (acetamiprid, imidacloprid, thiacloprid) than the honey bee (maximum 18 times; Fig. C.1).
Consequently, the honey bee is a sufficient surrogate species to assess acute toxicity of most pesticides. In some cases (e.g. neonicotinoids) it might be necessary to increase the assessment factor to >10 to achieve a proper level of safety in lower tier risk assessment. To distinguish these substance classes that are relatively more harmful to wild bees than honey bees, a comprehensive ecotoxicological database should be established that includes a representative amount of species and pesticides. Such a database would be helpful for policy-makers to determine protective assessment factors and also for choosing suitable additional test species, if necessary. Moreover, regulatory reporting standards should be improved. Our search for honey bee endpoints from the registration process proved to be complicated. We partly received contrasting information from several sources. A solution for this problem would be the creation of a transparent and publicly available database of regulatory data. Those data could be then complemented by non-regulatory study results to further not only the open science idea but also establish a more transparent regulation process.
Despite only rarely providing additional safety for lower tier risk assessment it should be noted that the proposed test species may be more valuable surrogates in more realistic experimental setups in higher tier risk assessment. Due to their ecological differences to the honey bee, populations of *O. bicornis*/*O. cornuta* and *B. terrestris* may react quite differently in (semi-)field studies. Such divergent effects have been shown in a Swedish field study where clothianidin/beta-cyfluthrin treatment of oilseed rape had no detectable adverse effects on honey bee colonies, yet substantial impact on *O. bicornis*' and *B. terrestris*' population development (Rundlöf et al., 2015). Therefore, they are good representatives to measure ecological impact of pesticides on solitary and bumble bees in large field studies such as Peters et al. (2016) and Sterk et al. (2016).

C.6 CONCLUSION

For the majority of substances we tested, the honey bee was more sensitive than *O. bicornis*. We, therefore, agree with Heard et al. (2017) that *A. mellifera* is a sufficient proxy for other bee species in laboratory acute mortality testing as long as an appropriate assessment factor is applied. Dividing the honey bee endpoint by a factor of 10 proved to be protective for *O. bicornis* for 87% of all tested insecticides. There might be exceptions (e.g. neonicotinoids) where this assessment factor needs to be increased. In our dataset, *O. bicornis* was at most 18 times more sensitive than the honey bee. However, an assessment factor should be carefully chosen after consulting a comprehensive bee acute toxicity database. Furthermore, it is still necessary to investigate less well-known issues such as effects of pesticides mixtures (Robinson et al., 2017; Sgolastra et al., 2016), prolonged pesticides exposure (Heard et al., 2017) or effects of pesticide adjuvants (Fine et al., 2017) on wild and managed bee species.

Our study provides further evidence that *O. bicornis* is rarely an adequate surrogate species to improve lower tier risk assessment (Uhl et al., 2016). Unnecessary acute studies with non-sensitive species should not be conducted. Only sensitive species should be chosen as additional surrogates to reduce overall uncertainty. However, we agree that the proposed test species can be appropriate in higher tier risk assessment. In complex field settings, ecological differences between the honey bee, bumble bees and solitary bees are more relevant (Gradish et al., 2019; Rundlöf et al., 2015; Sgolastra et al., 2019). Therefore, such realistic experiments are better suited to evaluate the overall impact of pesticides on bee species. Consequently, we believe that (semi-)field data should be relied upon to a greater extent than laboratory results in bee risk assessment.

C.7 ACKNOWLEDGMENTS

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C.8 AUTHOR CONTRIBUTIONS

Conceptualisation PU CAB.
Data curation PU.
Formal analysis PU RSS OA.
Funding acquisition PU CAB.
Investigation RSS OA PU.
Methodology PU RSS OA.
Project administration PU CAB.
Resources PU RSS OA CAB.
Software PU RSS OA.
Supervision PU CAB.
Validation PU.
Visualisation PU.
Writing - original draft PU.
Writing - review & editing PU RSS OA CAB.

C.9 REFERENCES

Alan Wood (2018). *Compendium of Pesticide Common Names*. URL: http://www.alanwood.net/pesticides/.

Arena, M. and F. Sgolastra (2014). "A meta-analysis comparing the sensitivity of bees to pesticides". *Ecotoxicology* 23 (3), 324–334.

Bonmatin, J.-M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, Mitchell, E A D, D. A. Noome, N. Simon-Delso, and A. Tapparo (2015). "Environmental fate and exposure; neonicotinoids and fipronil". *Environmental science and pollution research international* 22 (1), 35–67.

Botías, C., A. David, J. Horwood, A. Abdul-Sada, E. Nicholls, E. Hill, and D. Goulson (2015). "Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees". *Environmental science & technology* 49 (21), 12731–12740.

Chauzat, M.-P., A.-C. Martel, N. Cougoule, P. Porta, J. Lachaize, S. Zeggane, M. Aubert, P. Carpentier, and J.-P. Faucon (2011). "An assessment of honeybee colony matrices, Apis mellifera (Hymenoptera: Apidae) to monitor pesticide presence in continental France". *Environmental Toxicology and Chemistry* 30 (1), 103–111.

Dmochowska-Ślęzak, K., K. Giejdasz, M. Fliszkiewicz, and K. Żółtowska (2015). "Variations in antioxidant defense during the development of the solitary bee Osmia bicornis". *Apidologie* 46 (4), 432–444.

EFSA (2013). "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)". *EFSA Journal* 11 (7), 3295.

Elias-Neto, M., A. L. O. Nascimento, A. M. Bonetti, F. S. Nascimento, S. Mateus, C. A. Garófalo, and M. M. G. Bitondi (2014). "Heterochrony of cuticular differentiation in eusocial corbiculate bees". *Apidologie* 45 (4), 397–408.

European Commission (2014). *Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of flupyradifurone in Regulation (EU) 1107/2009*.

Fine, J. D., D. L. Cox-Foster, and C. A. Mullin (2017). "An Inert Pesticide Adjuvant Synergizes Viral Pathogenicity and Mortality in Honey Bee Larvae". *Scientific Reports* 7, 40499.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray (2015). "Bee declines driven by combined stress from parasites, pesticides, and lack of flowers". *Science* 347 (6229), 1255957.

Gradish, A. E., J. van der Steen, C. D. Scott-Dupree, A. R. Cabrera, G. C. Cutler, D. Goulson, O. Klein, D. M. Lehmann, J. Lückmann, B. O'Neill, N. E. Raine, B. Sharma,

and H. Thompson (2019). "Comparison of Pesticide Exposure in Honey Bees (Hymenoptera: Apidae) and Bumble Bees (Hymenoptera: Apidae): Implications for Risk Assessments". *Environmental Entomology* 48 (1), 12–21.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. de Kroon (2017). "More than 75 percent decline over 27 years in total flying insect biomass in protected areas". *PLOS ONE* 12 (10), e0185809.

Heard, M. S., J. Baas, J.-. L. Dorne, E. Lahive, A. G. Robinson, A. Rortais, D. J. Spurgeon, C. Svendsen, and H. Hesketh (2017). "Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species?" *Science of The Total Environment* 578, 357–365.

Julius Kühn-Institut (2018). *PAPA - Panel Pflanzenschutzmittel-Anwendungen*. Last accessed 2018-03-19. URL: http://papa.julius-kuehn.de/.

Klein, A.-M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke (2007). "Importance of pollinators in changing landscapes for world crops". *Proceedings of the Royal Society B: Biological Sciences* 274 (1608), 303–313.

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp, and J. S. Pettis (2010). "High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health". *PloS one* 5 (3), e9754.

Ollerton, J. (2017). "Pollinator Diversity: Distribution, Ecological Function, and Conservation". *Annual Review of Ecology, Evolution, and Systematics* 48, 353–76.

Ollerton, J., R. Winfree, and S. Tarrant (2011). "How many flowering plants are pollinated by animals?" *Oikos* 120 (3), 321–326.

Peters, B., Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (Osmia bicornis)". *Ecotoxicology* 25 (9), 1679–1690.

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Version 3.4.4 (2018-03-15). Vienna, Austria: R Foundation for Statistical Computing. URL: https://www.R-project.org/.

Rader, R., I. Bartomeus, L. A. Garibaldi, Garratt, Michael P. D., B. G. Howlett, R. Winfree, S. A. Cunningham, M. M. Mayfield, A. D. Arthur, G. K. S. Andersson, R. Bommarco, C. Brittain, L. G. Carvalheiro, N. P. Chacoff, M. H. Entling, B. Foully, B. M. Freitas, B. Gemmill-Herren, J. Ghazoul, S. R. Griffin, C. L. Gross, L. Herbertsson, F. Herzog, J. Hipólito, S. Jaggar, F. Jauker, A.-M. Klein, D. Kleijn, S. Krishnan, C. Q. Lemos, S. A. M. Lindström, Y. Mandelik, V. M. Monteiro, W. Nelson, L. Nilsson, D. E. Pattemore, N. de O. Pereira, G. Pisanty, S. G. Potts, M. Reemer, M. Rundlöf, C. S. Sheffield, J. Scheper,

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C. Schüepp, H. G. Smith, D. A. Stanley, J. C. Stout, H. Szentgyörgyi, H. Taki, C. H. Vergara, B. F. Viana, and M. Woyciechowski (2015). "Non-bee insects are important contributors to global crop pollination". *Proceedings of the National Academy of Sciences* 113 (1), 146–151.

Ritz, C. and J. C. Streibig (2005). "Bioassay analysis using R". *Journal of Statistical Software* 12 (5). Version 3.0-1, 1–22. URL: http://CRAN.R-project.org/package=drc/.

Robinson, A., H. Hesketh, E. Lahive, A. A. Horton, C. Svendsen, A. Rortais, J. L. Dorne, J. Baas, M. S. Heard, and D. J. Spurgeon (2017). "Comparing bee species responses to chemical mixtures: Common response patterns?" *PLOS ONE* 12 (6), e0176289.

Roessink, I., van der Steen, Jozef J. M., and N. Hanewald (2016). *Solitary bee, Acute Contact Toxicity Test*. ICPPR workgroup non-Apis bees.

Rundlöf, M., Andersson, Georg K S, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone, and H. G. Smith (2015). "Seed coating with a neonicotinoid insecticide negatively affects wild bees". *Nature* 521, 77–80.

Sánchez-Bayo, F. and K. A. Wyckhuys (2019). "Worldwide decline of the entomofauna: A review of its drivers". *Biological Conservation* 232, 8–27.

SANCO (2002). *Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC*. European Commission Health & Consumer Protection Directorate-General and Directorate E-Food Safety, plant health animal health and welfare and international questions and E1 - Plant health.

Sattler, C., H. Kächele, and G. Verch (2007). "Assessing the intensity of pesticide use in agriculture". *Agriculture, Ecosystems & Environment* 119 (3-4), 299–304.

Sgolastra, F., S. Hinarejos, T. L. Pitts-Singer, N. K. Boyle, T. Joseph, J. Lūckmann, N. E. Raine, R. Singh, N. M. Williams, and J. Bosch (2019). "Pesticide Exposure Assessment Paradigm for Solitary Bees". *Environmental Entomology* 48 (1), 22–35.

Sgolastra, F., P. Medrzycki, L. Bortolotti, M. T. Renzi, S. Tosi, G. Bogo, D. Teper, C. Porrini, R. Molowny-Horas, and J. Bosch (2016). "Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species". *Pest Management Science* 73 (6), 1236–1243.

Słowińska, M., J. Nynca, J. Wilde, B. Bąk, M. Siuda, and A. Ciereszko (2015). "Total antioxidant capacity of honeybee haemolymph in relation to age and exposure to pesticide, and comparison to antioxidant capacity of seminal plasma: Apidologie". *Apidologie* 47 (2), 227–236.

Smirle, M. J. and M. L. Winston (1988). "Detoxifying enzyme activity in worker honey bees: An adaptation for foraging in contaminated ecosystems". *Canadian Journal of Zoology* 66 (9), 1938–1942.

Sterk, G., B. Peters, Z. Gao, and U. Zumkier (2016). "Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (Bombus terrestris)". *Ecotoxicology* 25 (9), 1666–1678.

Uhl, P., O. Awanbor, R. S. Schulz, and C. A. Brühl (2018). "Raw data - Ecotoxicological tests with Osmia bicornis and 16 insecticides". *figshare. Fileset*. URL: https://figshare.com/articles/Raw_data_-_Ecotox_tests_with_16_insecticides/6143945/9.

Uhl, P., L. A. Franke, C. Rehberg, C. Wollmann, P. Stahlschmidt, L. Jeker, and C. A. Brühl (2016). "Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment". *Scientific Reports* 6, 34439.

van der Sluijs P., J., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D. W. Gibbons, C. Giorio, V. Girolami, Van der Sluijs, J P, Bijleveld van Lexmond, M F I J, J.-M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. McField, P. Mineau, Mitchell, E. A. D., C. A. Morrissey, D. A. Noome, L. Pisa, J. Settele, N. Simon-Delso, J. D. Stark, A. Tapparo, H. van Dyck, J. van Praagh, P. R. Whitehorn, and M. Wiemers (2015). "Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning". *Environmental Science and Pollution Research* 22 (1), 1007.

Wood, T. and D. Goulson (2017). *The Environmental Risks of neonicotinoid pesticides: a review of the evidence post-2013*. Greenpeace France.

C

C.S1 SUPPORTING INFORMATION

Table C.S1.: Overview of all tested insecticides and test dates. For a detailed account of raw data from all tests see Uhl et al. (2018).

Study code	Insecticide (a.i.)	Product	Application date
Ro_Ob_ALPHA_1	alpha-cypermethrin	FASTAC® SC	2016-07-08
OA_Ob_BCYF	beta-cyfluthrin	Bulldock®	2017-07-30
OA_Ob_DEL	deltamethrin	Decis® Forte	2017-07-30
Ro_Ob_ETOFEN_1	etofenprox	Trebon® 30 EC	2016-07-08
Ro_Ob_LAMBDA_1	lambda-cyhalothrin	Karate® Zeon	2016-05-28
OA_Ob_ZCYP	zeta-cypermethrin	Fury® 10 EW	2017-07-30
OA_Ob_ACE	acetamiprid	Mospilan® SG	2017-08-06
Ro_Ob_IMI_1	imidacloprid	Confidor® WG 70	2016-05-28
Ro_Ob_THIA_1	thiacloprid	Calypso®	2016-08-03
CW_Ob_DIM	dimethoate	PERFEKTHION®	2015-05-21
OA_Ob_DIM	dimethoate	PERFEKTHION®	2017-06-08
OA_Ob_CHL	chlorpyrifos	Pyrinex®	2017-08-06
Ro_Ob_CHLORAN_1	chlorantraniliprole	Coragen®	2016-08-04
Ro_Ob_FLUPY_1	flupyradifurone	Sivanto® SL 200 G	2016-08-04
OA_Ob_IND	indoxacarb	AVAUNT® 150 EC	2017-06-29
Ro_Ob_PIRI_1	pirimicarb	Pirimor®	2016-07-08
Ro_Ob_SPINO_2	spinosad	SpinTor®	2016-08-03

C.S1.1 *Data collection of regulatory honey bee endpoints*

Honey bee acute contact LD50 values were obtained from registration documents which included EC review reports EFSA conclusion and EC rapporteur state draft/renewal assessment reports (DAR/RAR). If data were avaiblable from DAR/RAR they were taken from these documents. If otherwise EC review reports and EFSA conclusion were consulted (Table C.S2). In two cases, values could not be procured from publicly available documentation but were provided in a personal communication from the German Environment Agency (UBA). Within these regulatory documents, formulated products are often called by code names. We matched those code names to the commercial names of the products to the best of our knowledge. In cases where honey bee endpoints were only available on a per formulated product basis, those values were converted to per active ingredient basis using the density at 20°C from safety data sheets. To avoid possible mistakes and validate the collected data, all endpoints were counterchecked with information that was provided by German national authorities, EFSA and manufacturers (Table C.S3).

Table C.S2.: Data sources of honey bee acute endpoints for all tested insecticides.

Insecticide (a.i.)	Product	Data source	Comment
acetamiprid	Mospilan® SG	RAR (2015)	
alpha-cypermethrin	FASTAC® SC	personal communication UBA	
beta-cyfluthrin	Bulldock®	RAR (2017)	modelled from 48 h mean mortality data
chlorantraniliprole	Coragen®	DAR (2008)	
chlorpyrifos	Pyrinex®	RAR (2017)	
deltamethrin	Decis® Forte	personal communication UBA	validated by Mark Miles (Bayer Crop Science)
dimethoate	PERFEKTHION®	RAR (2017)	
etofenprox	Trebon® 30 EC	NA	
flupyradifurone	Sivanto® SL 200 G	DAR (2014)	
lambda-cyhalothrin	Karate® Zeon	RAR (2013)	
imidacloprid	Confidor® WG 70	DAR (2005)	
indoxacarb	AVAUNT® 150 EC	RAR (2016)	
pirimicarb	Pirimor®	RAR (2017)	comparable value to Pirimor® according to RAR
spinosad	SpinTor®	RAR (2017)	
thiacloprid	Calypso®	EC review report (2004)	validated by Mark Miles (Bayer Crop Science)
zeta-cypermethrin	Fury® 10 EW	DAR (2006)	

Table C.S3.: Different organisations that aided with data collection and contact at the respective institutions.

Organisation	Contact
German Environment Agency (UBA)	Dirk Süßenbach
German Federal Office of Consumer Protection and Food Safety (BVL)	Rolf Forster
European Food Safety Authority (EFSA)	Csaba Szentes
Bayer Crop Science	Mark Miles
Dow AgroSciences	Anne Alix
Syngenta	Robert Spatz

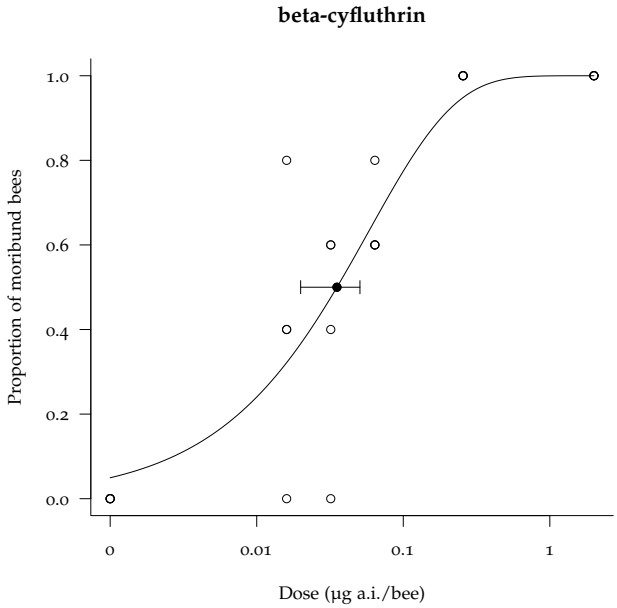


Figure C.S1.: Dose-response curve from *O. bicornis* 48h contact toxicity test with beta-cyfluthrin. Study code: OA_Ob_BCYF.

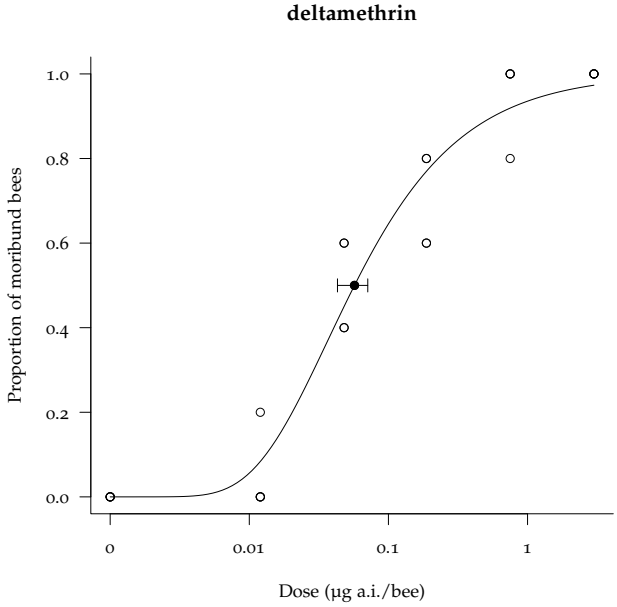


Figure C.S2.: Dose-response curve from *O. bicornis* 48h contact toxicity test with deltamethrin. Study code: OA_Ob_DEL.

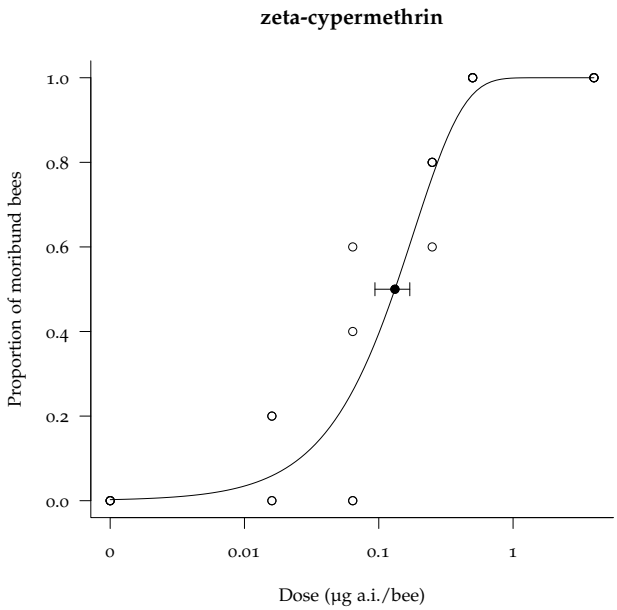


Figure C.S3.: Dose-response curve from *O. bicornis* 48h contact toxicity test with zeta-cypermethrin. Study code: OA_Ob_ZCYP.

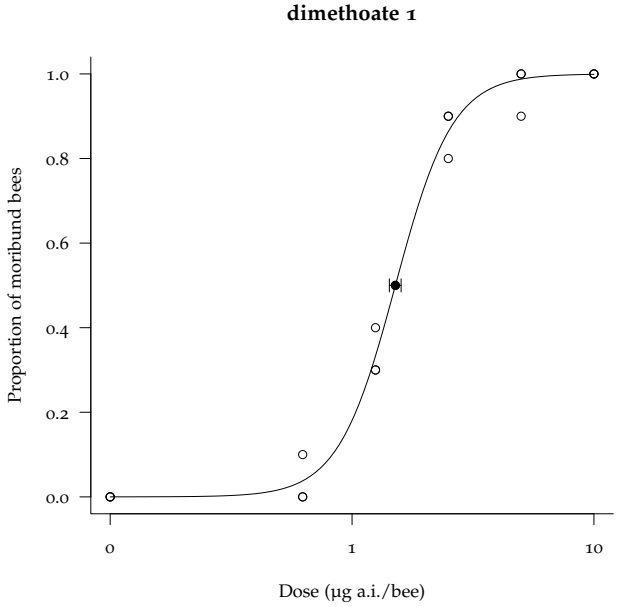


Figure C.S4.: Dose-response curve from *O. bicornis* 48h contact toxicity test with dimethoate. Study code: CW_Ob_DIM.

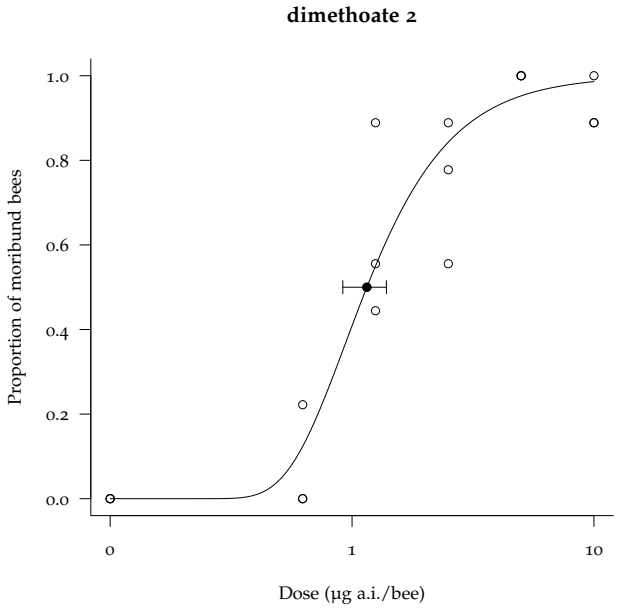


Figure C.S5.: Dose-response curve from *O. bicornis* 48h contact toxicity test with dimethoate. Study code: OA_Ob_DIM.

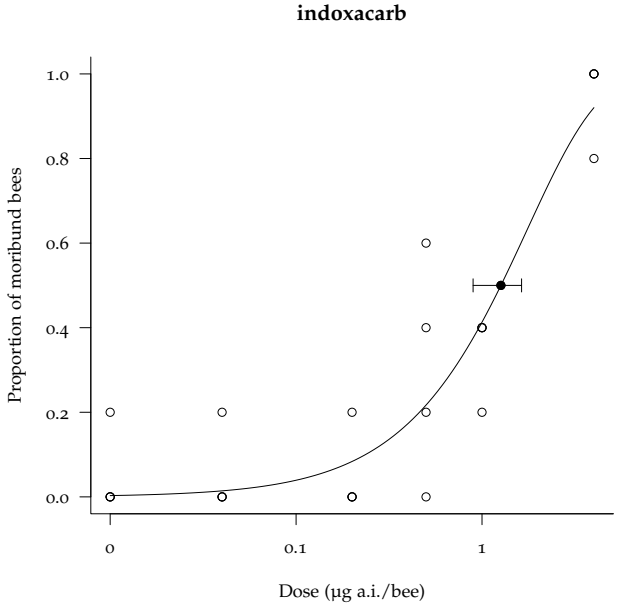
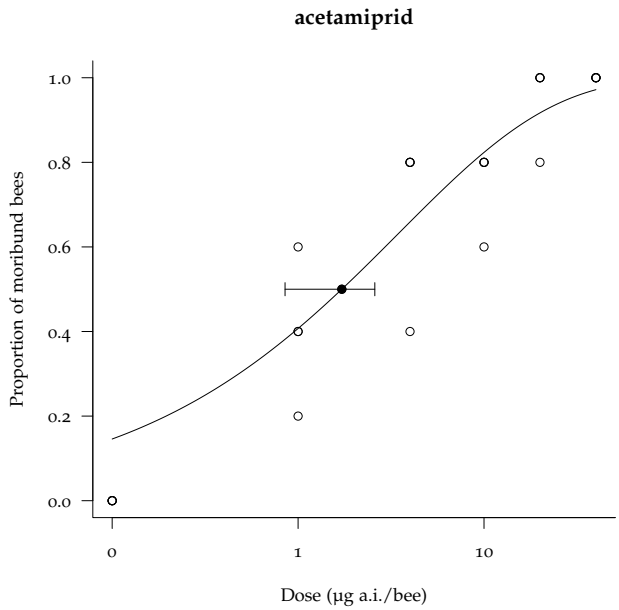


Figure C.S6.: Dose-response curve from *O. bicornis* 48h contact toxicity test with indoxacarb. Study code: OA_Ob_IND.



C

Figure C.S7.: Dose-response curve from *O. bicornis* 48h contact toxicity test with acetamiprid. Study code: OA_Ob_ACE.

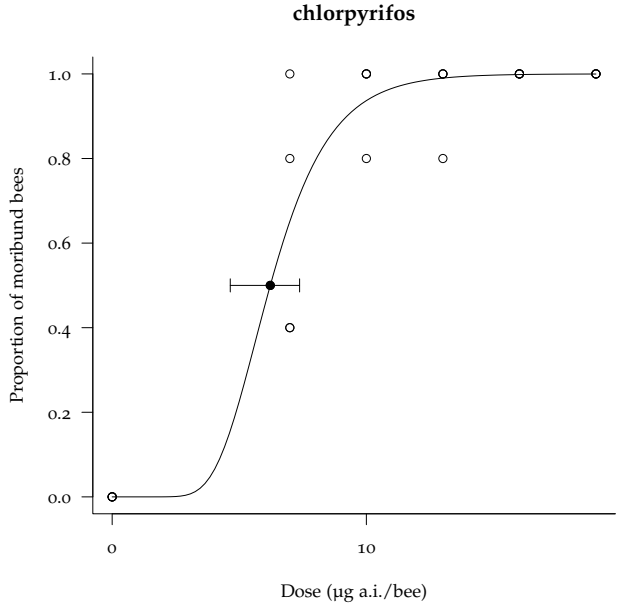


Figure C.S8.: Dose-response curve from *O. bicornis* 48h contact toxicity test with chlorpyrifos. Study code: OA_Ob_CHL.

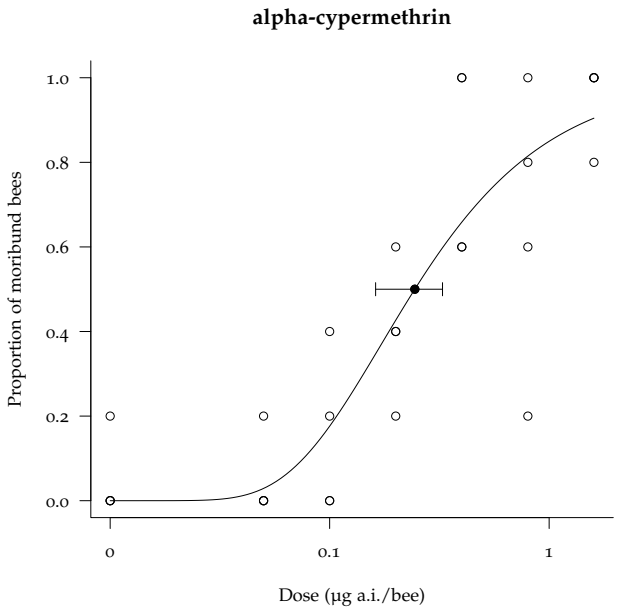


Figure C.S9.: Dose-response curve from *O. bicornis* 48h contact toxicity test with alpha-cypermethrin. Study code: Ro_Ob_ALPHA_1.

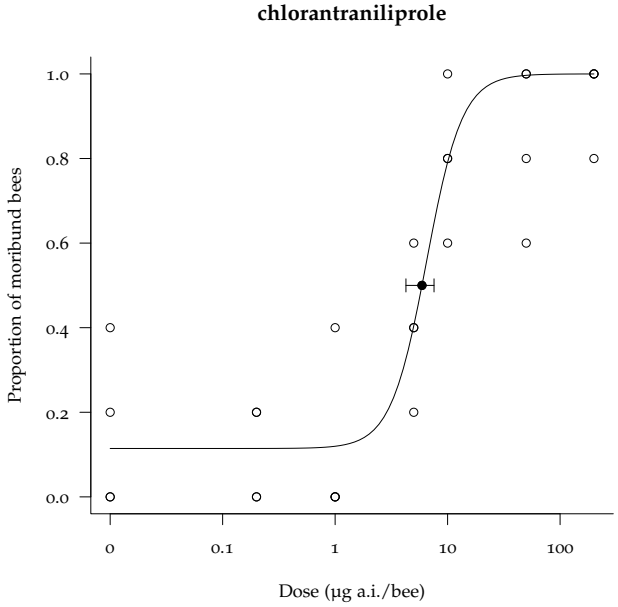


Figure C.S10.: Dose-response curve from *O. bicornis* 48h contact toxicity test with chlorantraniliprole. Study code: Ro_Ob_CHLORAN_1.

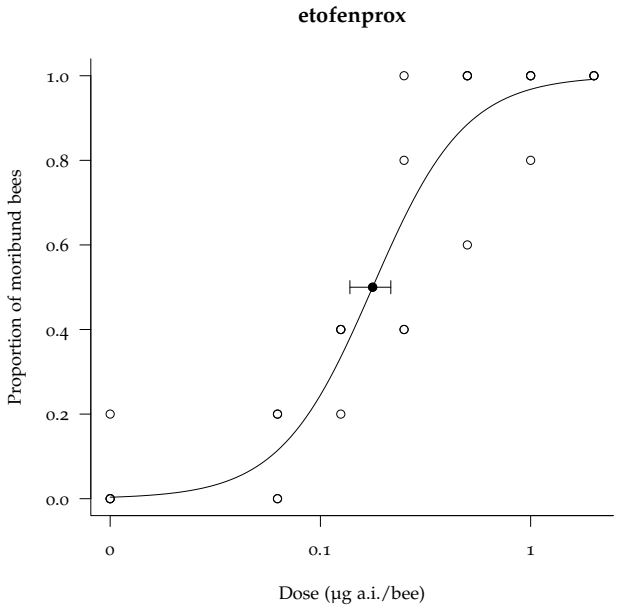


Figure C.S11.: Dose-response curve from *O. bicornis* 48h contact toxicity test with etofenprox. Study code: Ro_Ob_ETOFEN_1.

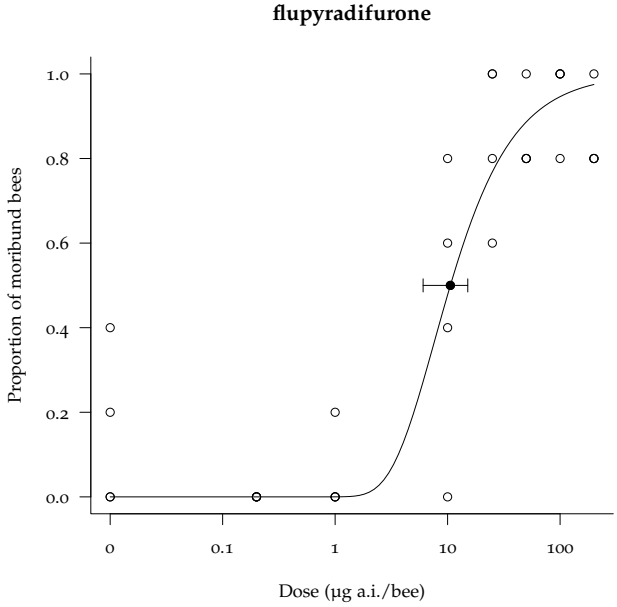


Figure C.S12.: Dose-response curve from *O. bicornis* 48h contact toxicity test with flupyradifurone. Study code: Ro_Ob_FLUPY_1.

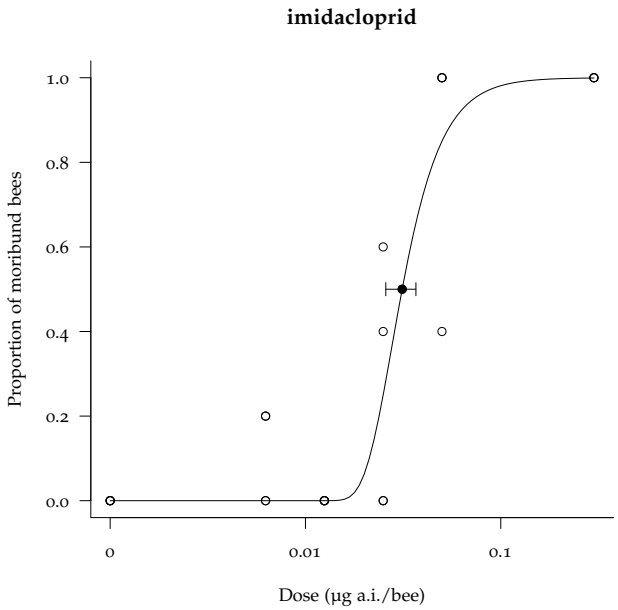


Figure C.S13.: Dose-response curve from *O. bicornis* 48h contact toxicity test with imidacloprid. Study code: Ro_Ob_IMI_1.

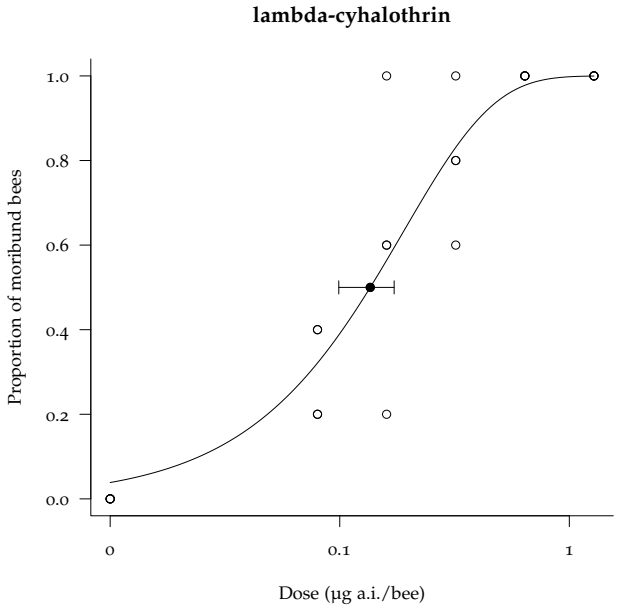


Figure C.S14.: Dose-response curve from *O. bicornis* 48h contact toxicity test with lambda-cyhalothrin. Study code: Ro_Ob_LAMBDA_1.

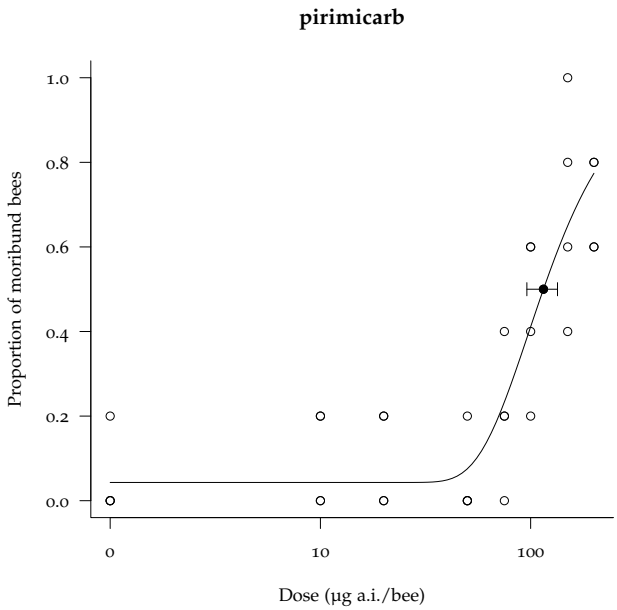


Figure C.S15.: Dose-response curve from *O. bicornis* 48h contact toxicity test with pirimicarb. Study code: Ro_Ob_PIRI_1.

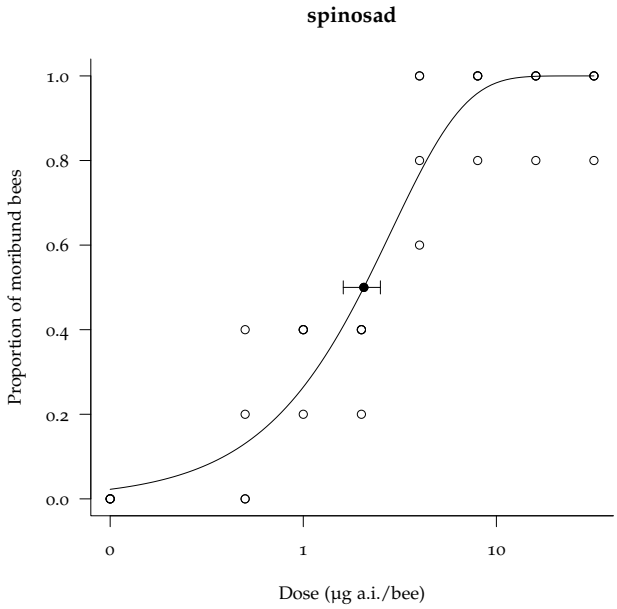


Figure C.S16.: Dose-response curve from *O. bicornis* 48h contact toxicity test with spinosad. Study code: Ro_Ob_SPINO_2.

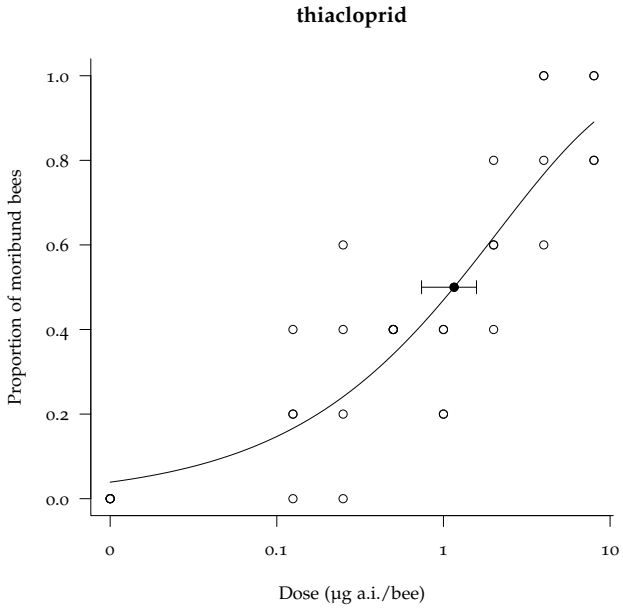


Figure C.S17.: Dose-response curve from *O. bicornis* 48h contact toxicity test with thiacloprid. Study code: Ro_Ob_THIA_1.

C.S1.2 *ICPPR solitary bee acute contact toxicity test protocol*

Solitary bee, Acute Contact Toxicity Test
Version: March 2016

ICPPR workgroup non-Apis bees
Eds. Ivo Roessink, Jozef J.M. van der Steen, Nicole Hanewald

INTRODUCTION

1. This test guideline is a laboratory test method, designed to assess the acute contact toxicity of pesticides and other chemicals to adult solitary bees. It is based principally on the OECD guidelines for the testing of chemicals 214 [1] and Methods to determine the acute oral and contact LD_{50} of pesticides for bumble bees (*Bombus terrestris* L.)[2] and results of the discussions regarding ring testing solitary bees during the meeting of the ICPPR non-Apis testing working group, March 6th, 2014 in Niefern, Germany, February 19th, 2015 in Limburgerhof, Germany and February 29th, 2016 in Braunschweig, Germany.

INITIAL CONSIDERATIONS

2. In the assessment and evaluation of toxic characteristics of substances, determination of acute contact toxicity in solitary bees may be required, e.g. when exposure of these bees to a given chemical is likely. The acute contact toxicity test is carried out to determine the inherent toxicity of pesticides and other chemicals. The results of this test should be used to define the need for further evaluation. In particular, this method can be used in step-wise programmes for evaluating the hazards of pesticides to bees, based on sequential progression from laboratory toxicity tests to semi-field and field experiments [1]. Pesticides can be tested as either active ingredients (a.i.) or as formulated products.
3. The effect of pesticides on solitary bees depends on the body size of the test subject. As solitary bee workers between different species, within one 'colony' of one species and between 'colonies' can have significantly different sizes and related weights, they have a different surface to volume ratio. This affects the susceptibility of these individuals to plant protection products. Smaller bees have a greater surface to volume ratio and have less weight [3][4]. For practical reasons not the surface to volume ratio of solitary bees is assessed but instead the bees are weighed. In this way the LD_{50} can be calculated as µg PPP bee^{-1} and µg PPP gram bee^{-1} which will make the evaluation of the LD_{50} for non-apis bees more consistent.
   To avoid great variation in susceptibility in one test, solitary bees of an average size / weight must be selected and tested.
4. The method is tested on *Osmia* sp. and may be adjusted for other solitary bees.
5. Definitions used are given in the Annex.

PRINCIPLE OF THE TEST

6. Adult female solitary bees are exposed to a range of doses of the test substance dissolved in appropriate carrier, by direct application to the dorsal side of thorax (droplets). The test duration is at max 96 h. If the mortality rate is increasing between 24 and 48h whilst control mortality remains at an accepted level, i.e. <10%, it is appropriate to extend the duration of the test to a maximum of 96 h. Mortality is recorded daily and compared with control values. The results are analysed in order to calculate the LD_{50} at 24, 48h, 72h, and 96h (see Annex for definitions).

Note that for this ring test the full test duration of 96h is required.

VALIDITY OF THE TEST

7. For a test to be valid the following conditions apply:
   - The average mortality for the total number of controls must not exceed 10 % at the end of the test.
   - The LD_{50} of the toxic standard Dimethoate 40% meets the specified range. As solitary bees differ more in size / weight than honeybees, a larger variation in LD_{50} values can be observed. For *Osmia* the LD_{50} of Dimethoate approximates 1.5 µg a.i./bee. For other solitary bee species the LD_{50} may be significantly different.

Note that depending on the results of the ring test the control mortality criterion might be changed to 15 to 20% in accordance to other non-target arthropod testing.

DESCRIPTION OF THE METHOD

Collection of bees

8. Newly emerged female bees (preferably of modal size) are selected for the test. Cocoons containing females are generally of larger size than those containing males. Do not manipulate the cocoons in order to facilitate hatching and/or sexing of the bees (i.e. do not open or cut prior to hatching). When hatching proper sized cocoons in a flight cage, any males still present will emerge earlier than the females and should be removed from the cage. Emerged females should be non-mated and meconium-free and are to be stored in the refrigerator at 5 ºC until enough bees have been collected to populate the test. Note that this can take up to 4 days since 30 bees per treatment group are required.

Number of bees per treatment group

9. Thirty (30) non-mated meconium-free solitary bee females.

Number of doses

10. Per test the bees are treated with 5 doses of the test substance: two between the presumed LD_{100} and LD_{50}, one at the presumed LD50 and two between the presumed LD_{50} and LD_0, a negative control (in case a solvent is used) and at least three concentrations of the positive control.

Note that in the current ring test, dimethoate (positive control substance when testing other chemicals) is tested so no positive control is required in the current ring test.

Number of replicates

11. An acute contact LD_{50} consists of three [5] replicates in parallel to be executed as 3 x 10 bees from the same geographic pool/supplier. However, good results have also been obtained by participants using 6x5 bees. Both designs are considered adequate. At all times the origin, normal flight period in the year and wintering conditions of the cocoons of the bees used in the test must be specified in the raw data.

Note that in the current test only group housing i.e. 3x10 or 6x5 bees per replicate will be tested.

Test cages

12. Easy to clean and well-ventilated cages are used. Any appropriate material can be used, e.g. stainless steel, wire mesh, plastic, disposable plastic cages, et cetera. The size of test cages should be appropriate to the number of bees, i.e. providing adequate space and feeding opportunity (i.e. all individuals should have access to the sugar solution). This can be arranged by using bigger cages with multiple feeders or using less bees per cage, but increasing the number of cages per treatment level. In principle, however, groups of 10 bees per cage are tested. Provide cage enrichment like a piece of gauze and/or (filter)paper, since *Osmia* bees like to play around/have hiding places. Food should be available *ad libitum* and feeders should be placed on the ground of the test cage (*Figure 1*).

Figure 1. Some examples of test cages with feeders positioned on the ground.

Note that feeders positioned on the ground appear to work better than suspended feeders. Hence in the ring test, feeders need to be positioned on the ground. Feeders containing a reservoir with some kind of wick or cotton from which the Osmia feed give good results, but good results are also obtained with feeders equipped with a flower petal. Participants are asked to fine-tune their choice feeders with the coordinator before testing so that a balanced ring test using both feeder types can be performed.

Preparations of bees

13. The collected bees are anaesthetized by chilling by putting them for at least 30 minutes at 4-5 °C or using an ice bath. Cold storage can be prolonged but the amount/duration of anaesthetic used and times of exposure should be minimised. Note that using CO_2 for anaesthesia can result in mortality for *Osmia* species and should therefore be avoided. All bees are weighed before application of the test substance to determine the average weight, standard deviation and min-max weight of animals used in the test. Moribund bees should be rejected and replaced by healthy bees before starting the test.

Preparation of doses

14. All test item doses will be dissolved in water. Add Triton X-100 (0.1%) as surfactant or any other low toxic surfactant which equally distributes the droplet on the bee body.

Note that in this ring test Triton X should be used as a surfactant. When testing formulated products, the test substance is dissolved in water.

Housing and keeping of the solitary bees

15. The bees are kept under light: dark conditions (16:8h) in a climate room at a temperature of 22 ± 2°C and a relative humidity of 60 ±10%. During the test the bees have access to sucrose solution 50% (w/w) ad libitum.
16. Per test cage ten bees will be housed (3x10). If using the 6x5 option, five bees per test cage will be used.

Handling and feeding conditions

17. Handling procedures, including treatment and observations may be conducted under (day)light.

Test item ring test

18. The ring test will be performed using Dimethoate 40% (e.g. Dimethoate 400 EC).

Test concentrations ring test

19. The proposed test-range for the ring test is: control, 0.5, 1.0, 2.0, 4.0, 8.0 µg active ingredient / bee

Note that this range has been slightly adapted, compared to the range used in 2015.

Administration of doses

20. Anaesthetized bees are individually treated by topical application. The bees are randomly assigned to the different test substance doses and controls. A volume of 2 µL of solution containing the test substance at the suitable dose should be applied with a micro-applicator to the dorsal side of the thorax of each bee between the neck and wing base. After application, the bees are allocated to test cages in groups of 10 bees and supplied with sucrose solutions 50% ad libitum.

Residue analyses test substance

21. At minimum, the stock solution, the lowest, and the highest test concentrations are analysed for Dimethoate levels. Till analysis, the solution of the test substance is stored in the freezer (-18 ºC).

PROCEDURE

Test and control groups

22. The number of doses and replicates tested should meet the statistical requirements for determination of LD_{50} with 95% confidence limits. Normally, five doses in a geometric series, with a factor not exceeding 2.2, and covering the range for LD_{50}, are required for the test. However, the number of doses has to be determined in relation to the slope of the toxicity curve (dose versus mortality) and with consideration taken to the statistical method which is chosen for analysis of the results. A range-finding test enables the choice of the appropriate doses (not applicable for the current dimethoate ring test).

Note that participants are asked to send in their raw data in the distributed format so that all data can be processed in an uniform manner.

23. A minimum of three replicate test groups, each of 10 bees, should be dosed with each test concentration (not applicable for the current dimethoate ring test).

24. A minimum of three replicate cages, each containing 10 bees, should be used with each test dose. Note that when 6 replicates are used, each can contain 5 bees.

Exposure

Test conditions

25. The bees should be held under light: dark conditions (16:8h) in a climate room at a temperature of 22 ± 2°C and a relative humidity of 60 ±10%. During the test the bees have access to sucrose solution 50% (w/w) ad libitum.

Duration

26. The duration of the test is 96 h.

Observations

27. Mortality is recorded at 4 h after dosing and thereafter at 24h, 48 h, 72 h, and 96 h. All abnormal behavioural effects observed during the testing period should be recorded.

DATA AND REPORTING

Data

28. Data should be summarised in tabular form, showing for each treatment group, as well as control and toxic standard groups, the number of bees used, mortality at each observation time and number of bees with adverse behaviour. Analyse the mortality data by appropriate statistical methods (e.g. probit analysis, moving-average, binomial probability) [5, 6]. Plot dose-response curves at each recommended observation time (i.e. 24h, 48h, 72h, and 96h) and calculate the slopes of the curves and the median lethal doses (LD_{50}) with 95% confidence limits. Corrections for control mortality could be made using Abbott´s correction or Scheider Orelli [7, 8]. LD_{50} should be expressed in µg of test substance per bee and µg of test substance per gram bee.

Note that participants are asked to send in their raw data in the distributed format so that all data can be processed in an uniform manner.

Test report

29. The test report must include the following information:

Test substance:

- physical nature and relevant physical-chemical properties (e.g. stability in water, vapour pressure);
- chemical identification data, including structural formula, purity (i.e. for pesticides, the identity and concentration of active ingredient (s)).

Test bees:

- scientific name, race, approximate age (in weeks), collection method, date of collection;
- all relevant information on colonies used for collection of test bees, including health, any adult disease, any pre-treatment, etc.

Test conditions:

- temperature and relative humidity of experimental room;
- housing conditions including type, size and material of cages;
- methods of administration of test substance, e.g. carrier solvent used, volume of test solution applied, anaesthetics used;
- test design, e.g. number and test doses used, number of controls; for each test dose and control, number of replicate cages and number of bees per cage;
- date of test.

Results:

- results of preliminary range-finding study if performed;
- raw data: mortality at each concentration tested at each observation time;
- graph of the dose-response curves at the end of the test;
- LD_{50} values, with 95% confidence limits, at each recommended observation time, for test substance and toxic standard;
- statistical procedures used for determining LD_{50};
- mortality in controls;
- other biological effects observed and any abnormal responses of the bees;
- any deviation from the Test Guideline procedures and any other relevant information.

Note that participants are asked to send in their raw data in the distributed format so that all data can be processed in an uniform manner.

LITERATURE

1. OECD, *Honeybees - acute oral toxicity test*, in *OECD Guidelines for the testing of chemicals No. 213*1998, Organization of Economic Co-operation and Development: Paris.
2. Steen. J.J.M. van der, C. Gretenkord, and H. Schaefer. *Methods to determine the acute oral and contact LD50 of pesticides for bumble bees (Bombus terrestris L.)* in *Proceedings ICPBR 6th Symposium on the Hazard of Pesticides to Bees* 1996. Braunschweig, Germany.
3. Johansen, C.A., et al., *Pesticides and Bees.* Environmental Entomology, 1983. **12**(5): p. 1513-1518.
4. Steen, J.J.M.v.d. *The effect of the size of the solitary bee (Bombus terrestris L.) on the susceptibility to the pesticide dimethoate 40%* in *Proceedings 7th International Symposium of the ICPBR Bee Protection Group co-organised by INRA and ACTA 1999:213-216. INRA Editions RD 10-78026 Versailles Cedex, France. ISBN 2-7380-0966-2*. 2001.
5. Finney, D.J., *Probit Analysis. 3rd ed., Cambridge, London and New York.* 1971, Cambridge, London and New York. 333.
6. Litchfield, J.T. and F. Wilcoxon, *A simplified method of evaluating dose-effect experiments.* Journal of Pharmacology and Experimental Therapeutics, 1949. **96**(2): p. 99-113.
7. Schneider - Orelli, O., *Entomologisches Praktikum : Einfuehrung in die land- und forstwirtschaftliche Insektenkunde*. 1947, Aarau: Sauerlaender.
8. Abbott, W.S., *A method for computing the effectiveness of an insecticide.* Jour. Econ. Entomol., 1925. **18**: p. 265-267.

ANNEX
DEFINITIONS

Acute contact toxicity is the adverse effects occurring within a maximum period of 96 h of a topical application of a single dose of a substance.

Dose is the amount of test substance applied. Dose is expressed as mass (µg) of test substance per test animal (µg/bee).

LD_{50} (median lethal dose) contact, is a statistically derived single dose of a substance that can cause death in 50 per cent of animals when administered by contact. The LD_{50} value is given in µg of test substance per bee and per gram bee. For pesticides, the test substance may be either an active ingredient (a.i.) or a formulated product containing one or more than one active ingredient.

Mortality: an animal is recorded as dead when it is completely immobile.

Additionally to the LD_{50} the test duration time in which the LD_{50} is calculated, 24, 48, 72 or 96 h is presented.

C.S1.3 *References*

Uhl, P., O. Awanbor, R. S. Schulz, and C. A. Brühl (2018). "Raw data - Ecotoxicological tests with Osmia bicornis and 16 insecticides". *figshare. Fileset*. URL: https://figshare.com/articles/Raw_data_-_Ecotox_tests_with_16_insecticides/6143945/9.

C

D ACUTE TOXICITY DATA OF ADDITIONAL BEE SPECIES

D.1 COMBINED DATA OF APPENDIX B AND UNPUBLISHED RESULTS

Table D.1.: Sensitivity of several European bee species to the dimethoate formulation Perfekthion®. Table adapted from Appendix B. Species were sorted by LD50 value (µg a.i./bee) in ascending order. Where multiple LD50 values were available they were summarised using the geometric mean. Fresh and dry weight-normalised values were calculated by dividing the LD50 by the respective weight. LD50 values for *A. mellifera*, *O. cornifrons* and *O. lignaria* were extracted from peer-reviewed literature (Appendix B Supporting Information Table B.S7). LD50 values for *A. gallica*, *B. lapidarius*, *C. hederae* ♂ and *L. politum* are unpublished results that were generated after the publication of Appendix B using an identical study design (master thesis, Schnetzer (2017)). Female or worker bees were tested if not otherwise specified.

Species	LD50	95% CI	Mean fresh weight	LD50 Fresh weight-normalised	95% CI	Mean dry weight	LD50 Dry weight-normalised	95% CI
	[µg a.i./bee]		[mg]	[µg a.i./g bee]		[mg]	[µg a.i./g bee]	
L. politum	0.02	0.01 – 0.03	3.6	6.35	3.45 – 9.25	NA	NA	NA – NA
O. cornifrons	0.09	0.02 – 0.20	NA	NA	NA – NA	22.2	4.05	0.90 – 9.01
L. malachurum	0.12	0.09 – 0.14	11.0	9.80	7.84 – 11.76	NA	NA	NA – NA
A. mellifera	0.18	NA – NA	99.7	1.82	NA – NA	17.0	10.66	NA – NA
C. hederae ♂	0.23	0.22 – 0.25	42.9	5.45	5.02 – 5.88	NA	NA	NA – NA
B. lapidarius	0.31	0.03 – 0.59	143.5	2.16	0.22 – 4.11	NA	NA	NA – NA
A. gallica	0.68	0.39 – 0.97	126.2	5.36	3.07 – 7.65	NA	NA	NA – NA
A. flavipes	0.73	0.07 – 1.39	47.3	15.44	1.57 – 29.31	21.6	33.78	3.44 – 64.11
C. hederae ♀	1.14	0.72 – 1.57	105.5	10.84	6.83 – 14.85	43.4	26.35	16.61 – 36.09
O. lignaria	1.21	1.05 – 1.57	92.3	13.11	11.38 – 17.01	29.4	41.16	35.71 – 53.40
O. bicornis ♂	1.71	1.37 – 2.04	37.7	45.27	36.31 – 54.22	17.6	96.90	77.73 – 116.07
O. bicornis ♀	4.29	3.72 – 4.91	93.6	45.89	39.80 – 52.47	30.4	141.46	122.68 – 161.73
B. terrestris	5.13	4.10 – 6.15	205.0	25.00	20.00 – 30.00	55.8	91.87	73.49 – 110.2

D.2 REFERENCES

Schnetzer, N. (2017). "Sensitivity of different wild bee species towards dimethoate and imidacloprid and potential pesticide exposure of bee communities in the agriculture landscape". Landau: Universität Koblenz-Landau.

PUBLICATIONS AND CONTRIBUTIONS

This cumulative dissertation includes three scientific publications:

Appendix A
Uhl, P. & Brühl, C. A. (2019). "The impact of pesticides on flower-visiting insects: A review with regard to European risk assessment". *Environmental Toxicology and Chemistry*, 38(11), 2355-2370, doi: 10.1002/etc.4572.

Contributions:
Philipp Uhl (90%): Conception, literature search, information synthesis, manuscript writing
Carsten A. Brühl (10%): Conception, manuscript editing

Appendix B
Uhl, P., Franke, L. A., Rehberg, C., Wollmann, C., Stahlschmidt, P., Jeker, L., & Brühl, C. A. (2016). "Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment". *Scientific Reports*, 6, 34439, doi: 10.1038/srep34439.

Contributions:
Philipp Uhl (65%): Experimental work, data analysis, result discussion, manuscript writing
Lea A. Franke (10%): Experimental work, data analysis, manuscript writing
Christina Rehberg (2.5%): Experimental work
Claudia Wollmann (2.5%): Manuscript writing
Peter Stahlschmidt (6%): Conception, experimental work, manuscript editing
Lukas Jeker (4%): Conception, manuscript editing
Carsten A. Brühl (10%): Conception, manuscript editing

Appendix C
Uhl, P., Awanbor, O., Schulz, R. S. & Brühl, C. A. (2019). "Is *Osmia bicornis* an adequate regulatory surrogate? Comparing its acute contact sensitivity to *Apis mellifera*". *PLOS ONE*, 14(8), e0201081, doi: 10.1371/journal.pone.0201081.

Contributions:
Philipp Uhl (75%): Conception, experimental work, data analysis, result discussion, manuscript writing
Osarobo Awanbor (5%): Experimental work, data analysis
Robert S. Schulz (10%): Experimental work, data analysis, manuscript editing
Carsten A. Brühl (10%): Conception, manuscript editing

This thesis also includes unpublished data from a master thesis that I co-supervised:

Appendix D
Schnetzer, N. (2017). "Sensitivity of different wild bee species towards dimethoate and imidacloprid and potential pesticide exposure of bee communities in the agriculture landscape". Master thesis. University of Koblenz-Landau.

In addition to the peer-reviewed articles, I wrote multiple chapters (1 to 6) of a technical report as part of a project financed by the German Environment Agency (UBA):

Bereswill, R., Krichbaum K., Meller, M., Schmidt, K., Brühl, C. A., **Uhl, P.**, and Topping, C. J. (2019). *Protection of wild pollinators in the pesticide risk assessment and management*. Final Report. Project No. (FKZ) 3715 64 409 0. Texte 54/2019. German Environment Agency. Free access at: https://www.umweltbundesamt.de/publikationen/protection-of-wild-pollinators-in-the-pesticide

TEACHING INVOLVEMENTS

During my PhD studies at the University Koblenz-Landau, Campus Landau, I co-supervised research projects in the course of several master theses. These studies are listed below and they were partly integrated into my PhD research. First supervisor of all of these theses was Dr. habil. Carsten Brühl.
In addition, I held several lectures for master students as part of a seminar on "Risk assessment and risk management" at the University of Koblenz-Landau.

Co-supervised projects:

Eschenbach, E. (2016): "Influence of pesticides on bumble bees in laboratory and semi-field design". Master thesis. University of Koblenz-Landau

Wollmann, C. (2016): "Ecology of the red mason bee *Osmia bicornis* under the influence of pesticide stress and food limitation". Master thesis. University of Koblenz-Landau

Schulz, R. S. (2016): "Potential exposure and acute effects of frequently used agricultural insecticides on the wild bee species *Osmia bicornis*". Master thesis. University of Koblenz-Landau

Schnetzer, N. (2017): "Sensitivity of different wild bee species towards dimethoate and imidacloprid and potential pesticide exposure of bee communities in the agriculture landscape". Master thesis. University of Koblenz-Landau

Awanbor, O. (2018): "Comparative Analysis of the Effect of commonly used Agricultural Pesticides on the European wild bee species *Osmia bicornis*; Investigation of susceptible routes of exposure". Master thesis. University of Koblenz-Landau

DECLARATION

I, the author of this work, declare that this thesis is my own, independent work and has not been submitted in any form for the award of another degree at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. The contributions of the co-authors of my peer-reviewed publications have been clearly indicated. I am aware that a violation of the aforementioned conditions can have legal consequences.

Landau, February 26, 2020

Philipp Uhl

Philipp Uhl

Environmental Scientist

honest
thorough
enthusiastic

* 29.01.1987
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Personal information

Date of birth	29.01.1987
Nationality	German

Education

2014 – 2020 **University of Koblenz-Landau**, Landau
Doctorate studies in Environmental Sciences (grade: 1.3)

Topic *Insecticide effects on wild bees and the general impact of pesticide use on European wild pollinators*

I investigated the effects of common insecticides on several wild bee species in laboratory experiments. I also planned a semi-field experiment to study the effects of a neonicotinoid insecticide on the red mason bee *Osmia bicornis* as part of a Deutsche Forschungsgemeinschaft (DFG) research proposal. Furthermore, I synthesized the available literature on the impact of pesticides on wild pollinators in a report for the German Environment Agency (R+D project FKZ 3715 64 409 0).

2007 – 2013 **University of Koblenz-Landau**, Landau
Diploma Environmental Sciences (grade: 1.4)

Topic Diploma thesis: *Sublethal Effects of Imidacloprid on Interactions in a Tritrophic System*

I studied the laboratory effects of sublethal doses of a neonicotinoid insecticide on a three-step food chain consisting of wild strawberry, wood cricket and nursery web spider.

1999 – 2006 **Carl-Friedrich-Gauss Gymnasium**, Frankfurt (Oder)
School with focus on natural sciences and mathematics

Work experience

2014 – 2019 **PhD student**, *University of Koblenz-Landau*, Landau
As part of the doctorate studies: Project planning and management, data collection and analysis, writing peer-reviewed scientific articles and technical reports, knowledge management and communication, supervision of bachelor and master students.

2011 **Intern**, *IES (Innovative Environmental Services) Ltd.*, Witterswil, Switzerland
3 month internship in terrestrial ecotoxicology. Field and laboratory studies under GLP, mainly honey bee tunnel and field studies.

2010 **Student assistant**, *University of Koblenz-Landau*, Landau
Image recognition of flowering Common buttercup (*Ranunculus acris*) and analysis of flowering area.

2006 – 2007 **Voluntary ecological year as civil service**, *Kompetenzzentrum Wasser Berlin gGmbH*, Berlin
Basic chemical analytic work in a sewage treatment plant for the SCST project (Sanitation Concepts for Seperate Treatment), assistant work in groundwater well service for pigadi GmbH.

Publications

2019 Uhl, P & Brühl, CA: The impact of pesticides on flower-visiting insects: A review with regard to european risk assessment. *Environmental Toxicology and Chemistry* 38(11): 2355–2370. doi: 10.1002/etc.4572

2019 Uhl P, Awanbor O, Schulz RS & Brühl CA: Is *Osmia bicornis* an adequate regulatory surrogate? Comparing its acute contact sensitivity to *Apis mellifera*. *PLOS ONE* 14(8): e0201081. doi: 10.1371/journal.pone.0201081

2019 Bereswill R, Krichbaum K, Meller M, Schmidt K, Brühl CA, Uhl P & Topping CJ: Protection of wild pollinators in the pesticide risk assessment and management. Final Report. Project No. (FKZ) 3715 64 409 0. Texte 54/2019. German Environment Agency. url: `https://www.umweltbundesamt.de/publikationen/protection-of-wild-pollinators-in-the-pesticide`

2016 Uhl P, Franke LA, Rehberg C, Wollmann C, Stahlschmidt P, Jeker L & Brühl CA: Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment. Scientific Reports 6: Article number: 34439. doi: 10.1038/srep34439

2015 Uhl P, Bucher R, Schäfer RB & Entling MH: Sublethal effects of imidacloprid on interactions in a tritrophic system of non-target species. Chemosphere 132: 152-158. doi: 10.1016/j.chemosphere.2015.03.027

Conference contributions

Talks

2017 Uhl P, Franke LA, Rehberg C, Schnetzer N, Schulz RS, Wollmann C, Stahlschmidt P, Jeker L & Brühl CA: Interspecific sensitivity of wild bee species and the relevance of the honey bee as a surrogate organism for pollinators. 27th annual SETAC Europe meeting (Society of Environmental Toxicology and Chemistry). Brussels, Belgium, 7-11 May 2017

2017 Uhl P, Bucher R, Schäfer RB & Entling MH: Trophic pesticide effects in terrestrial settings: A case study using a three non-target species model system. 6th SETAC Young Environmental Scientists Meeting. Stockholm, Sweden, 16-20 February 2017

Posters

2018 Uhl P, Bereswill R, Meller M, Süßenbach D, Topping CJ & Brühl CA: The impact of pesticides on flower-visiting insects: State of knowledge and research opportunities. 48th annual meeting of the Ecological Society of Germany, Austria and Switzerland (GfÖ). Vienna. Austria. 10-14 September 2018

2017 Uhl P, Bereswill R, Krichbaum K, Meller M, Süßenbach D, Topping CJ & Brühl CA: Effect and exposure assessment of flower-visiting insects in Europe: Progress, deficits and research opportunities. 22nd annual SETAC GLB meeting (Society of Environmental Toxicology and Chemistry, German Language Branch). Neustadt. Germany. 12-14 November 2017

2017 Uhl P, Bereswill R, Krichbaum K, Meller M, Schulz RS, Süßenbach D, Topping CJ & Brühl CA: Exposure of flower-visiting insects to pesticides in the European agricultural landscape. 27th annual SETAC Europe meeting (Society of Environmental Toxicology and Chemistry). Brussels, Belgium, 7-11 May 2017

Software

Basic	ArcGIS, Inkscape, GIMP, ImageJ, eCognition, CMS
Advanced	Linux, LibreOffice, Microsoft Windows, Microsoft Office, R, LaTeX, Ethovision

Languages

German	First language	
English	Advanced	*fluent*
French	Basic	*school knowledge*

COLOPHON

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