Extrapolating acute bee sensitivity to insecticides using a phylogenetically informed interspecies scaling framework

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Abstract

Plant protection products, including insecticides, are important for global food production. Historically, research of the adverse effects of insecticides on bees has focused on the honeybee (*Apis mellifera*), while non-*Apis* bee species remained understudied. Consequently, sensitivity assessment of insecticides for the majority of bees is lacking, which in turn hinders accurate risk characterization and consequently bee protection. Interspecies sensitivity extrapolation based on body weight offers a potential solution to this problem, but in the past such approaches have often ignored the phylogenetic background and consequently non independence of species used in such models. Using published data on the sensitivity of different bee species to commonly used insecticides, their body weight and phylogenetic background I build interspecies scaling models (ISMs) applying a phylogenetically informed framework. In addition, I compared, the relative sensitivity of the standard test species *Apis mellifera* to other bee species to evaluate their protectiveness when used as standards screening bee species in the risk assessment process. I found that overall 1) body weight is a predictor of bee sensitivity to insecticides for a range of insecticide classes and 2) *A. mellifera* is the most sensitive standard test species currently available and consequently a suitable surrogate species for ecotoxicological risk assessment.

Introduction

Insects, in particular bees, play a vital role in pollinating wild plants as well as crops [1]. This ecosystem service provided by both domesticated (e. g. *A. mellifera*) and wild bees, is essential for maintaining the functionality of non-managed (e g. biodiversity), as well as food production in agricultural landscapes [2, 3]. Due to their ecological and economical importance, reports of wild bee population declines in some areas have raised concerns regarding the sustainability of pollination services [4-6]. Several stressors including changes in land use, intensified agricultural practice and bee diseases are being discussed as drivers for this dynamic [7-9]. Within this broad discussion, the use of PPPs, in particular insecticides, and their potential adverse side effects on wild bee populations has emerged as a controversially discussed topic [9].

In order to accurately estimate the potential risk for bees following insecticide application in an agricultural setting, a robust risk assessment is needed [10]. A prerequisite for such a process is accurate hazard (sensitivity) characterization (e g. lethal doses or rates) of insecticides for the bees in question, which together with realistic exposure estimates, provides the basis for a robust estimation of the actual risk bees face. However, besides a few well-studied model bee species (e g. *A. mellifera* and *Bombus terrestris*) little is known about the response of the majority of bees to insecticides to date. As *A. mellifera* is currently used as the main surrogate species for non-Apis bees risk assessment around the globe, this lack of comparative sensitivity data generates uncertainty regarding the protectiveness of this approach [11-13].

The problem of limited toxicological information and the need for inter-species extrapolation, in the context of risk assessment, is not new and different approaches have been developed to address it in other areas including toxicology [14-16]. A straightforward method is cross species sensitivity extrapolation based on body weight,

which is current practice in other areas (e g. vertebrates) of PPP risk assessment (e.g. US EPA wildlife risk assessment [17]). In vertebrates, it is assumed and generally accepted that toxicity, similarly to other fundamental traits (e g. metabolic rate), is closely linked to the size and consequently weight of an organism with smaller ones being more susceptible [16, 18]. As many scaling relationships seems to apply to most living organisms [18-20] it is very likely that toxicity scaling extends beyond vertebrates and can be applied to bees as well.

Besides size, other factors likely play a role in shaping the sensitivity of an organism to a given substance. Such factors include extrinsic ones such as the type and properties of the substance (e g. water solubility) and intrinsic properties like receptor composition affecting molecule binding strength [21] and effectiveness of detoxification pathways [16, 21, 22]). Such intrinsic factors are likely linked to the organisms' evolutionary history (phylogeny) as more closely related species are often more similar in their traits compared to more distantly related ones [23]. This important and potentially confounding factor for such inter-species analyses has long been recognized [24], but only recently have authors started to directly address this issue in their ecotoxicological studies [25-28].

In this paper I use a phylogenetically informed analytical framework to build inter-species scaling models (ISMs) using acute contact toxicity for the currently most commonly used insecticide classes and bee body weight to investigate, 1) whether scaling relationships can be used to extrapolate bee sensitivity based on their body weight, 2) if scaling relationships differ between different classes and 3) to which degree scaling relationships are shaped by the underlying phylogenetic relationship among species. In a last step 4), I investigate the relative sensitivity of bees of the Apis

genus compared to other bees and discuss its consequences for surrogate bee species selection in the context of ecotoxicological risk assessment.

Materials and methods

Data collection

Between June 2017 and July 2019 I conducted a literature search used Google Scholar and Scopus (last search June 2019) and the search terms bee* AND tox* AND LD50. I screened the returned publications and their respective reference section for relevant publications. A paper was considered relevant if it met the following criteria: 1) reporting acute contact toxicity data (LD_{50}/bee) following direct exposure paradigm similar to the OECD 214 guidelines [29] (2) testing an insecticides (active substance or formulation) and 3) using a bee species (Apiformes) as test species. In case all criteria where met the results were included in the analysis.

Data selection and aggregation

All reported substance specific LD_{50} were categorized according to their mode of action (MoA [30]) and the four currently most commonly used insecticides were selected for further analysis [31-34]. The neonicotinoids were split between cyano- (c-) and nitro- (n-) substituted neonicotinoids, as it is known that bee species' responses can drastically differ between these two classes [22, 35, 36]. This resulted in five insecticide classes for analysis: acetylcholinesterase inhibitors (AChE-inhibitors), pyrethroids, c- and n-substituted neonicotinoids and organochlorines. After an initial screen one paper Del Sarto et al. [37] was excluded from further analysis because the results reported for *A. mellifera* deviated drastically (up to 700 times higher LD_{50}) from all other results reports for the same substance-species pair.

For each publication in the remaining dataset I calculated the geometric mean per chemical class resulting in one endpoint per data source (paper) and chemical class. As quality and study design drastically varies between publications this approach was chosen to limit the disproportionate influence of individual studies on the overall results without excluding data. For the same reason I calculated the geometric mean as the arithmetic mean is highly susceptible to outliers [38]. In a last step, I calculated the geometric mean per bee species for all insecticide classes which are the values used in the final analysis. For all bee species present in the final dataset I collected data on bee wet weight in milligram as a predictor variable for bee sensitivity. In cases where I did not find species-specific weight data or multiple reported values for a species, I used the average reported weight for the genus or the species respectively. The final dataset thus contained bee species, insecticide class, bee weight and LD_{50} values. Because I assumed a power law or power law like association between the variables commonly encountered in body mass associated traits including sensitivity to xenobiotics [18-20], all used acute contact LD_{50} values and bee wet weights were $\log_{10}$ transformed prior to analysis.

Phylogeny

For all bee species represented in the dataset I constructed an initial phylogeny using the “rotl” R package [39]. “rotl” uses data from the open tree of life project (https://tree.opentreeoflife.org) to reconstruct the phylogenetic relationship of the input species. Using this phylogeny as a back bone I used recent publications on bee phylogeny [40-44] to resolve some remaining polytomies. Because there are no consistent branch length information available for the resulting tree I Grafen-transformed [45] the tree using the R package “ape” [46].

Phylogenetic signal

In a first step I tested for phylogenetic signal in both bee weight and LD_{50} of the five insecticide classes separately. Phylogenetic signal is used as an indicator to see if more closely related species are more similar in the respective trait than expected by chance. I used the phylosig() function in the R package “phytools” using the “K” estimation method [47].

Interspecies scaling models (ISM)

A linear model (LM) was fit for every insecticide class independently with LD_{50} values as the response, bee wet weights as the predictor and number of publications as a weighting factor in order to account for the uneven sampling coverage between species (see Tab S1). In order to assess the robustness of the model outputs (e g. slope) the same models were 1) fitted without the weighting factor and 2) with the weighting factor but excluding *A. mellifera*. Function fitting was performed using ordinary least square regression implemented in the *lm* function in R. Residuals were inspected visually for normality (QQ plot), homogeneity of variances (residuals versus predicted plot).

Phylogentic ISM diagnostic

Following the procedures outlined by [48], I checked the residuals of each final linear model for phylogenetic signals using the phylosig() function in the “phytools” package [47] using the “K” method. In cases where a significant phylogenetic signal is detected it is suggested [48] to use a phylogenetically controlled general least square regression (PGLS) as implemented in e g. the “caper” package [49].

Weight corrected LD_{50} & relative sensitivity

In order to visualize and compare the LD_{50} across different insecticide classes and bee species I calculated the weight-corrected LD_{50} (LD_{50}/g bee wet weight) and tested if they exhibited phylogenetic signal and compared the results to the primary analysis of the corresponding uncorrected LD_{50} values. In order to assess the overall sensitivity of a given species across insecticide classes, I calculated the standardized (z-transformed) LD_{50}/g bee for all bee species, from now on referred to as relative sensitivity, for all five insecticide classes. This approach is necessary because different subset of bees have been sampled for the insecticide classes (Tab. S1) and if uncontrolled for one might simply compare the median size of bees tested per insecticide class instead of the actual toxicity (See Fig. S1 A & B). For all species where I had information on more than three insecticide classes, I additionally calculated their overall median standardized relative sensitivity.

Graphics

All analyses were conducted in R v 3.5.1 [50]. Graphs and trait mapping on the phylogeny were done using ggplot2 [51] and phytools [47], respectively.

Results

In total, I collected data from 55 data sources containing 343 reports of acute toxicity LD_{50} measurements. The final data set provided information for 28 bee species belonging to 12 genera spanning five of the seven currently recognized bee families [41]. However, the data was heavily skewed towards AChE-inhibitors (N = 164) and the model species *Apis mellifera* (N = 131). For a summary of the results and associated references see Tab. S1 & 2, ESM 1 & 2).

Phylogenetic signal

I found clear evidence for phylogenetic signal for bee weight ($K = 0.71$, $p = 0.001$) and the LD_{50} for AChE-inhibitors ($K = 0.3$, $p = 0.016$) suggesting that more closely related species are more similar for these two traits. In contrast I did not find evidence for a phylogenetic signal for any of the other insecticide class specific LD_{50} measurements (all $K < 0.6$, all $p > 0.32$).

Interspecies scaling models

In four out of five insecticide classes, I found evidence that bee LD_{50} are positively associated with body weight (all $R^2 > 0.27$ see Fig. 1A-C & E) meaning that heavier bees have higher LD_{50} (are less sensitive). This relationship was statistically significant for three of the classes (AChE-inhibitors, c-neonicotinoids and pyrethroids) and a trend for n-neonicotinoids ($p = 0.09$). For organochlorines I found no clear indication for an association ($R^2 = 0.12$, $p = 0.36$ Fig. 1D). When looking at the estimated regression slopes, I find that c-neonicotinoids likely scale allometrically with body weight (the 95% CI of slope did not overlap with 1) while the slope for all other insecticide classes was consistent with isometric scaling (95% CI of slope overlap with 1). The slope estimates were robust when removing *A. mellifera* or using an unweighted model (see Tab. S3). Except for organochlorines the 95% CI did not overlap with 0 further supporting an overall positive association between bodyweight and LD_{50} (see Tab. S3).

Phylogenetically controlled interspecies scaling models

I found no indication for phylogenetic signal in the model residuals (all $p > 0.32$ indicating that fitting a linear model without correcting for phylogenetic autocorrelation can be considered robust in this case [48] which rendered a phylogenetically controlled approach (e g. PGLS) unnecessary.

Weight corrected LD_{50} & relative sensitivity

When visualizing the weight-corrected LD_{50} (log_{10} LD_{50} / g bee) values of bee species across the different chemical groups, I found that at least some species of the *Apis* genus are always among the most sensitive (Fig. 2 A-E). While in some cases non-*Apis* bee species are more sensitive compared to Apis bees e g. *Scaptotrigona bipunctata* (see Fig 2A), *Megachile rotundata* (see Fig. 2B) and *Melipona scutellaris* (see Fig. 2E) it has to be noted that these values are based on single studies making these estimates less reliable (see Fig.S2). A notable exception to this overall picture were c-neonicotinoids (Fig. 2D) where *A. mellifera* was among the most resilient species together with *B. terrestris.*

When looking at the relative sensitivity, I found that overall bees of the *Apis* genus were the most sensitive (Fig. 2F, Tab.2) when considering all insecticide classes. In contrast bees of the genus *Bombus, Osmia and Trigona* rank among the more resilient bees found in this analysis (Fig.2, Tab.2 & Tab. S4). When looking at the neotropical stingless bees we find a diverse picture *Scaptotrigona* being more sensitive compared to other bees such as *Trigona*. However, these results are based on a very limited number of studies (Fig. S2E). For none of the insecticide classes I found significant phylogenetic signal for the weight corrected LD_{50} (All $p > 0.34$).

Discussion

The available data indicates that body weight, similarly to vertebrates [15, 16], is an important and often strong predictor of acute bee sensitivity towards insecticides. However, the strength of the scaling relationship (slope) can differ between insecticide classes. When taking body weight into account, *Apis mellifera* bees seem particularly susceptible to a range of common insecticide classes (with the notable exception of c-

neonicotinoids for *A. mellifera*), while *Osmia* and *Bombus* bees appear comparatively resilient.

Weight corrected LD_{50} Relative sensitivity & Phylogenetic patterns

When plotting the weight corrected LD_{50} measurements and the relative sensitivity on the phylogeny an interesting pattern emerged. In the majority of cases members of the Apis genus are among the most sensitive species present in the data set (see Fig. 2). The idea that *Apis* bees are particularly sensitive has been present in the literature for some time with some evidence supporting it [11-13, 52, 53]. It is tempting to speculate about the underlying cause for this phenomenon. One might argue that domestication and the strong anthropogenic driven selection for certain traits (e.g. increased honey yields and low aggression) might have unintentionally resulted in increased sensitivity to xenobiotics. However, the fact that not only the domesticated honeybee *A.mellifera*, but all members of the genus including feral ones (*e g. Apis florea*) share this increased susceptibility hints towards a different explanation. In contrast to most other bees all species of the *Apis* genus are highly eusocial [54-56]. This means that these species live in groups and reproduction is monopolized by a single female (queen) with all other members of the colony being non-reproductive helpers (workers; see [55]). Currently standard ecotoxicological toxicity testing is done using the worker class and several studies have shown that reproductive status strongly affects the bees' behavior and physiology including sensitivity to some toxic substances [57]. For example, *A.mellifera* workers have been reported to be up to 54 (mean 15) times more sensitive to some xenobiotics than queens [58]. While worker sterility might be part of the explanation of the elevated sensitivity compared to many solitary and therefore reproductively active bees (e g. *Osmia bicornis*), it might not fully explain why workers other semi- and eusocial worker bees (e g. *Trigona spinipe* and *B. terrestris*) appear resilient to a range

of insecticide classes (Fig. 2). However, in both case worker bees are known to reproduce and lay unfertilized eggs which develop into males [59, 60] in particular once the queen is absent [61] similarly to the standard test conditions in the laboratory [62, 63]. If reproductive activity provides some protection from adverse effects of insecticides (e g. improved detoxification) this might explain the observed elevated resilience. It might be a promising approach to investigate and compare the reproductive status of workers of other eusocial bee species in order to better understand the observed patterns of sensitivity.

In addition to this physiological explanation there is some genomic evidence suggesting that *A. mellifera*, and by extension likely additional members of the *Apis* genus, lack some substantial detoxifying enzymes (including glutathione-S-transferases, cytochrome P450 monooxygenases and carboxyl/cholinesterases) which are essential for detoxifying a range of xenobiotics including insecticides [53]. This line of evidence is supported by recent findings which pinpoint members of the P450 family as key determinates of bee sensitivity to different insecticides [22, 35, 36]. When taken together these results support the previous suggestion that *A. mellifera* is sensitive [11] and consequently a sensible option [12] to use as standard surrogate test species for contact scenarios during the risk assessment process.

Scaling patterns and scaling coefficients

Our results suggest that it is in principal possible to predict the acute sensitivity of bee species to insecticides based on their body weight. While this holds true for four out of five chemical classes of insecticides investigated, the organochlorines are a potential exception. It is possible that organochlorine acute toxicity in bees doesn’t scale with body weight at all, but given that many scaling relationships hold across a wider

taxonomic range [16, 19, 20, 64], it is more likely that this relationship is obscured by possible outliers likely caused by experimental conditions which is a substantial problem when overall sampling size is low. In order to resolve this issue, additional species that are not included in the current data set could be tested and/or outlier species have to be re-tested by independent labs.

However, when looking at the slope associated large confidence intervals it becomes clear that there is still a substantial amount of uncertainty associated with the estimated scaling coefficients (Tab.1 & Tab. S4) likely due to low sampling size and variation in data quality. Before such approaches can be considered for actual risk assessment purposes more work is needed to reduce the uncertainties associated with these models and test if its predictions hold for bee species not present in the current data set. In order to effectively improve the model predictions, it would be fruitful to focus future exploratory bee toxicity testing on 1) species- insecticide class combinations not present in the data set and 2) focus primarily on bee species occupying the edges of weight the parameter space (very small and very large bees) in order to limit the need of model extrapolation outside its parameter space.

Risk assessment considerations

Accurate sensitivity (hazard) characterization is at the heart of current ecotoxicological risk assessment [65]. In the case of bees, the honeybee has traditionally been used as a surrogate test species for this purpose. However, the relative sensitivity of honeybees compared to other bee species is not well characterized and consequently it is unclear if it is a suitable surrogate system for the approximately 20 00 bee species worldwide [54].

There have been two approaches to improve overall sensitivity characterization for non *Apis* bees: 1. Develop new model test species (e g. *Bombus terrestris* and *Osmia bicornis*) for lower tier screening or 2) better characterize the relative sensitivity of *A. mellifera* in relation to other bees and develop and appropriately conservative risk assessment based on the honeybee [11-13].

Our study suggests that the second approach would be feasible as *Apis* (including *A. mellifera*) worker bees, compared to other bees, appear to be highly sensitive to a range of different insecticide classes in acute contact scenarios. In combination with the extensive experience and resulting robust test methodology makes *A. mellifera* solid choice as screening level test species. In contrast the relative sensitivity data indicates that the two recently developed model species *Bombus terrestris* and *Osmia bicornis* for the European risk assessment process are comparatively resilient to a range of insecticides, which in turn means that including them in the screening step will likely not make the risk assessment more protective for bees from a sensitivity perspective [12, 13, 66, 67].

One could imagine a scenario, once robust scaling relationships have been established, to use scaling coeffects to extrapolate the sensitivity of an insecticide class to other bees based on their bodyweight and the respective honeybee sensitivity. This approach would circumvent the problem that it is unlikely to ever test enough bee species to adequately cover non Apis bee sensitivity (~20 000 species globally) and limit excessive bee testing necessary to cover all regulatory requirements.

Merging ecotoxicological and evolutionary thinking

When conducting comparative analyses, it is important to consider the non-independence of the different species due to their phylogenetic associations [24]. As

Revell [44] pointed out, the often used two-step-approach: 1. testing for a phylogenetic signal in the response or predictor variable and in case of a significant signal apply 2. phylogenetically controlled approaches (PIC, PGLS etc.) is misguided and does not address the underlying technical problem of correlated model residuals potentially violating model assumptions [48]. Revell rightly suggested to only control for phylogeny if the model residuals show phylogenetical signal violating LM assumptions. However, in case of limited sampling this approach will likely suffer from low power to detect phylogenetic signal. In such cases an evolutionary perspective becomes important when deciding the analytical approach. In a first step it should always be considered if we expect phylogenetic signal to occur. One important aspect in this regard is the scale of the analysis. When comparing more distantly related taxa it becomes more likely to find more pronounced differences in a trait of question and consequently phylogenetic signal. In the case of insecticides, we can observe such pattern for n-neonicotinoids. When comparing mammals and insects (phyla) we find clear sensitivity differences with mammals being less susceptible compared to insects [21]. This effect is likely driven by compound specific variation in the binding strength to the receptor [21]. However, when looking within insects (order) or even at lower taxonomic levels (e g. Apiformes) we find less pronounced absolute differences. In such a case it would make sense to use phylogenetically controlled approaches in the former, but less so in the latter case.

In the case of this analysis I found that both bee sensitivity (LD_{50}) to AChE-inhibitors and bee weight exhibit phylogentic signal while the weight corrected LD_{50} values do not. This suggests that the phylogenetic signal found for AChE-inhibitors sensitivity is likely driven by bee body weight. This finding, in combination with the lack of phylogenetic signal in the model's residuals, supports the use of a simple LM for the analysis [48]. However, it also shows the importance of using an evolutionary

framework when deciding on the most appropriate analysis for a given data set. Without such considerations one might be tempted to simply fit LMs, even when LM assumptions are violated, which could facilitate the misinterpretation of such data [48].

In recent years an increasing number of comparative statistical tools as well as phylogenetic information became available to researchers (e g. “rotl”, “phytools” and “ape” [39, 46, 47]). It is now technically feasible to conduct such analyses while addressing the potential pitfalls associated with inter-species analysis. When carefully thinking about the analytical approach these tools will increase the robustness and reliability of inter-species inference, which is particularly important and exciting for larger data sets. In the context of ecotoxicology the awareness for the need of a phylogenetic informed analytical framework has been recognized [25-27, 68] and the tools available will facilitate the further integration of evolutionary thinking in ecotoxicology.

Conclusions and future directions

1. When taking body weight into account, we find across most insecticide classes, genus *Apis* (including *A. mellifera*) seem particularly sensitive to a range of insecticides during contact exposure.

2. This pattern (see 1), emerging on a genus level, the clear association of bee weight and phylogeny and the association between AChE enhibitors phylogeny all highlights the importance of taking the evolutionary background of species into account when interpreting inter-species results.

3. Our results show that similarly to other biological systems, body weight is an important predictor of bee acute contact sensitivity to a range of insecticide classes and could be used to extrapolate bee sensitivity to non-tested species.

4. In order to improve the sensitivity prediction based on bodyweight it is important to focus future exploratory bee sensitivity testing afford. This afford should be focused on 1) so far untested species- insecticide class pairs 2) independent confirmation of extrema results ("potential outliers") and 3) on species on end of the weight spectrum (heavy and light) to minimize the necessity to extrapolate outside the parameter space.

5. In the future, I hope that toxicity data for other exposure pathways (oral, chronic and developmental) will become available which in turn will provide a more complete picture of bee sensitivity across the phylogeny and ultimately could improve the reliability of bee risk assessment.

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Figure legends

Figure 1: Depicts the association between the log_{10} transformed bee body weight and sensitivity for the five insecticide classes (LM regression line and 95%CI). We show this association for AChE inhibitors (A), pyrethroids (B), n-neonicotinoids (C), c-neonicotinoids (D) and organochlorines (E). The numerical results for the LM can be found in Table 1.

Figure 2: Depicts the log_{10} transformed LD_{50} per gram bee for the five insecticide classes plotted on the bee phylogeny: AChE inhibitors (A), pyrethroids (B), n-neonicotinoids (C), c-neonicotinoids (D) and organochlorines (E). In the last penal the median relative sensitivity (z-transformed $LD_{50\ per}$ gram bee) for all species where data on more than 2 insecticide classes was available (all relative sensitivity values can be found in Table S4).

Figure S1: Comparison of uncorrected (A) and weight corrected (B) sensitivity of bees to exposure of five different classes of insecticides.

Figure S2: Depicts the association between the log_{10} transformed bee body weight and sensitivity for the five insecticide classes (similar to Fig. 1) We show this association for AChE inhibitors (A), pyrethroids (B), n-neonicotinoids (C), c-neonicotinoids (D) and organochlorines (E). Circle sizes indicate the number of bees species samples for this data point.

Figure 1:

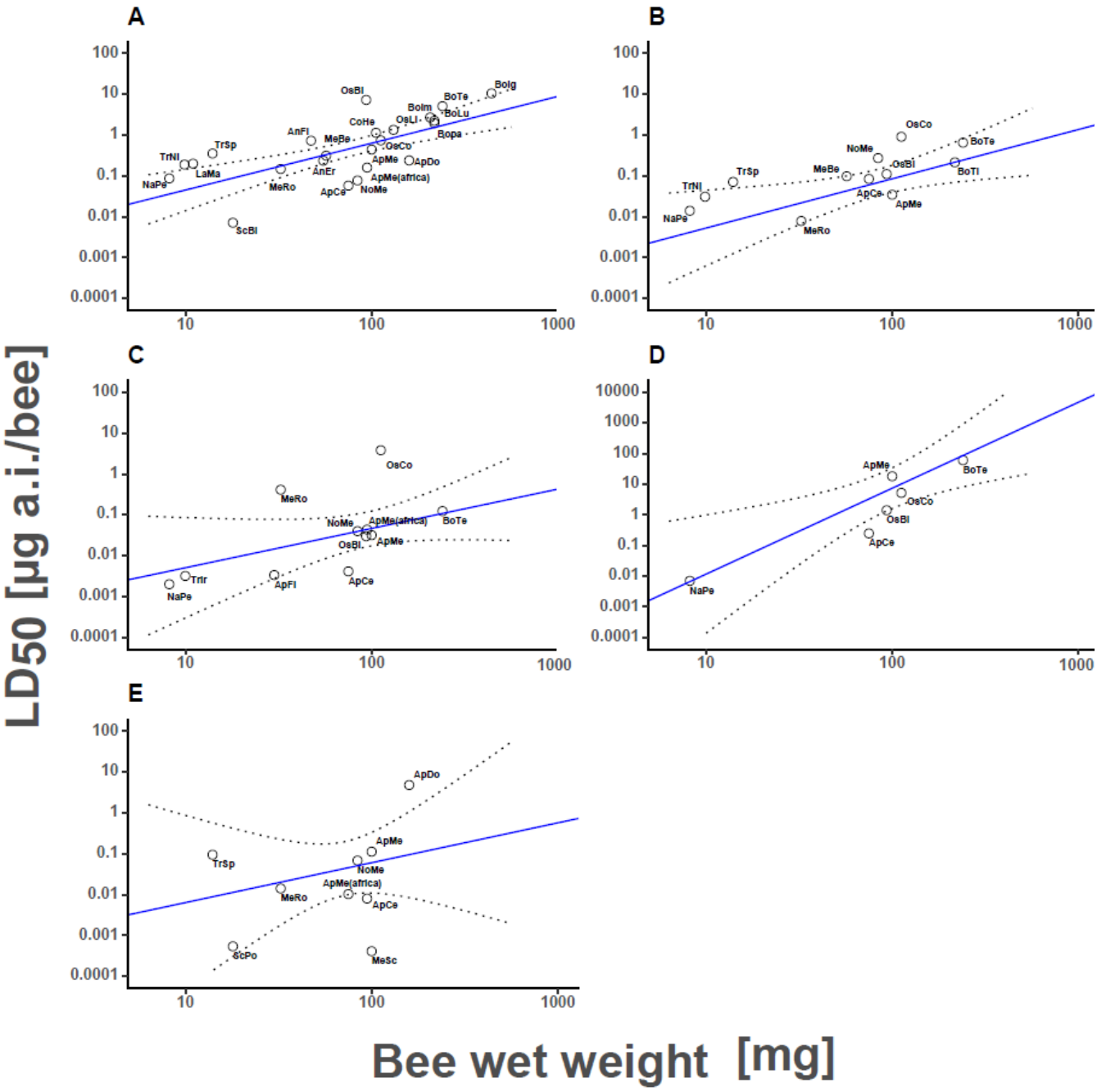
A
B
C
D
E
LD50 [µg a.i./bee]
Bee wet weight [mg]
OsBl
BoTe
BoIg
BoIm
BoLu
CoHe
OsLl
AnFl
MeBe
BoPa
OsCo
TrNl
TrSp
ApMe
ApDo
LaMa
AnEr
NaPe
MeRo
ApMe(africa)
ApCe
NoMe
ScBl
BoTl
TrIr
ApFl
ScPo
MeSc

Figure 2:

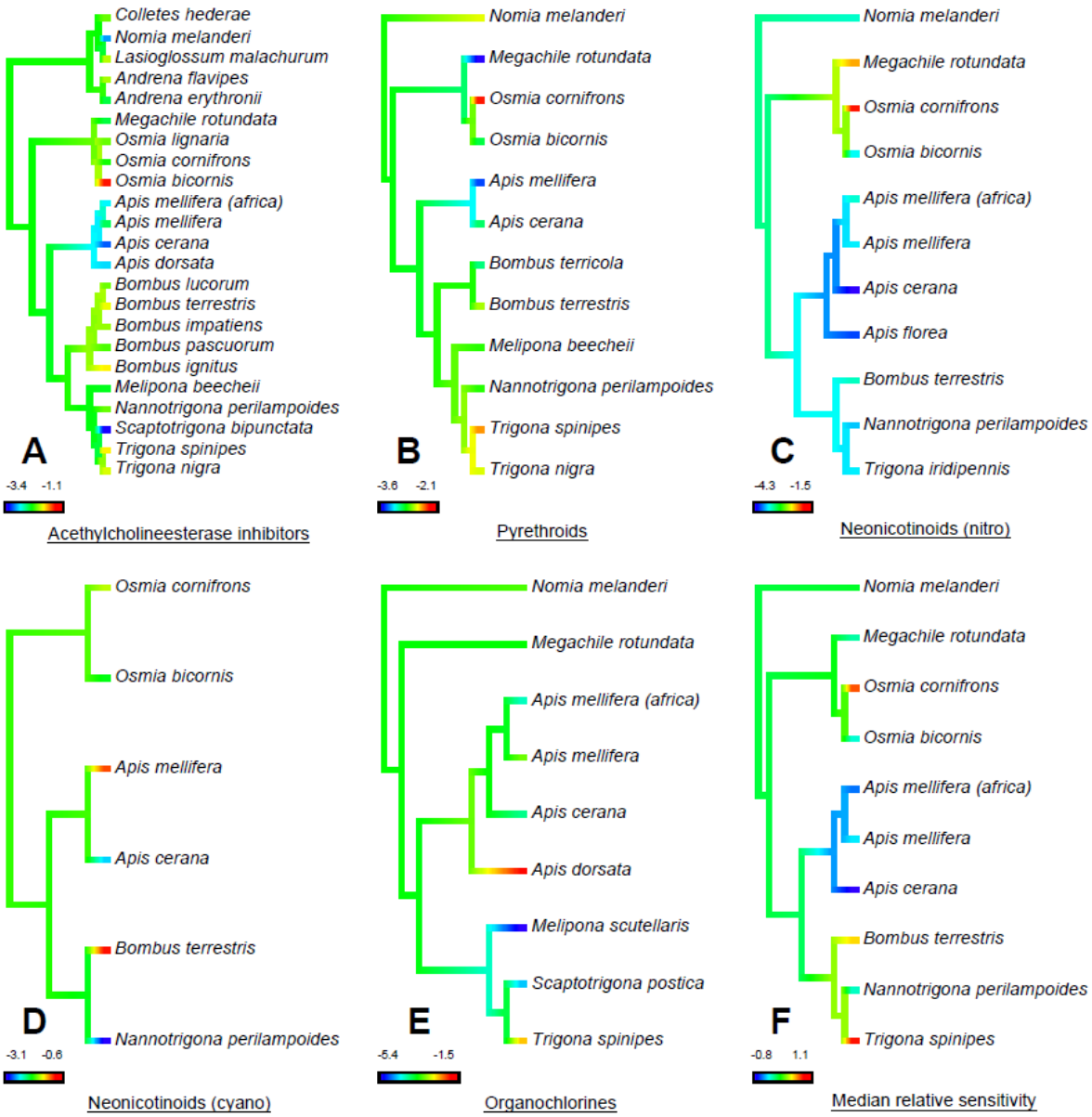
Colletes hederae
Nomia melanderi
Lasioglossum malachurum
Andrena flavipes
Andrena erythronii
Megachile rotundata
Osmia lignaria
Osmia cornifrons
Osmia bicornis
Apis mellifera (africa)
Apis mellifera
Apis cerana
Apis dorsata
Bombus lucorum
Bombus terrestris
Bombus impatiens
Bombus pascuorum
Bombus ignitus
Melipona beecheii
Nannotrigona perilampoides
Scaptotrigona bipunctata
Trigona spinipes
Trigona nigra
A
-3.4 -1.1
Acethylcholineesterase inhibitors
Nomia melanderi
Megachile rotundata
Osmia cornifrons
Osmia bicornis
Apis mellifera
Apis cerana
Bombus terricola
Bombus terrestris
Melipona beecheii
Nannotrigona perilampoides
Trigona spinipes
Trigona nigra
B
-3.6 -2.1
Pyrethroids
Nomia melanderi
Megachile rotundata
Osmia cornifrons
Osmia bicornis
Apis mellifera (africa)
Apis mellifera
Apis cerana
Apis florea
Bombus terrestris
Nannotrigona perilampoides
Trigona iridipennis
C
-4.3 -1.5
Neonicotinoids (nitro)
Osmia cornifrons
Osmia bicornis
Apis mellifera
Apis cerana
Bombus terrestris
Nannotrigona perilampoides
D
-3.1 -0.6
Neonicotinoids (cyano)
Nomia melanderi
Megachile rotundata
Apis mellifera (africa)
Apis mellifera
Apis cerana
Apis dorsata
Melipona scutellaris
Scaptotrigona postica
Trigona spinipes
E
-5.4 -1.5
Organochlorines
Nomia melanderi
Megachile rotundata
Osmia cornifrons
Osmia bicornis
Apis mellifera (africa)
Apis mellifera
Apis cerana
Bombus terrestris
Nannotrigona perilampoides
Trigona spinipes
F
-0.8 1.1
Median relative sensitivity

Figure S1:

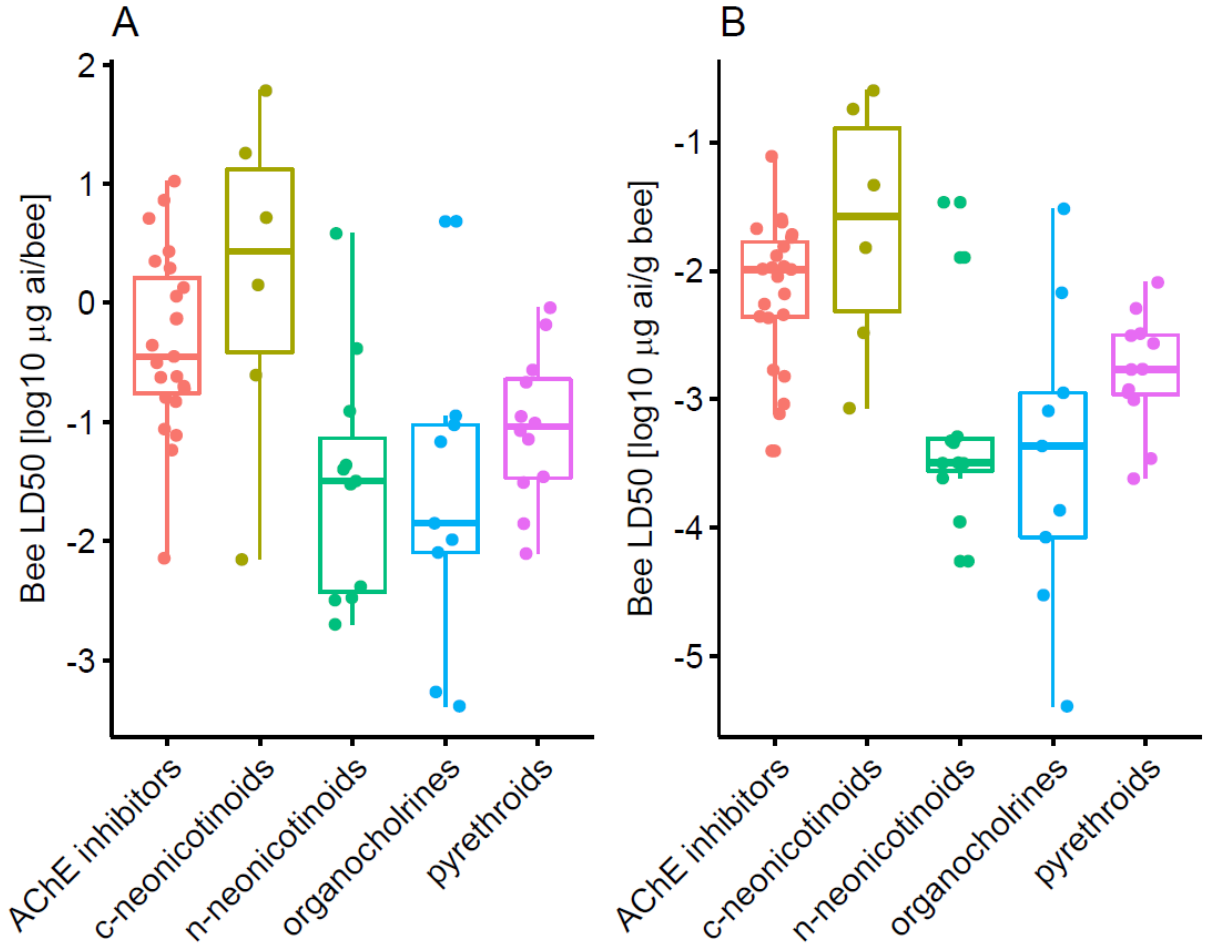
A
B
Bee LD50 [log10 µg ai/bee]
Bee LD50 [log10 µg ai/g bee]
2
1
0
-1
-2
-3
-1
-2
-3
-4
-5
AChE inhibitors
c-neonicotinoids
n-neonicotinoids
organocholrines
pyrethroids

Figure S2:

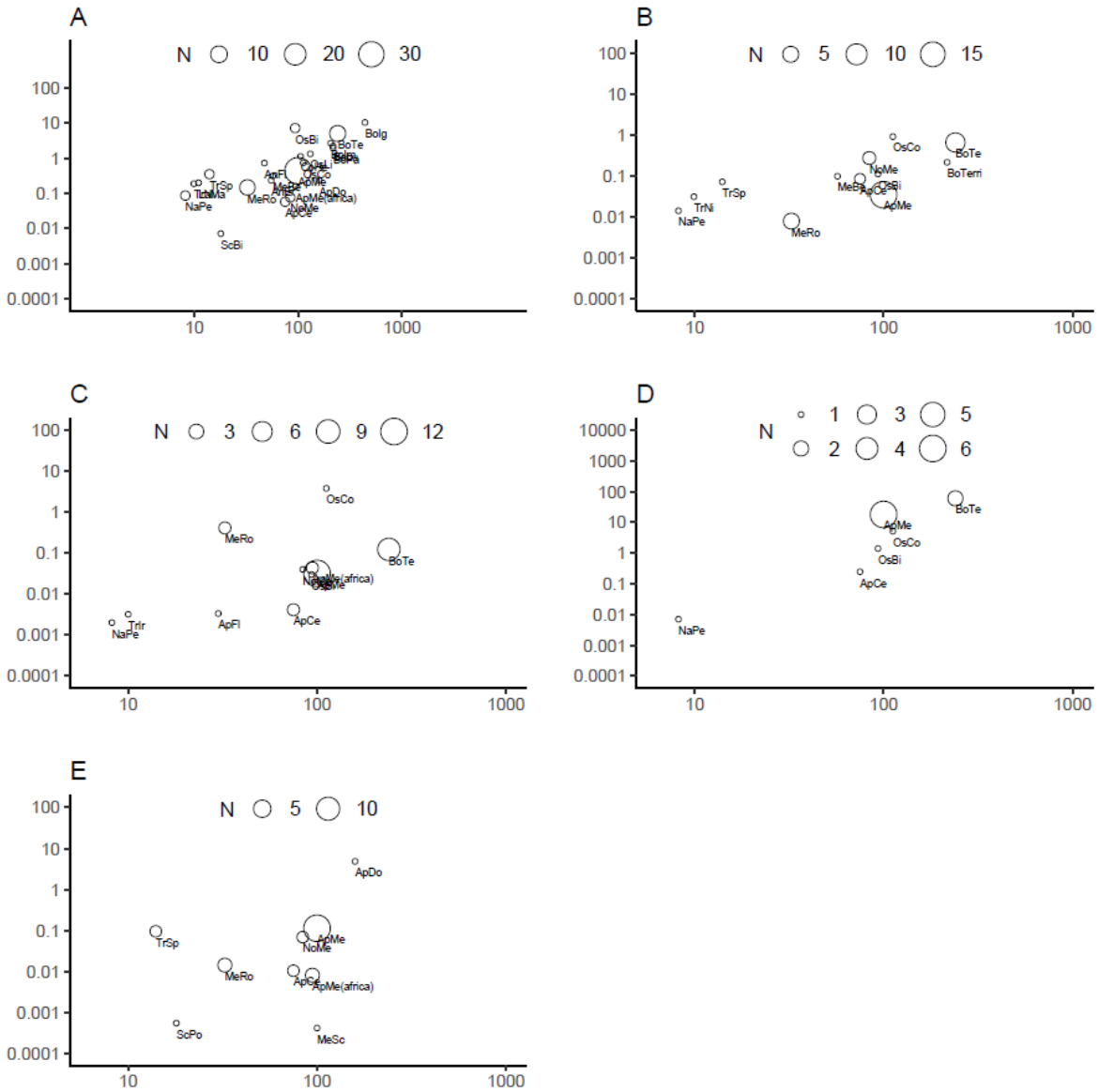
A
N 10 20 30
OsBi
BoIg
BoTe
TrSp
NaPe
MeRo
ApMe(africa)
ApCe
ScBi
B
N 5 10 15
OsCo
BoTe
BoTerri
NoMe
TrSp
TrNi
NaPe
MeRo
ApMe
C
N 3 6 9 12
OsCo
MeRo
BoTe
ApMe(africa)
TrIr
NaPe
ApFl
ApCe
D
N 1 3 5 2 4 6
BoTe
ApMe
OsCo
OsBi
ApCe
NaPe
E
N 5 10
ApDo
TrSp
ApMe
NoMe
MeRo
ApCe
ApMe(africa)
ScPo
MeSc
100
10
1
0.1
0.01
0.001
0.0001
10000
1000
10
100
1000

Table 1: Summary results of the LM fit to the log_{10} bee weight and sensitivity. We present samplesize (number of bee species), slope and 95% CI (Upper bound | Lower bound), R^2 and associated P values

MoA	N	slope (95%CI)	R^2	P
AChE	25	1.15 (1.65 | 0.65)	0.51	0.0001
Pyrethroids	12	1.21 (2.05 | 0.37)	0.46	0.016
n-Neonicotinoids	11	0.96 (2.00 | 0.00)	0.27	0.09
c-Neonicotinoids	6	2.80 (4.16 | 1.44)	0.81	0.014
Organochlorides	9	0.98 (2.98 | -1.02)	0.12	0.36

Table 2: Standardized relative sensitivty of bee species where informtaion for more than three MoA

Scientific Name	Relative sensitivity	N
Apis_cerana	-0.83	5
Apis_mellifera	-0.31	5
Apis_mellifera_(africa)	-0.54	3
Bombus_terrestris	0.69	4
Megachile_rotundata	-0.13	4
Nannotrigona_perilampoides	-0.21	4
Nomia_melanderi	0.10	4
Osmia_bicornis	-0.23	4
Osmia_cornifrons	0.95	4
Trigona_spinipes	1.07	3

Table S1: Summary statistics of the collected dataset regarding number bee species, substances and results found.

	Number (N)			
Chemical group	bee species	substances	data sources	endpoints reported
AChE Inhibitors	25	34	31	164
Pyrethroids	12	16	21	63
nAChR antagonist (nitro)	11	4	21	52
nAChR antagonist (cyano)	6	2	9	17
Organochlorines	9	7	16	47
total (unique)	29	63	98	343
Andrena erythronii				5
Andrena flavipes				1
Apis cerana				41
Apis dorsata				2
Apis florea				2
Apis mellifera				131
Apis mellifera (africa)				6
Bombus ignitus				1
Bombus impatiens				1
Bombus lucorum				3
Bombus pascuorum				3
Bombus terrestris				34
Bombus terricola				6
Colletes hederae				1
Lasioglossum malachurum				1
Megachile rotundata				33
Melipona beecheii				3
Melipona scutellaris				1
Nannotrigona perilampoides				7
Nomia melanderi				20
Osmia bicornis				13
Osmia cornifrons				5
Osmia lignaria				3
Scaptotrigona bipunctata				2
Scaptotrigona postica				1
Tetragonista fibrigi				2
Trigona iridipennis				2

Trigona nigra	3
Trigona spinipes	10

Table S2: Wet weight of bees in mg and associated references the full list is found in ESM 2.

Scientific Name	wet weight [mg]	reference
Andrena erythronii	55	Helson et al. 1994
Andrena flavipes	47.3	Uhl et al. 2016
Apis cerana	75	Thompson 2016. Yasuda 2017 (citing Sasaki 1999)
Apis dorsata	159	Tan 2007 Apido
Apis florea	30	Kumar and Regupathy 2005
Apis mellifera	100	Steiner et al. 2012
Apis mellifera (africanized)	94.5	BASF (unpublished)
Bombus ignitus	440	BASF (unpublished)
Bombus impatiens	206	BASF (unpublished)
Bombus lucorum	217	Bombus mean
Bombus pascuorum	217	Bombus mean
Bombus terrestis	240	BASF (unpublished)
Bombus terricola	217	Bombus mean
Colletes hederae	105.5	Uhl et al. 2016
Lasioglossum malachurum	11	Uhl et al. 2016
Megachile rotundata	32.5	Klostermayer 1973, Thompson 2017 (Helson 1994)
Melipona beecheii	57	Valdovinos-Nunez et al. 2009
Melipona scutellaris	100.0	Lourenco et al. 2012
Nannotrigona perilampoides	8.2	Valdovinos-Nunez et al. 2009
Nomia melanderi	84	Lourenco et al. 2012
Osmia bicornis	93.4	Uhl et al. 2016 (female)
Osmia cornifrons	112	Uhl et al. 2016
Osmia lignaria	131	Bosch et al. 2010
Scaptotrigona bipunctata	18	Hardfelder & Engels 1992 Insec Soc (Scaptotrigona postica)
Scaptotrigona postica	18	Hardfelder & Engels 1992 Insec Soc (Scaptotrigona postica)
Tetragonista fibrigi	3.4	Grüter et al. 2012 PNAS (Teragonista angustula fibrigi)
Trigona iridipennis	10	Kumar and Regupathy 2005
Trigona nigra	9.9	Valdovinos-Nunez et al. 2009
Trigona spinipes	14	Macieira and Hebling-Beraldo 1989

Table S3: Assessment of the robustness of the slope estimates for each mode of action for the implemented linear models ("main model": including weights = number of publications all data). I compared it to 1) the estimates of a model without weights ("excluding weights") and 2) to a model including weights after the removal of *Apis mellifera* ("excluding *A. mellifera"*).

MoA	main model	excluding weights	excluding *A. mellifera*
AChE	1.15 (1.65 | 0.65)	1.04 (1.54 | 0.54)	1.23 (1.73 | 0.73)
Pyrethroids	1.21 (2.05 | 0.37)	0.89 (1.45 | 0.33)	1.35 (1.97 | 0.73)
n-Neonicotinoids	0.96 (2 | 0)	1.28 (2.48 | 0.08)	0.98 (2.06 | -0.10)
c-Neonicotinoids	2.80 (4.16 | 1.44)	2.66 (3.72 | 1.60)	2.69 (3.59 | 1.79)
Organochlorides	0.98 (2.98 | -1.02)	1.1 (3.52 | -1.32)	0.33 (2.63 | -1.97)

Table S4: Standardized (z-transformed) relative sensitivity values of bees (per gram) for the five investigated insecticide classes.

Scientific Name	abbrev.	AChE Inhib.	Pyrethroids.	n-Neonicotinoid	c-Neonicotinoid.	Organochlorines
Andrena erythronii	AnEr	-0.36				
Andrena flavipes	AnFl	0.63				
Apis cerana	ApCe	-1.71	-0.37	-1.23	-0.83	-0.36
Apis dorsata	ApDo	-1.18				1.62
Apis florea	ApFl			-0.86		
Apis mellifera	ApMe	-0.34	-1.51	-0.31	0.96	0.41
Apis mellifera (africa)	ApMe(africa)	-1.09		-0.12		-0.54
Bombus ignitus	BoIg	0.97				
Bombus impatiens	BoIm	0.50				
Bombus lucorum	BoLu	0.32				
Bombus pascuorum	BoPa	0.21				
Bombus terricola	BoTi		-0.49			
Bombus terrestris	BoTe	0.88	0.50	-0.06	1.10	
Colletes hederae	CoHe	0.35				
Lasioglossum malachurum	LaMa	0.76				
Megachile rotundata	MeRo	-0.32	-1.87	1.62		0.06
Melipona beecheii	MeBe	-0.17	0.05			
Melipona scutellaris	MeSc					-1.65
Nannotrigona perilampoides	NaPe	0.34	0.04	-0.45	-1.43	
Nomia melanderi	NoMe	-1.57	0.67	-0.10		0.29
Osmia bicornis	OsBi	1.89	-0.31	-0.31	-0.15	
Osmia cornifrons	OsCo	-0.03	1.56	2.14	0.35	
Osmia lignaria	OsLi	0.31				
Scaptotrigona bipunctata	ScBi	-2.22				
Scaptotrigona postica	ScPo					-0.92
Trigona nigra	TrNi	0.80	0.63			
Trigona spinipes	TrSp	1.02	1.11			1.07
Trigona iridipennis	TrIr			-0.31		