Assessment of the Vulnerability to Pesticide Exposures Across Bee Species

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Bee vulnerability to pesticide exposures

Assessment of the vulnerability to pesticide exposures across bee species

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Abstract: In many countries, the Western honey bee is used as surrogate in pesticide risk assessments for bees. However, uncertainty remains in the estimation of pesticide risk to non-*Apis* bees because their potential routes of exposure to pesticides, life histories, and

ecologies differ from honey bees. We applied the vulnerability concept in pesticide risk assessment to 10 bee species including the honey bee, two bumble bee species, and seven solitary bee species with different nesting strategies. The trait-based vulnerability considers the evaluation of a species both at the level of the organism (exposure and effect) and the population (recovery) that goes beyond the sensitivity of individuals to a toxicant assessed in standard laboratory toxicity studies by including effects on populations in the field. Based on expert judgement, each trait was classified by its relationship to the vulnerability to pesticide exposure, effects (intrinsic sensitivity), and population recovery. The results suggested that the non-*Apis* bees included in our approach are potentially more vulnerable to pesticides than the honey bee due to traits governing exposure and population recovery potential. Our analysis highlights many uncertainties related to the interaction between bee ecology and the potential exposures and population-level effects of pesticides, emphasizing the need for more research to identify suitable surrogate species for higher-tier bee risk assessments.

Introduction

Bees play an important role in crop pollination but can also be adversely affected by exposure to pesticides used in agricultural landscapes. To protect bees in agricultural systems, they are explicitly considered in risk assessments of pesticides (e.g., European

Food Safety Authority 2013; U.S. Environmental Protection Agency 2014; Australian Pesticides and Veterinary Medicines Authority 2017; Cham et al. 2017). At present, the Western honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) is used as a surrogate species to assess the risk of plant protection products to bees because its biology, ecology, and behavior is well-known, hives are extensively managed for crop pollination and honey production and individual bees are amenable to use in laboratory toxicity studies (Hinarejos et al. 2019). However, there are more than 20,000 described bee species worldwide (Michener 2007; Ascher and Pickering 2017), and the majority of bee genera and species are solitary (Michener 2007; Sgolastra et al. 2019). Bee species differ widely in their ecology. For instance, bees may be strictly solitary, with a single female who mass provisions each of her offspring in individual cells that will be sealed off and never revisited. Depending on the species, nests are built in existing cavities above ground or below ground in chambers newly excavated by the female (e.g., Hurd et al. 1974; Johansen et al. 1978; Bosch et al. 2001). The solitary lifestyle contrasts with eusocial bees (like the honey bee) with one queen and tens of thousands of sterile workers that progressively feed thousands of offspring in a single colony (Winston 1987) (Note that throughout the paper, we use "colony" to refer to colonies of social bees (here: honey bees and bumble bees), not nest aggregations of solitary bees.) Additionally, some bee species may portray aspects of both solitary and social lifestyles, while a few others are cleptoparasites and lay their eggs in nest cells made by a bee of another species (Michener 2007). The vast majority of bee species use pollen and nectar as food source, and some species are oligolectic, i.e., specialized in foraging on flowers of few, closely related plant species whilst others are polylectic, collecting pollen and nectar from a wide

range of plant species (Wcislo and Cane 1996). Phenology also varies widely, particularly among solitary bees. In many cases, solitary species are only active during a few weeks of the year, and their cocooned offspring remain dormant in the nest until the following nesting season. Reproductive solitary females may produce less than one to a couple of eggs per day compared to over a thousand eggs per day laid by honey bee queens (Winston 1987; Michener 2007; Bosch et al. 2008). The variety of phenotypic or ecological characteristics (traits) of these many species may result in differences in sensitivity and exposure routes of non-*Apis* bees in comparison to honey bees (Hinarejos et al. 2019; Rubach et al. 2011; Uhl and Brühl 2019).

In 2017, a workshop hosted by the U.S. Environmental Protection Agency was held to assess whether the honey bee is a good surrogate for evaluating risks of exposure to pesticides across bee species, including other social and solitary bees (2017 Workshop on Pesticide Exposure Assessment Paradigm for non-*Apis* bees; hereafter referred to as "Pesticide Exposure Workshop") (Bireley et al. 2019; Boyle et al. 2019; Cham et al. 2019; Gradish et al. 2019; Hinarejos et al. 2019; Sgolastra et al. 2019). It was concluded that there are differences in life history traits of non-*Apis* bees compared to honey bees that may translate into different levels of exposure. For instance, materials used for nesting (soil, leaves, wax) may be differently contaminated by agrochemicals and varying amounts of pollen and nectar consumed by adults and larvae may result in different exposures and effects on a solitary bee (complete loss of reproductive potential and community-level population reduction) compared to harmful effects or death of a non-reproductive and replaceable workers in social bee colony (Kopit and Pitts-Singer 2018; Sgolastra et al. 2019). However, the Pesticide Exposure Workshop participants did not

address how the identified life history traits for the different types of bees translate to vulnerability to pesticide exposure and effects.

The species vulnerability concept accounts for the exposure of a species to a pesticide, the intrinsic sensitivity to the pesticide, and the recovery of the population from the exposure (i.e. population resilience) (van Straalen 1994). For the estimation of the relative risk posed to species or populations by a pesticide, the trait-based vulnerability assessment assumes: 1) there are phenotypic and ecological characteristics (traits) of an organism that govern whether it is likely to come into contact with a toxicant and to what level (exposure), 2) the sensitivity level of an organism if exposure occurs (effect), and 3) the potential for population recovery from impacts of the toxicant exposure (De Lange et al. 2009; Rubach et al. 2011; van Straalen 1994). Because trait-based vulnerability analysis considers the evaluation of a species both at the organism (exposure and effect) and the population (recovery) levels, it goes beyond the sensitivity of individuals to a toxicant assessed in standard toxicity studies in the laboratory, focusing on exposure and effects on populations in the field. The concept has been mainly applied to aquatic invertebrates (De Lange et al. 2009; Rico and van den Brink 2015), and to higher levels of organization, e.g., ecosystems (De Lange et al. 2010).

In the current paper, we compared the vulnerability of several bee species with different traits to the honey bee and identified the potentially most vulnerable species to two pesticide application scenarios. Vulnerability analyses generally are based on those traits of the system that were identified as important in the context of a stressor. We used and expanded on bee traits and exposure routes identified at the Pesticide Exposure Workshop as important in the context of pesticide risk assessment. We then asked several

experts to independently judge the importance of each characteristic in relation to pesticide risk. Based on these expert judgements, we ranked the species according to their vulnerability to two pesticide application scenarios. We used the bee species previously identified in the Pesticide Exposure Workshop as model species. The species selection does not capture the diversity of all bee species but focuses on species that are at least partially managed for crop pollination, represent different nesting strategies and for which at least some information is available to characterize their traits. The selected bee species occur in temperate agricultural regions, mainly in North America and Europe. The selection includes solitary bee species with different nesting strategies (*Megachile rotundata, Nomia melanderi* and four species of *Osmia*) and two bumble bee species (*Bombus impatiens* and *B. terrestris*) to compare to the honey bee. We also included an unmanaged, ground-nesting solitary bee, *Eucera* (*Peponapis*) *pruinosa*, due to its importance for pollination of cucurbit crops in North America (Hurd et al. 1974). Trait values for these species were compiled from the literature. We conducted a qualitative ranking of bee species' vulnerabilities based on traits related to pesticide exposure, intrinsic sensitivity, and population-level characteristics. The ranking was predominantly informed by traits related to the bees' potential of exposure to pesticides. Laboratory-based toxicological studies for some compounds and bee species other than the honey bee suggest that bee body size may be related to organism-level sensitivity to toxicants (Arena and Sgolastra 2014; Thompson 2016). Body size was the trait used in the vulnerability analysis to represent intrinsic sensitivity to pesticides. However, to gain a broader understanding of bee vulnerability in the field, our analysis includes traits beyond this intrinsic (organism-level) sensitivity to a toxicant, i.e. traits relevant to exposure and

population-level recovery potential. In applying the vulnerability concept, we also identified the influence of certain traits on bee vulnerability and uncertainties in their relationship to vulnerability in the context of two pesticide application scenarios.

Methods

Bee species

We conducted the vulnerability analysis with 10 bee species. The species were chosen based on the selection from the Pesticide Exposure Workshop. The following three criteria were applied during the Pesticide Exposure Workshop to select potential surrogate bee species: 1) they should be commercially reared, leading to the availability of sufficiently large managed populations; 2) they can be handled in the laboratory as well as in field studies; and 3) their behavioral and life history traits are representative of other species in the taxonomic and ecological group (Sgolastra et al. 2019). The importance of the selected species for crop pollination also means that they can potentially be exposed to pesticides applied in the agricultural landscape. The range of behaviors and life histories of the selected bee species result in different pathways by which bees can potentially be exposed to pesticides. Bees may be exposed via direct spray during pesticide application, residues in pollen and nectar, and contact with materials used for nesting, such as soil, leaves or wax, dependent on nesting strategy. A detailed description of the interaction between bee traits and potential for exposure is provided by Sgolastra et al. (2019).

Because of the current use of the honey bee for risk assessments, including in the European Union and the U.S. (European Food Safety Authority 2013; US Environmental Protection Agency. 2014), to represent insect pollinators, it was included in the

vulnerability analysis as the reference species. Two bumble bee species (*Bombus impatiens* Cresson and *B. terrestris* L., Apidae) were included following the selection from the Pesticide Exposure Workshop (Gradish et al. 2019). Bumble bees are important crop pollinators, and the two chosen species are reared commercially. *B. impatiens* is native to North America and *B. terrestris* to Europe. Bumble bees are social, but with much smaller colonies than honey bees. Colonies do not overwinter, but rather over-wintered, mated queens establish new colonies in the following year and often build nests underground in existing cavities (Cnaani et al. 2002; Goulson et al. 2002). For solitary bees, four species of *Osmia* (*O. lignaria* Say, *O. cornifrons* Radoszkowski, *O. cornuta* Latreille, *O. bicornis* (= *rufa*) L., Megachilidae) were included along with *Megachile rotundata* F. (Megachilidae) and *Nomia melanderi* Cockerell (Halictidae) (Sgolastra et al. 2019). *O. lignaria* is native to North America, *O. cornuta* and *O. bicornis* are European species and *O. cornifrons* is native to East Asia (but is also commercially available in North America). *M. rotundata* is native to Europe and Southwestern Asia but was introduced to North America where it is widely distributed and also commercially available for alfalfa pollination. *N. melanderi*, a native species in the Western United States, is managed for alfalfa pollination. These solitary bee species collect pollen and nectar from floral resources to create a mass food provision for each egg they lay. Each egg with its provision is sealed in an individual cell within a nest (Michener 2007). The four species of *Osmia* nest above-ground in existing cavities, e.g., hollow plant stems, and use soil or mud to cap each cell and the end of the nest (Bosch et al. 2001, 2008). *M. rotundata* (a leaf-cutting bee) lines each of many nest cells with leaf pieces before filling it with provision; each cell and nest is capped with leaf discs (Tasei and Masure 1978;

Kemp and Bosch 2000; Gemmill-Herren and Strohm 2014). *N. melanderi* is a ground-nesting species, a trait it has in common with the majority of solitary bee species (Michener 2007). *N. melanderi* nesting aggregations are managed by farmers for alfalfa pollination in the Western USA (Johansen et al. 1978; Sgolastra et al. 2019). For our approach, we added the squash bee *Eucera (Peponapis) pruinosa* Say (Apidae), which is native to North America, to extend the representation of solitary ground-nesting bees. Squash bees are not managed but important pollinators of cucurbit crops and are commonly found nesting close to squash, pumpkin and gourd fields (cultivated *Cucurbita* species) (Hurd et al. 1974; Julier and Roulston 2009), with potential of exposure to pesticides (Willis Chan et al. 2019; Willis Chan and Raine 2021). In the Pesticide Exposure Workshop, stingless bees (Meliponini) were included due to their importance as crop pollinators in tropical and subtropical regions (Cham et al. 2019). However, we did not include stingless bees in our approach because they comprise a wide variety of species, and the five species highlighted by Cham et al. (2019) as potential surrogates for risk assessments of stingless bees lack key life history and other data necessary for valid evaluation.

Bee traits

Vulnerability analyses generally are based on several characteristics or traits of the system, e.g., species or ecosystems, that are identified as important in the context of the stressor (De Lange et al. 2010). Expert judgement is then applied to score the importance of each characteristic in relation to risk. For the bee species included in the vulnerability analysis, we compiled trait-level data for 16 traits (Supplemental Material A). Sgolastra et al. (2019) identified 15 bee traits with implications for pesticide risk assessment, 13 of

which are included in our analysis. Two traits were excluded in this analysis. First, the trait "amenability to nest in confined conditions" was listed by Sgolastra et al. (2019) because it informs whether the species can be used for pollination in greenhouses and semi-field studies for pesticide risk assessments. However, the trait does not directly inform whether a species may or may not be vulnerable to pesticide exposure and was, therefore, excluded from our approach. Second, adult food was identified as important in the context of pesticide risk assessment because the amounts of pollen and nectar consumed by adult females affects the potential for exposure from residues in those plant matrices. However, lack of data on nectar and pollen consumption rates in most species prevented us from using the trait in our analysis.

In addition to those traits included in the list from Sgolastra et al. (2019), we identified three other traits as important based on trait-based analysis of bees conducted in contexts other than pesticide risk assessments (Bommarco et al. 2010; Ekroos et al. 2013; Hopfenmüller et al. 2014; De Palma et al. 2015; Forrest et al. 2015; Pisanty and Mandelik 2015). De Palma et al. (2015) found that "voltinism" affected bee abundance in interaction with land use pressures which were categorized according to the major land use at a site. The traits "emergence time" of bumble bee queens and "colony size" were included by Ekroos et al. (2013) in their analysis of impacts of landscape heterogeneity on bumble bees, butterflies and hoverflies. They found that the two traits were correlated in bumble bees and interacted with landscape heterogeneity, impacting bumble bee abundance. Emergence time in solitary and bumble bees may also be important in the context of pesticide risk assessment due to the potential temporal overlap of bee activity and pesticide application. Thus, we added emergence time of queen/females, colony size,

and voltinism to the list proposed by Sgolastra et al. (2019). All traits included in our vulnerability analysis, as well as the average trait values for selected bee species, are listed in Table 1. Traits interacting with the potential for exposure to pesticides were strongly represented in our analysis (10 out of 16 traits), a single trait related to intrinsic effects (body size) and 5 traits relevant to the potential for population recovery were included (Table 1).

Trait values compiled from literature are often expressed as ranges, either due to reported measured ranges or due to varying values reported by different sources. For the vulnerability analysis, single trait values for each trait and species are necessary to calculate the relative vulnerability to a chemical. Therefore, we applied averages of reported ranges or deviating values. If the emergence (or start of foraging activity) in spring was reported generically, we assumed it occurs in March. Data for three traits were missing for *O. cornifrons*. We used the average of the values compiled for the other *Osmia* species in our analysis to fill in these gaps. In addition, no report of body length in *N. melanderi* was found, but only the intertegular distance (ITD). We estimated the length of the species at 10 mm based on the reported ITD relative to ITDs reported for the other bee species in our analysis. Note that the fecundity and the body length in social bee species (honey bees, *B. impatiens* and *B. terrestris*) reflect the averages of worker eggs produced by queens per day and worker sizes, respectively. Finally, detailed data on adult food could not be found for any of the focal species except honey bees. While active females of all species are known to consume nectar and pollen, quantities or ratios are not reported (Michener 2007; Cane et al. 2017). Therefore, despite its importance in the context of pesticide exposure and effects, we removed adult food as a trait in the

vulnerability analysis due to lack of data. In the Supplemental Material A, the detailed trait values compiled from literature are presented.

Pesticide scenarios

The analysis of the vulnerability of bee species is specific to a pesticide use scenario. Pesticides differ in their timing and type of application and in their chemical characteristics (e.g., mode of action, persistence, systemicity) that can influence how and when they might intersect with bees (Kopit and Pitts-Singer 2018). Accordingly, bee traits may interact differently with different use scenarios but, in general, vulnerability is relative to both the increased likelihood of exposure and the potential for an adverse effect impacting the population (rather than only the sensitivity of individuals, i.e., pesticide species sensitivity). In the current analysis, we aimed to capture potential differences in vulnerability due to general pesticide use characteristics rather than conducting an analysis for a specific compound. We chose two generic pesticide application scenarios to represent the highest potential for impacting bees: A) pre-bloom soil treatment with a systemic insecticide on a bee attractive crop, and B) foliar application of a non-systemic insecticide during crop bloom on a bee attractive crop. When highly systemic pesticides, which include most neonicotinoids, are applied to soil, they are taken up by the treated plants and remain in the plants and the treated soil over extended time periods (Blacquière et al. 2012; Jeschke et al. 2011; Lundin et al. 2015). Residues of these primarily xylem-mobile pesticides can reach all plant tissues, including pollen and nectar. In addition to pollen and nectar, bees may be exposed through interaction with the treated plants (not surface contact) and soil. Foliar applications of non-systemic pesticides (e.g, organophosphate, pyrethroid, and growth regulator

insecticides) during crop bloom may lead to exposure of bees through collection and consumption of pollen and nectar from treated crops. In addition, foraging bees may be directly exposed by overspray or interaction with the surface of treated plants or soil shortly after pesticide application.

Expert judgement

To estimate the relationship between traits and bee vulnerability, we relied on the expert judgement of the relative importance of each trait with respect to bee risk of pesticide exposure and effects. Experts were identified as scientist with expertise in bee biology and pesticide risk assessment, demonstrated by their previous publications in this field. From this limited group of scientists, seven were successfully recruited to contribute to the current paper as experts and all are co-authors (MF, HT, FS, TP, CE, TP, SH). The judgements included assigning weights between 0 and 5 to each trait. A weight of 0 means that the trait is assumed not to influence vulnerability to the pesticide, a value of 5 means that the trait is assumed to be highly important for the vulnerability to the pesticide. Weights were assigned by each expert separately for each pesticide scenario accounting for possible differences in the relationship between traits and the vulnerability specific to pesticide and application type. Expert judgements sorted categorical trait values from assumed lowest to highest vulnerability for each pesticide scenario. Traits with numerical values were assigned increasing or decreasing vulnerability to scenario-specific pesticide exposure and effect with increasing trait value. All judgements by experts can be found in the Supplemental Material B.

From the expert judgements, average assignments were derived. For categorical traits, the most frequent order of trait levels (from least to most vulnerable) was applied in the

average assignment for each pesticide application scenario. Similarly, the most frequent assignment of 'decrease' or 'increase' in vulnerability with increasing continuous trait value was used. Mean vulnerability weights ($w_{(i,s)}$; see Equation 3) across experts were used for the average assignments.

Relative vulnerability of bee species

For the calculation of the relative vulnerability of the bee species to pesticide exposure and effects, we followed the methodology introduced by De Lange et al. (2009). For each pesticide scenario, categorical trait values were converted to numerical trait values according to their order from lowest to highest vulnerability. The categorical bee traits used in the current analysis either had two or three categorical values, i.e., numerical values applied were either [1, 2] or [1, 2, 3] (Table 1).

With all trait values numerical, each trait value was standardized to the range of the trait values across species included in the assessment, converting trait values linearly to values between 0 and 1. We used the minimum and the maximum trait value for standardization whereby the lowest trait value was converted to 0, and the highest trait value to 1. This represents a deviation from the standardization applied by De Lange et al. (2009): for their analysis, they scaled trait values to their maximum, not to the range, scaling the range from 0 to the maximum trait value irrespective of the minimum observed trait value. We assumed that it is important to represent the range (defined by minimum and maximum values) rather than only the maximum values because not all traits can take a value of 0, e.g., foraging distance or body length.

If the trait-based vulnerability was assigned to increase with increasing trait level (including all converted categorical traits), the standardized vulnerability $v_{(i,s)}$ of trait i in

species s was calculated from the trait value $t_{(i,s)}$ and the range of trait values t_i across all species (Equation 1).

(1) $v_{(i,s)} = \dfrac{\left(t_{(i,s)} - \min(t_i)\right)}{\left(\max(t_i) - \min(t_i)\right)}$

If a decreasing vulnerability was assigned with an increasing trait value $t_{(i,s)}$ of trait i in species s, the standardized vulnerability $v_{(i,s)}$ was calculated using Equation 2.

(2) $v_{(i,s)} = 1 - \dfrac{\left(t_{(i,s)} - \min(t_i)\right)}{\left(\max(t_i) - \min(t_i)\right)}$

We are illustrating the calculation of $v_{(i,s)}$ using the example of the trait 'body length.' Across the bee species included in the analysis, the body length varied between a minimum of 9 mm (*M. rotundata*) and a maximum of 14 mm (*O. cornuta* and *B. terrestris*). According to the expert judgement, the trait-specific vulnerability decreases with increasing body length. Accordingly, the species with the minimum body length has the highest vulnerability for this trait: $v_{(body\ length, M.rotundata)} = 1$. Correspondingly, the species with the maximum body length have the lowest relative vulnerability in the trait: $v_{(body\ length, O.cornuta)} = 0$. Females of *E. pruinosa* have an average body length of 13.25 mm, which results in $v_{(body\ length, P.pruinosa)} = 1 - \dfrac{(13.25 - 9)}{(14 - 9)} = 0.15$.

The vulnerability weight $w_{(i,s)}$ was applied to the standardized vulnerability values $v_{(i,s)}$ as a factor. The relative vulnerability of each species v_s was calculated as the sum of all weighted trait-level vulnerabilities divided by the sum of all weights applied (Equation 3).

(3) $v_s = \dfrac{\sum_i \left(v_{(i,s)} w_{(i,s)}\right)}{\sum_s w_{(i,s)}}$

The resulting species-specific relative vulnerability v_s had a value between 0 and 1. The species included in the analysis were ranked by their relative vulnerability. The highest ranked species corresponded to species with the highest vulnerability in the context of the pesticide scenario, and the lowest ranked species to the lowest vulnerability. The vulnerability values do not reflect measures of absolute vulnerability to a pesticide application scenario, but exclusively allow to estimate the *relative* vulnerability between the species included in the analysis.

Comparison of individual expert opinions

We analyzed discrepancies in our expert judgements for each pesticide scenario to identify uncertainties in the relationship between traits and vulnerabilities. For each pesticide scenario, we considered our assignments divergent for a given trait if the order of categorical trait values or the decision on increasing or decreasing vulnerability with increasing continuous trait value differed. In addition, we compared each expert's judgements between the two pesticide scenarios. Differences in judgements between scenarios reveal which traits are assumed to play varying roles dependent on pesticide use.

Results

Relative vulnerability of bee species

In both pesticide application scenarios and across all expert opinions, the honey bee ranked as least vulnerable based on the assessed traits (Table 2 and Table 3, Supplemental Material B). The average expert judgement across traits resulted in *E. pruinosa* ranking as the most vulnerable species to pesticide exposure in both scenarios. While the relative vulnerability of the honey bee (0.28 in pesticide scenario A and 0.19 in

scenario B) was clearly distinguished from the second-most vulnerable species in both pesticide scenarios, the relative vulnerabilities amongst the other species were comparatively more similar to each other, ranging between 0.44 and 0.62 in scenario A (Table 2) and 0.52 and 0.68 in scenario B (Table 3). The vulnerability ranking of the bee species included in the current analysis resulted from the combined assessment of each trait's relationship to vulnerability. Accordingly, the consistent ranking of the honey bee as the least vulnerable species is expected to be linked to low vulnerabilities relative to all other species in several traits. Correspondingly, the high vulnerability ranking of *E. pruinosa* should be expected to be linked with high relative vulnerability in multiple traits.

The trait-level relative vulnerabilities are shown in Figure 1 for pesticide application scenario A (pre-bloom soil treatment with a systemic insecticide) and Figure 2 for scenario B (foliar application of a non-systemic insecticide during crop bloom). The honey bee's trait-level vulnerabilities were ranked zero in multiple traits where all other bee species included in the analysis received a vulnerability score larger than zero in both scenarios. These traits include foraging range, nesting period, larval food (jelly), colony size, fecundity (daily egg laying rate) and larval feeding period. In addition, the honey bee was assessed with the lowest vulnerability score across traits impacting potentials for external exposure except for trophallaxis, larval food provisioning and emergence time in scenario A (Figure 1). In scenario B, emergence time represented the only trait related to external exposure that indicate a non-zero relative vulnerability in honey bees (Figure 2). The opposite end of the vulnerability spectrum, *E. pruinosa*, was assessed with higher vulnerability values with respect to its foraging range and flower preference compared to

all other species in the analysis. In addition, it ranked high across trait-level vulnerabilities related to external exposure, displaying an opposite pattern of vulnerabilities compared to honey bees in both scenarios (Figures 1 and 2).

The pesticide scenario resulted in different rankings of the species other than *E. pruinosa* and honey bees. In scenario A, the vulnerability ranking resulted in intermixed solitary cavity (*Osmia*) and ground-nesting bees (*E. pruinosa*, *N. melanderi*) along with the ground-nesting social *Bombus* species. The leafcutting bee *M. rotundata* ranked as the least vulnerable other than the honey bee (Table 2). *M. rotundata* shows a comparable pattern of relative trait-level vulnerabilities as the *Osmia* species but with lower vulnerability values for several traits (see Figure 1). It is the only bivoltine species in our analysis, a trait value judged to correspond to lower vulnerability than a single generation per year (univoltinism). In scenario B, all solitary species were ranked more vulnerable than the social species irrespective of their nesting habits (Table 3), with *B. terrestris* ranking as the least vulnerable species other than honey bees.

Comparison of individual expert opinions

Expert judgements on trait level order (for categorical traits) and trait value impact on vulnerability (for continuous traits) varied between experts as well as weights assigned to each trait. This resulted in variable rankings of species if conducted based on each single expert opinion. However, all individual expert opinions resulted in the honey bee's rank as least vulnerable in both scenarios. In scenario A, *M. rotundata* consistently ranked between rank 7 and 9 of the 10 species, suggesting relatively low vulnerability compared to the other solitary species and the two *Bombus* species. In scenario B, only the honey bee had a consistent ranking across expert opinions.

In scenario A, all experts agreed on the trait level order (for categorical traits) or the trait effect direction (for continuous traits) of nesting substrate (above-ground corresponding to lower vulnerability than below-ground), larval food (jelly corresponding to lower vulnerability than no jelly), emergence time (decreasing vulnerability with later emergence), body size (decreasing vulnerability with increasing length), colony size (decreasing vulnerability with increasing colony size in social bees), fecundity (decreasing vulnerability with increasing daily egg laying rate) and voltinism (decreasing vulnerability with increasing number of generations per year).

In scenario B, all experts agreed on the same set of traits with respect to the correlation with effects direction except for nesting substrate which was variable between experts. Instead, the nesting period was consistently judged to correlate negatively with vulnerability (i.e., decreasing vulnerability with increasing duration of nesting activity per year). The individual expert judgements and the rankings based on individual judgements can be found in Supplemental Material B.

Discussion

The vulnerability concept in chemical risk assessment was applied to a range of bee species representing social and solitary bees, and including cavity (honey bees, *M. rotundata* and four species of *Osmia*) and ground-nesting species (*N. melanderi*, *E. pruinosa* and two species of *Bombus*). This approach addresses the drivers of exposures explicitly along with intrinsic (individual-level) sensitivity and population recovery (Rubach et al. 2011). Trait data for the bees in this analysis were compiled from available literature sources (Supplemental Material A). We relied on the opinions of seven experts with expertise in bee biology and pesticide risk assessment and asked for their judgement

of trait-level vulnerabilities in the context of two hypothetical pesticide application scenarios: A) pre-bloom soil treatment with a systemic insecticide on a bee attractive crop, and B) foliar application of a non-systemic insecticide during crop bloom on a bee attractive crop. For both scenarios, the average expert judgements ranked the honey bee as the least vulnerable. This was also the case in each individual expert opinion, suggesting a high robustness of this result.

Relative vulnerability

The bee species ranked as the most vulnerable in our analysis was the solitary bee *E. pruinosa,* which is distinguished from all other bee species in our analysis by its specialization in a single plant family grown as crops (cucurbit crops, hence its common name, squash bee) and nesting in the soil in close proximity to those same crops, correlated with short foraging ranges. Despite its ranking as the most vulnerable species in both pesticide scenarios using average expert judgements, the squash bee was not consistently ranked most vulnerable based on individual expert opinions. Relative vulnerabilities were more similar amongst all non-*Apis* species in our analysis than between any of these species and the honey bee in pesticide application scenario B. In pesticide application scenario A, only *M. rotundata* had a relative vulnerability closer to honey bees than to the highest ranked *E. pruinosa*. Except for honey bees and *M. rotundata*, all bee species in our analysis overwinter and/or nest in the ground (*B. impatiens, B. terrestris*, *E. pruinosa*, and *N. melanderi*) or use mud or soil as cell partitions of their above-ground nests (*O. lignaria, O. cornuta, O. bicornis,* and *O. cornifrons*). As it was suggested in the Pesticide Exposure Workshop, the exposure routes stemming from the nesting matrix and the additional bee-collected nesting material

should be considered when conducting pesticide risk assessments for non-*Apis* bee species, although several key data gaps currently hinder the quantification of these routes of exposure.

Our analysis does not constitute a risk assessment that defines the level of risk from a pesticide to honey bees and the other bee species. Rather, we can only use it to estimate the *relative* vulnerability between the species included in the analysis. Accordingly, this analysis can provide insights into the species and highlight traits that may be important for risk assessments applicable across bee species. However, multiple expert opinions on the trait-level vulnerabilities to pesticides revealed the uncertainties in understanding how bee traits may interact with population-level risk. Several traits were categorized affecting vulnerability in opposite directions by different experts, i.e., whether an increasing trait value is correlated with increasing or decreasing vulnerability. For instance, a larger foraging range may result in dilution of pollen and nectar collected from an exposed source with food from other, non-exposed sources, but smaller foraging ranges could result in no encounter with exposed food sources at all. Similar lack of understanding applies to the vulnerability related to the strategy of larval food provisioning and the larval feeding period. Larger colonies were assumed to provide a buffer against bee mortality, but experts also raised the possibility that larger colonies could accumulate more exposed products in the hive due to the larger number of foragers.

Expert Judgements and Data Limitations

This variability in expert judgements reflects the general lack of understanding of how some bee traits influence vulnerability. Hemming et al. (2020) recommend a group size of 5-12 experts to derive aggregated judgements in approaches relying on expert

opinions. However, with the variability in judgements in our analysis, for a more definitive identification of the most uncertain traits, a considerably larger number of experts would have been needed. In addition, expert opinions may be influenced by different perspectives with respect to bee traits. Faced with the lack of knowledge about the life history traits, behavior, and exposure, experts may tend to assume higher potential vulnerability, which could result in the honey bee consistently ranking less vulnerable because it has been studied more extensively than any of the other species included in our analysis. Correspondingly, *E. pruinosa* is a species with low data availability and a high vulnerability rank.

The lack of data also increases the uncertainty of the trait values themselves. Values were compiled from multiple sources that reflect different study locations and designs. For instance, the larval development time strongly depends on temperature, resulting in potentially very different development times under controlled conditions in the laboratory compared to field conditions (Kemp and Bosch 2000; Bosch et al. 2008). The vulnerability concept assumes a linear relationship between trait values and vulnerability, but this may not hold for all traits. Lastly, traits may be correlated in many species (e.g., the foraging distance shows a correlation with body size; Greenleaf et al. 2007), adding uncertainty to the adequate relative weights of correlated traits.

Trait-based vulnerabilities may also be strongly variable based on temporal and spatial coincidence of bee activity and presence of pesticide residues in the landscape. Exposures through nesting matrices and materials depend on the location of the nests and the material sources relative to the application of a pesticide. For instance, a bee may have increased exposure if it nests within a treated field or collects nesting material from

treated soil or plant parts (Willis Chan et al. 2019). In contrast, a species' risk may be very low if nesting activity occurs exclusively in non-exposed areas. Correspondingly, phenological bee traits need to be understood in the context of the temporal presence of exposure in nesting and foraging habitats.

Use of the Vulnerability Concept

In previous trait-based analyses of bees that were not related explicitly to risks of pesticides, the selection of traits was strongly informed by the availability of trait-based data across bee species, resulting in a limited number of traits used (Bommarco et al. 2010; Ekroos et al. 2013; Hopfenmüller et al. 2014; De Palma et al. 2015; Forrest et al. 2015; Pisanty and Mandelik 2015). In our analysis, traits were included to inform the three aspects of the vulnerability concept: exposure, intrinsic sensitivity, and population-level recovery. While trait values could be identified from the literature for the bee species included in the analysis (see Supplemental Material A), the interaction with pesticide exposures and effects has not been studied comprehensively. This led us to rely on expert opinion in our analysis. Multiple traits were included based on their likely interaction with pesticide exposure, including traits related to nesting habits, food preferences and provisioning and phenology (Gradish et al. 2019; Sgolastra et al. 2019). Intrinsic sensitivity was only represented by body size in our analysis. Body size is indicated as one factor influencing sensitivity of individual bees in laboratory toxicity studies beyond which we have little understanding of the processes that drive observed differences in sensitivities among bee species (Arena and Sgolastra 2014; Thompson 2016; Heard et al. 2017; Carnesecchi et al. 2019). The population-level recovery potential is captured by traits related to sociality, fecundity and generation time (voltinism),

assuming that these traits represent the potential for buffering or recovery from stressor-induced mortalities (Rubach et al. 2011). Accounting for trait data availability and for their relevance for pesticide risk assessment, our vulnerability analysis was influenced more by external exposure and recovery potential traits than by those related to intrinsic sensitivity.

Other applications of the vulnerability concept in chemical risk assessment had a higher representation of traits related to intrinsic sensitivities (De Lange et al. 2009; Rico and Van den Brink 2015). Those analyses also included a larger variety of taxonomic groups, encompassing aquatic and terrestrial insects and vertebrates (De Lange et al. 2009) or various taxa of aquatic invertebrates (Rico and Van den Brink 2015). Consistent with our analysis, their results also suggest that the relative vulnerability strongly depends on both a taxon's traits and the characteristics of the chemical exposure and effects. Traits related to population recovery were important across analyses. As in other studies, we also found that the vulnerability ranking is dependent on the pesticide scenario applied. However, vulnerabilities amongst the non-*Apis* species in our analysis varied less than their vulnerability compared to the honey bee, irrespective of pesticide application scenario. Overall, even though our analysis was focused on species within a monophyletic group (Apiformes) with a smaller range of trait variability, the main findings from our analysis corresponded to those found in other studies.

In the context of pesticide risk assessments of bees, the vulnerability analysis of bees points to additional research needs with respect to higher-tier assessments. In risk assessments, a step-wise or tiered approach is applied in which tier 1 corresponds to a screening method, using very conservative assumptions about potential exposures and

effects on the organism level (European Food Safety Authority 2013; US Environmental Protection Agency 2014). Standard laboratory-based toxicity studies with honey bee larvae and adults are conducted in the context of tier 1 assessments. A growing number of laboratory-based toxicity studies with bee species other than the honey bee can inform cross-species analysis of intrinsic sensitivities and possible test designs with other species (Arena and Sgolastra 2014; Thompson 2016; Heard et al. 2017; Thompson and Pamminger 2019; Eeraerts et al. 2020; Ceuppens et al. 2021; Pamminger 2021). If tier 1 indicates that a risk cannot be excluded based on the conservative assumptions, higher-tier assessments must be conducted, including semi-field (tunnel) and field studies (Peters et al. 2016; Ruddle et al. 2018; Franke et al. 2021). The traits in the vulnerability analysis were mainly related to the relevance of exposure routes of bees in the field and the population-level recovery potential. These traits are not addressed in tier 1 risk assessments but rather become relevant in higher-tier assessments (Uhl and Brühl 2019). Accordingly, the results of the vulnerability analysis suggest that more research is needed to identify the most suitable surrogate bee species in higher-tier field-based studies.

Conclusions

The term "surrogacy" is often used in a context of extrapolating data from toxicity studies conducted on one organism, in this case the honey bee, to a broader range of species usually in the same taxa (e.g., honey bees and non-*Apis* bees). However, the population-level vulnerability of species to pesticides is influenced by not only intrinsic sensitivity, but also a range of ecological traits related to the likelihood of exposure and the potential of population recovery from effects. The results from our analysis suggested that the non-*Apis* bee species included are potentially more vulnerable to pesticides than the honey

bee, particularly with respect to traits governing exposure and population recovery potential. Ground-nesting bee species that specialize on potentially treated crops, represented in our analysis by the squash bee, *E. pruinosa*, may be most vulnerable to exposure and subsequent population-level effects.

The mechanistic link between bee traits and temporal and spatial exposure patterns in their habitats is not well understood but may be important for a realistic estimate of risk. Assessing these interactions empirically in the field across compounds and bee species may not be feasible, but mechanistic effect modeling may provide more detailed insights relevant for population-level risk assessments for bees (Hommen et al. 2015). The relationship between bee traits and their interaction with environmental factors, including exposures, can be conceptualized based on best available knowledge and implemented in mechanistic effect models, specifically population models (Raimondo et al. 2021). Such models would represent the interaction between traits as well as between traits and the environment, including varying pesticide scenarios that could not be fully captured in the vulnerability analysis presented in the current paper. This work could also help to quantify both nectar and pollen consumption and additional exposure routes for non-*Apis* bees in comparison to the conservative exposure estimates for honey bees (e.g., pollen and nectar sourced only from the treated crop, low sugar content of nectar; see Hinarejos et al 2019) currently used in risk assessments globally.

While the vulnerability analysis does not provide conclusions about actual risks from pesticides to the bee species, it emphasizes the major uncertainties that exist around vulnerability of non-*Apis* bee populations to pesticides. More research could elucidate the interaction between bee traits and the potential pesticide exposures, particularly with

respect to traits that differ considerably between the honey bee and non-*Apis* bees, e.g., consumption of pollen and nectar, the potential exposure from nesting substrates and materials, larval food composition and provisioning strategy as well as the role of sociality. Although the level of conservatism built into the honey bee exposure assessment may be adequate to cover the risk for other bee species in the first tiers of risk assessment (individual level), more research is needed to identify the most suitable test strategy for higher-tier bee risk assessment including the most suitable surrogate species and to inform colony or population modeling approaches.

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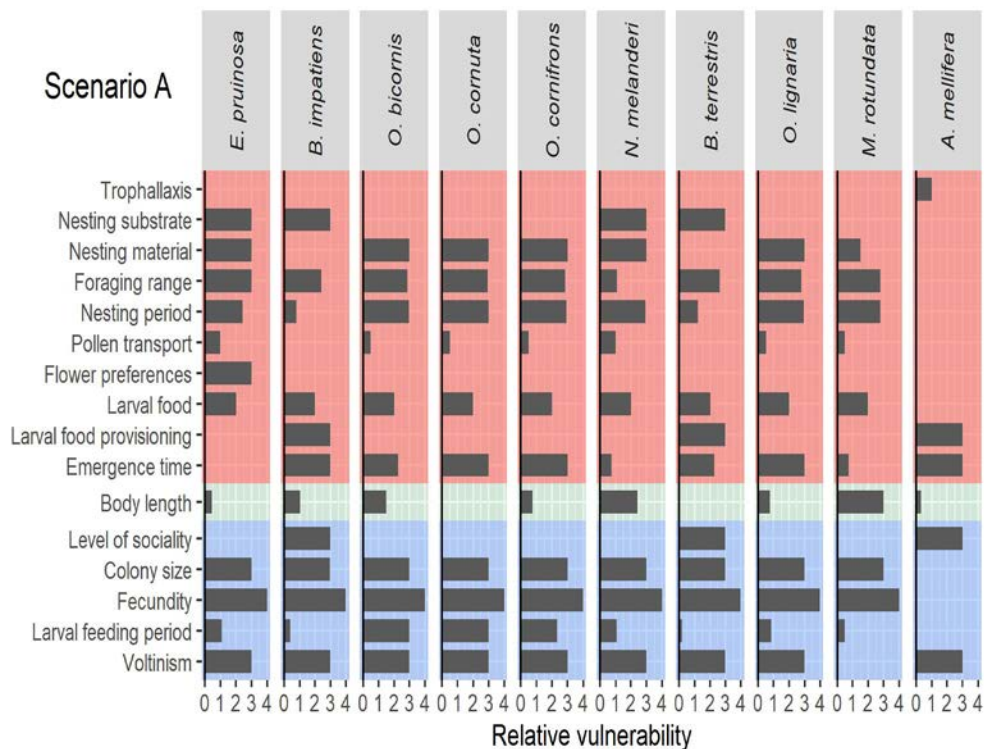
FIGURE CAPTIONS

Figure 1. Trait-level vulnerabilities for pesticide scenario A): pre-bloom soil treatment with a systemic insecticide on a bee attractive crop. Larger values signify higher trait-level vulnerabilities and are calculated from the species-specific trait value and average weight applied to the trait by the experts. The species are sorted from highest (left) to lowest (right) species-level vulnerability. Traits are grouped into traits important for external exposure (red), intrinsic sensitivity (green) and population recovery potential (blue).
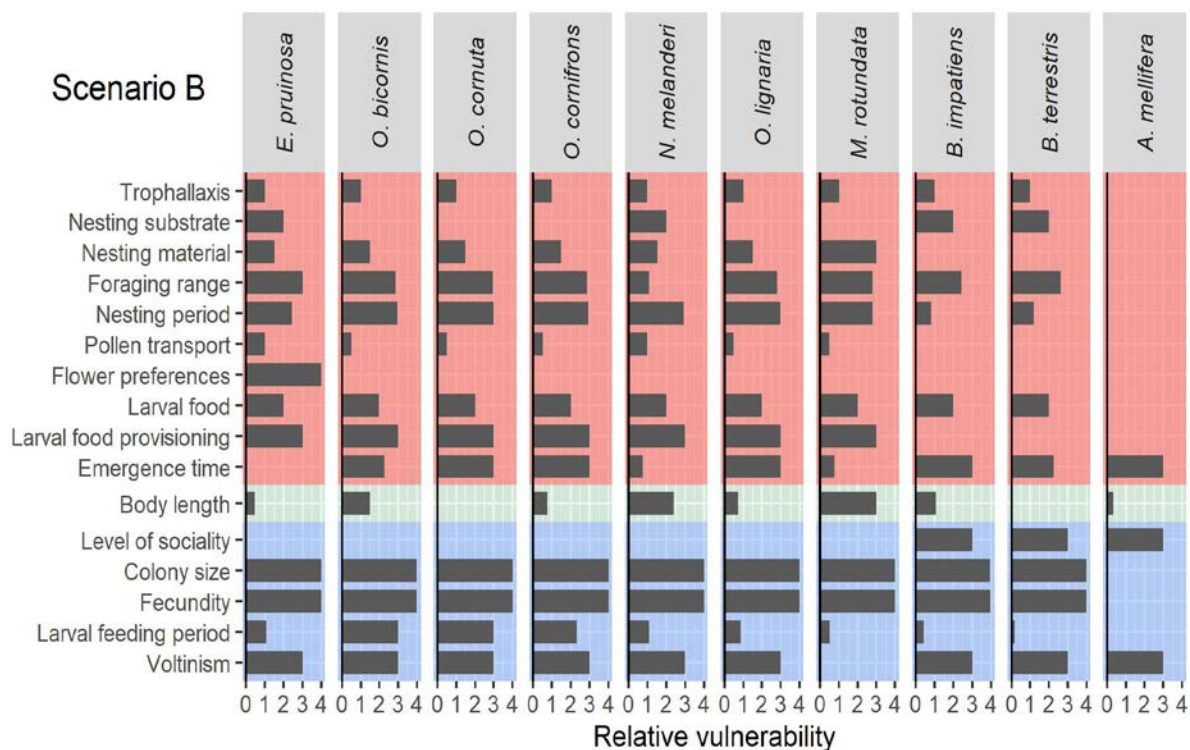
Figure 2. Trait-level vulnerabilities for pesticide scenario B): foliar application of a non-systemic pesticide during crop bloom on a bee attractive crop. Larger values signify higher trait-level vulnerabilities and are calculated from the species-specific trait value and average weight applied to the trait by the experts. The species are sorted from highest (left) to lowest (right) species-level vulnerability. Traits are grouped into traits important for external exposure (red), intrinsic sensitivity (green) and population recovery potential (blue).

Table 1. Trait compilation for the bee species included in the vulnerability analysis. Average trait values were used where ranges were reported in the literature. Missing values were filled in using the average across the related species. See Supplemental Material A for the detailed compilation of trait values, including literature references.

Species	External exposure										Intrinsic sensitivity	Population recovery potential				
	Trophallaxis	Nesting substrate	Nesting material	Foraging range (km)	Nesting period (months)	Pollen transport	Flower preferences	Larval food	Larval food provisioning	Emergence time (month of year)	Body length (mm)	Level of sociality	Colony size	Fecundity (eggs/day)	Larval feeding period	Voltinism

															(days)	
Apis mellifera	present	above-ground	wax	10	12	legs	polylectic	jelly	progressive	3	13.5	social	40000	1500	5.5	1
Bombus impatiens	absent	below-ground	wax	2.15	9	legs	polylectic	no jelly	progressive	3	12.25	social	375	6.7	9.1	1
Bombus terrestris	absent	below-ground	wax	1.45	7.5	legs	polylectic	no jelly	progressive	4	14	social	350	12	7	1
Megac	absent	ab ov	leav	0.95	1.6	abdo	polyle	no	mas-s-	6	9	solit	0	1.5	10	2

hile rotundata		e-ground	es			men	ctic	jelly	provisioning			ary				
Nomia melanderi	absent	below-ground	soil/mud	6.45	1.1	legs and abdomen	polylectic	no jelly	mass-provisioning	6	10	solitary	0	0.5	15	1
Osmia bicornis	absent	above-ground	soil/mud	0.7	1	abdomen	polylectic	no jelly	mass-provisioning	4	11.5	solitary	0	0.65	32	1
Osmia cornifrons	absent	above-grou	soil/mud	0.7	1	abdomen	polylectic	no jelly	mass-provisioni	3	12.75	solitary	0	1.55	26	1

		nd						ng							
Osmia cornuta	absent	above-ground	soil/mud	0.4	0.8	abdomen	polylectic	no jelly	mass-provisioning	3	14	solitary	0	1.5	32	1
Osmia lignaria	absent	above-ground	soil/mud	0.9	1	abdomen	polylectic	no jelly	mass-provisioning	3	12.75	solitary	0	2.5	13	1
Eucera (Peponapis) pruinosa	absent	below-ground	soil/mud	0.26	3	legs and abdomen	oligolectic	no jelly	mass-provisioning	7	13.25	solitary	0	1	15	1

Table 2. Ranking of bee species by their relative vulnerability for pesticide scenario A):

pre-bloom soil treatment with a systemic insecticide on a bee attractive crop.

Species	Average vulnerability across traits
Eucera (*Peponapis*) *pruinosa*	0.62
Bombus impatiens	0.61
Osmia bicornis	0.6
Osmia cornuta	0.58
Osmia cornifrons	0.58
Nomia melanderi	0.58
Bombus terrestris	0.58
Osmia lignaria	0.55
Megachile rotundata	0.44
Apis mellifera	0.28

Table 3. Ranking of bee species by their relative vulnerability for pesticide scenario B):

foliar application of a non-systemic pesticide during crop bloom on a bee attractive crop.

Species	Average vulnerability across traits
Eucera (*Peponapis*) *pruinosa*	0.68
Osmia bicornis	0.66
Osmia cornuta	0.64
Osmia cornifrons	0.64
Nomia melanderi	0.62
Osmia lignaria	0.61
Megachile rotundata	0.57
Bombus impatiens	0.55
Bombus terrestris	0.52
Apis mellifera	0.19