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Hazard/Risk Assessment

Comparing Sensitivity of Different Bee Species to Pesticides: A TKTD modeling approach

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Abstract: Risk assessment for bees is mainly based on data for honey bees; however, risk assessment is intended to protect all bee species. This raises the question of whether data for honey bees are a good proxy for other bee species. This issue is not new and has resulted in several publications in which the sensitivity of bee species is compared based on the values of the 48-h median lethal dose (LD50) from acute test results. When this approach is used, observed differences in sensitivity may result both from differences in kinetics and from inherent differences in species sensitivity. In addition, the physiology of the bee, like its overall size, the size of the honey stomach (for acute oral tests), and the physical appearance (for acute contact tests) also influences the sensitivity of the bee. The recently introduced Toxicokinetic–Toxicodynamic (TKTD) model that was developed for the interpretation of honey bee tests (Bee General Uniform Threshold Model for Survival [BeeGUTS]) could integrate the results of acute oral tests, acute contact tests, and chronic tests within one consistent framework. We show that the BeeGUTS model can be calibrated and validated for other bee species and also that the honey bee is among the more sensitive bee species. In addition, we found that differences in sensitivity between species are smaller than previously published comparisons based on 48-h LD50 values. The time-dependency of the LD50 and the specifics of the bee physiology are the main causes of the wider variation found in the published literature. *Environ Toxicol Chem* 2024;43:1431–1441. © 2024 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Bee sensitivity; General Uniform Threshold Model for Survival (GUTS); Modeling; Pesticides; ToxicoKinetic–ToxicoDynamic model (TKTD)

INTRODUCTION

The environmental risk assessment for bees is mainly based on acute test data for honey bees. In a first-tier approach, an assessment factor of 10 is applied to the honey bee data to protect other bee species (European Food Safety Authority [EFSA], 2012). This raises the question of whether data for honey bees can be used as a starting point and whether such data are indeed protective for other bee species with an assessment factor of 10. This question is not new and has resulted in several publications in which the sensitivity of bee species is compared, for example, Arena & Sgolastra (2014), Hardstone & Scott (2010), Thompson & Pamminger (2019), and Uhl et al. (2016). In these publications, the 48-h median lethal dose (LD50) values from acute test results were used for the comparison of species sensitivity. These tests are carried out in a similar way for different species and are based on the acute oral test for honey bees (Organisation for Economic Co-operation and Development [OECD], 1998a), or the acute contact test for honey bees (OECD, 1998b). A chronic oral test (OECD, 2017a) is also available, but this is not frequently used, and bee sensitivity comparisons based on chronic tests are only reported for a limited number of species and/or compounds (Baas et al., 2022; Heard et al., 2017).

The typical end result of both the acute oral and the acute contact test is a 48-h LD50. In an acute oral test, the bees take a few hours to eat contaminated food (usually after a short starvation period), which is then followed by an observation period

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in which effects on mortality are scored. In an acute contact test, usually the compound of interest is dissolved in a carrier solvent, like acetone, a 1- or 2-μL droplet is put on the ventral thorax of the bee, and then the observation period starts. The calculated LD50 is subsequently based on the assumption that the exposure concentration is constant over time for all tests and all species.

However, it has been shown that the exposure concentration is not constant in the observation period but declines over time (Haas et al., 2021; Hillier et al., 2013; Suchail et al., 2004; Zaworra, Koehler, Schneider, Lagojda, & Nauen, 2019). This implies that the LD50 value is only valid for the test it was derived from. The decline over time is likely to be different for different species and will depend on factors like the overall size of the bee, the size of the honey stomach (for acute oral tests), and the physical appearance of the bee. In addition, tests with relatively small bees, like *Megachile*, are compared with the approximately 30 times larger bees (on a weight basis) from the *Bombus* family, based on 48-h LD50s. Observed differences in 48-h LD50s may, therefore, result from differences in physiology, kinetics, and the inherent differences in species sensitivity (Ashauer & Escher, 2010; Jager et al., 2006).

Therefore, comparisons of LD50 values for calculating the sensitivity of bee species must be carried out with greater care. Also, a comparison of the sensitivity of various bees based on 48-h LD50s is likely to be biased and therefore not a proper method by default. An assessment based on chronic LD50s will be better, because in a chronic test the concentration is constant over time; the bees are fed contaminated food with a constant concentration during the entire observation period (taking out part of the differences in physiology). However, these data are scarce, and the requirement remains to separate kinetic effects from dynamic effects.

To overcome these types of issues, a toxicokinetic–toxicodynamic (TKTD) model was developed for the interpretation of bee tests, based on the General Uniform Threshold Model for Survival (GUTS; EFSA et al., 2018; Jager et al., 2011; the BeeGUTS model: Baas et al., 2022). This model allows for interpretation of the results of the acute oral, the acute contact, and the chronic oral tests within one consistent modeling framework with one set of parameter values. The most important parameter, describing the inherent sensitivity of bees, is the effect threshold (Baas & Kooijman, 2015; Heard et al., 2017; Jager et al., 2006). This parameter is a time- and test-independent TD parameter, reflecting the sensitivity of a species to chemicals. Thus this parameter is a more robust proxy for the sensitivity of bees than an LD50. The LD50 proved to be test dependent, as was shown by Baas and coworkers (2022) In addition, the LD50 proved to be strongly time dependent, and the time-dependence itself proved to be species and compound dependent, as was shown by Heard and coworkers (2017).

The TKTD approach has been applied, calibrated, and validated for honey bees, but so far the approach has not been applied to species other than honey bees. Therefore, the aim of our study was to apply, calibrate and possibly validate the BeeGUTS model for different bee species and to compare intrinsic sensitivities of bees within the TKTD framework.

METHODS

Modeling approach

In a standard approach whereby a 48-h LD50 is derived from an acute test, the assumption is that the exposure concentration is constant over time and that the exposure concentration is the driving force for effects. The exposure concentration combined with observation on survival at 48 h are input for the model, and the output is the 48-h LD50. With the BeeGUTS model the approach is very similar; the exposure concentration is still the driving force for effects and thus also the input for the model, combined with the observed survival pattern over time. The main differences are that the exposure concentration does not need to be constant over time but depends on the test and species and that survival is followed over time.

Acute contact test. In an acute contact test, the compound of interest is dissolved in a "carrier solvent" and applied as a droplet on the dorsal side of the thorax of the bee. The concentration on the bee declines over time (Haas et al., 2021; Zaworra et al., 2019). The decline proved to be remarkably similar for different compounds and can be described with a first-order process and the default value for the rate constant (the contact uptake availability rate constant [k_{ca}]) for honey bees is 0.4 d^{-1} (Baas et al., 2022).

Acute oral test. In an acute oral test, the bees are starved before the start of a test, and the compound of interest is administered via the food during a 3 to 4-h exposure period. Subsequently, the bees are fed noncontaminated food for the remaining observation period during which effects are observed. The assumption is that the toxicant is taken up in the honey stomach during the exposure period and that the concentration then declines over time with a first-order rate constant, which is governed by the volume of the honey stomach and the feeding rate. For honey bees with a honey stomach volume of approximately 40 μL and a feeding rate of approximately 25 μL/day, this leads to a default value for the honey stomach release rate (k_{sr}) of 0.675 d^{-1} (Baas et al., 2022).

Chronic test. In a chronic test, the bees are fed contaminated food from the start with a fixed concentration, so the concentration is assumed to be constant over time (Baas et al., 2022).

Short description of the BeeGUTS model

An elaborate description of the BeeGUTS model can be found in Baas et al. (2022). The model was evaluated, calibrated, and validated for honey bees based on chronic test results combined with acute oral and acute contact tests (Baas et al., 2022). Figure 1 shows a schematic of the model.

The TK part of a TKTD model describes the uptake of the compound of interest. Subsequently, the TD part links the uptake to effects by assuming a so-called death mechanism (Ockleford et al., 2018; Jager et al., 2011). Two different mechanisms are distinguished: the Stochastic Death (SD)

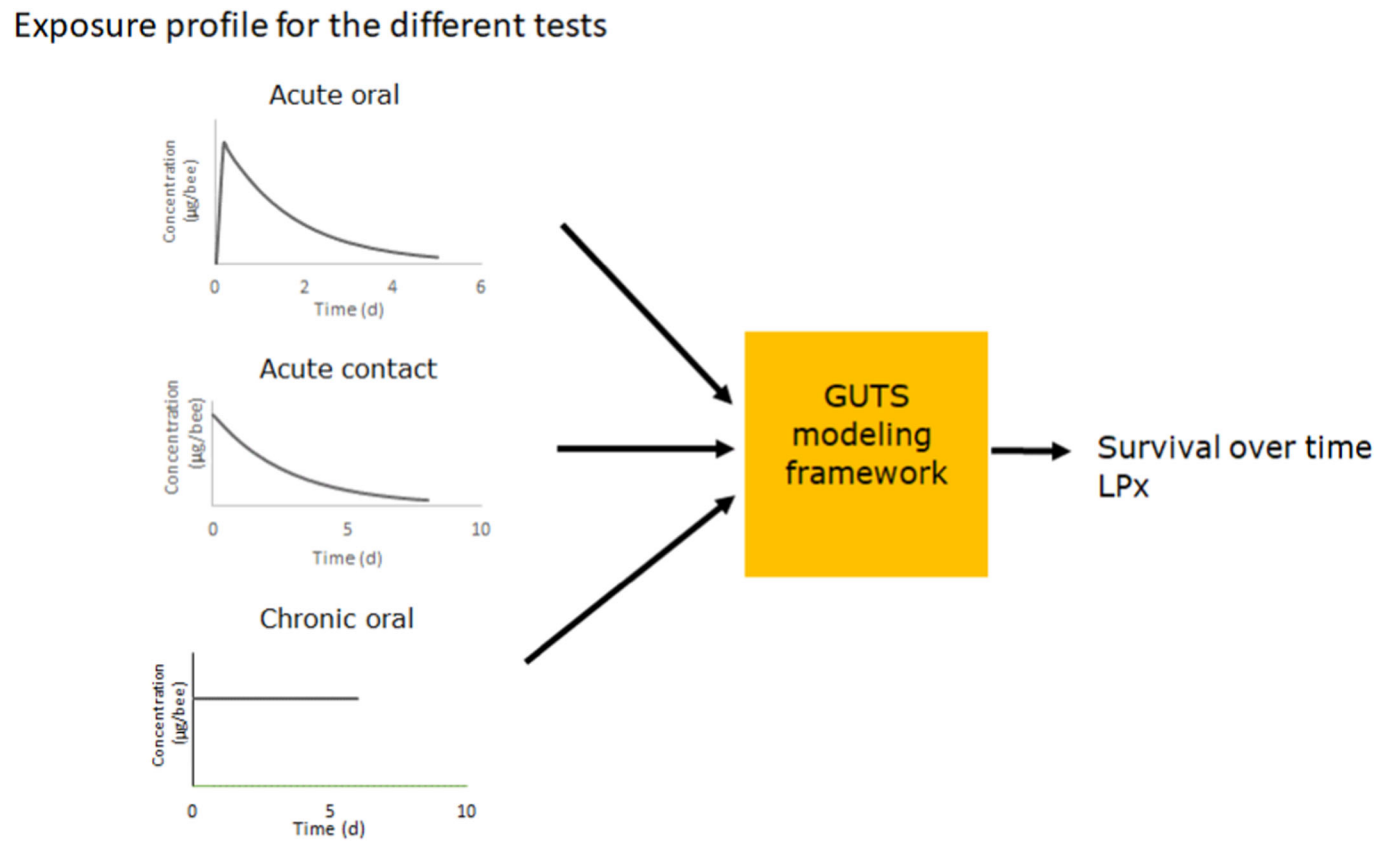

FIGURE 1: Model outline: the time-dependent exposure profiles for acute oral, acute contact, and chronic tests feed into the General Uniform Threshold Model for Survival (GUTS) framework with survival over time and LPx values as output. The effective concentration is determined by the test and the physiology of the bee. LPx, the factor by which an entire exposure profile needs to be multiplied to yield *x*% lethality by the end of the exposure.

model and the Individual Tolerance (IT) model. The different models have their own assumptions, but both models have the effect threshold as a parameter. It is current practice to run both models and take the results of the most conservative model for further assessment. More detailed information can be found in the references just mentioned.

Calibration and validation of the model for other bee species

The procedure is to calibrate the model (i.e., run the model and estimate parameter values) and check whether the goodness-of-fit parameters are within the requirements set out in the EFSA scientific opinion on TKTD modeling (Ockleford et al., 2018). If this is the case, these parameters are used to predict the effects with the time-variable exposure concentrations and also to check whether the requirements on goodness-of-fit parameters are met. For calibration, a data set with at least seven points in time is required. For validation of the model, an additional time-dependent exposure is required, preferably a repeated pulse exposure (Ockleford et al., 2018). These requirements applied to bee testing imply that a chronic test is perfectly suited for calibration of the model. Also, for validation of the model, at least one acute test is needed, preferably a combination of an acute oral test and an acute contact test. This implies that the values of k_{sr} and k_{ca} must be known for the species of interest for validation of the model, or they must be estimated from an independent source. An elaborate description of the calibration and validation procedures with the goodness-of-fit parameters can be found in the Supporting Information.

Calibration data for B. terrestris and O. bicornis. Limited data are available for non-*Apis* bee species. A search in the US Environmental Protection Agency ECOTOX database (carried out in February 2021) on all bee species combined produced a total of 2542 hits. Of all the entries, 86% were for species from the *Apis* family, 6% for the *Bombus* family, 2% for the *Megachile* family, a little over 1% for the *Osmia* family, and the remaining 5% for all other bee species combined. Nearly all reported endpoints are single-timepoint LD50s or no-observed-effect concentrations, and are therefore insufficient to calibrate and validate a TKTD model. However, an elaborate study on chronic effects was carried out at the Centre for Ecology and Hydrology in the United Kingdom for *Apis mellifera*, *B. terrestris*, and *O. bicornis* (Heard et al., 2017). The sensitivity of these species was evaluated for five different pesticides and two metals. These data were kindly made available and were combined with other available data to successfully calibrate the model for *B. terrestris* and *O. bicornis*.

Validation data for B. terrestris. It proved remarkably difficult to find direct data on the size of the honey stomach for bumble bees. The website Bumblebee.org has extensive data on all aspects of bumblebees and gives a range for the size of the honey stomach of 60 to 200 µL. A recent study by Pattrick and coworkers (2020) measured the volume that was eaten by foraging bumblebees, giving a range of 52 to 163 µL. Probably the honey stomach was not completely filled during foraging and therefore a default setting for the honey crop volume of 200 µL appears to be a reasonable assumption. A typical feeding rate is approximately 200 µL/day (Stabler et al., 2015), leading to a honey stomach release rate of 1.0 day^{-1} as a default setting for bumble bees. Note that the default setting should be treated as a first estimate that can be over-ruled if dedicated data are available. The k_{ca} value was assumed to be identical to that of the honey bee (0.4 d^{-1}).

Acute test data for *B. terrestris* used for validation of the model were made available by Bayer Crop Sciences (report 16796 for the Acute Oral data and report 17116 for the Acute Contact data). With these settings, the model could be successfully validated for *B. terrestris*.

Validation data for O. bicornis

For *O. bicornis* data, the size of the honey stomach could not be retrieved from the literature. In addition, *Osmia* species in general are somewhat smaller than honey bees and have more body hair, which might influence the values of k_{ca} and k_{sr}. We therefore used an alternative approach and derived these rate values from the available survival data. Acute contact and acute oral test data for dimethoate were made available by Wageningen University and Research (The Netherlands). These data were generated for a ring test on effects of dimethoate on *O. bicornis*. In addition, we used raw data for acute contact tests had been published by Uhl and coworkers (2019).

Acute oral exposure

To accurately describe acute oral uptake, an estimation must be made of the size of the honey stomach. It has been reported that some species of the *Osmia* family feed their larvae with pollen, and some feed with nectar (Kemp & Bosch, 2005). This indicates that several species of the solitary bees have the possibility of storing nectar for later use, probably similar to the honey stomach in an *Apis* bee species. However, the size of the honey stomach is unclear. Therefore, the honey stomach release rate (k_{sr}) was estimated in a three-step procedure: 1) the model was calibrated with the chronic data, which have a constant concentration over time; 2) the calibrated model was used to estimate the value of k_{sr} that gave the best fit with the Wageningen University and Research data; and 3) the model was subsequently validated with these parameter settings together with the Uhl et al. (2019) data.

This gave a best estimate of 1.5 d^{-1} for k_{sr}. With the known feeding rate, the honey stomach of *O. bicornis* was estimated to have a volume of 20 µL, or approximately half the size of that of a honey bee (see Supporting Information for a more detailed description of the data). Based on the size and biology of *O. bicornis*, this was considered a plausible volume for the honey stomach. It is striking that information on the physiology of a bee can be obtained solely from on survival data.

Acute contact exposure for O. bicornis

Because independent data on uptake over the chitin layer are missing, the combined chronic and acute data were used to estimate the value of the k_{ca}, in the same three-step approach used to derive the value for k_{sr}. This gave a best estimate of 2.0 d^{-1} for k_{ca}, which is substantially higher than that of the honey bee; we are not certain why. The more bristle-like hairs on *O. bicornis*, which give a larger surface area, might increase the decline rate in the concentration on the bee. When dedicated experimental data are available, they should be preferred over the default setting because this value is rather uncertain.

With these settings, the model could be validated successfully for *O. bicornis*.

k_{sr} and k_{ca} values for B. terrestris and O. bicornis species

Table 1 gives an overview of the k_{sr} and k_{ca} values, for interpretation of acute oral and acute contact tests for different species.

TKTD PARAMETERS DERIVED FROM PUBLISHED STUDIES

The model outline and the settings of the parameters for interpretation of acute tests is given in Section 2 of the Supporting Information, in which the model is applied to the different available studies and the parameters are estimated. The data published by Heard et al. (2017) and Baas et al. (2022; both studies only for honey bees) are the starting point for comparison of the sensitivities of different species of bees. The available data for the different compounds on honey bees were complemented as far as possible by data on different species of bees generated in our own laboratories and from the literature.

Bombus terrestris

Comparison of chronic data on Dimethoate. There are three independent sources for chronic tests for dimethoate: 1) the aforementioned data generated by Heard et al. (2017), 2) a

TABLE 1: Honey stomach release rate and contact availability rate constant that could be derived for different species

Species	Honey stomach release rate (k_{sr}) d^{-1}	Contact availability rate constant (k_{ca}) d^{-1}
Apis mellifera	0.675	0.40
Bombus terrestris	1.0	0.40
Osmia bicornis	1.5	2.0

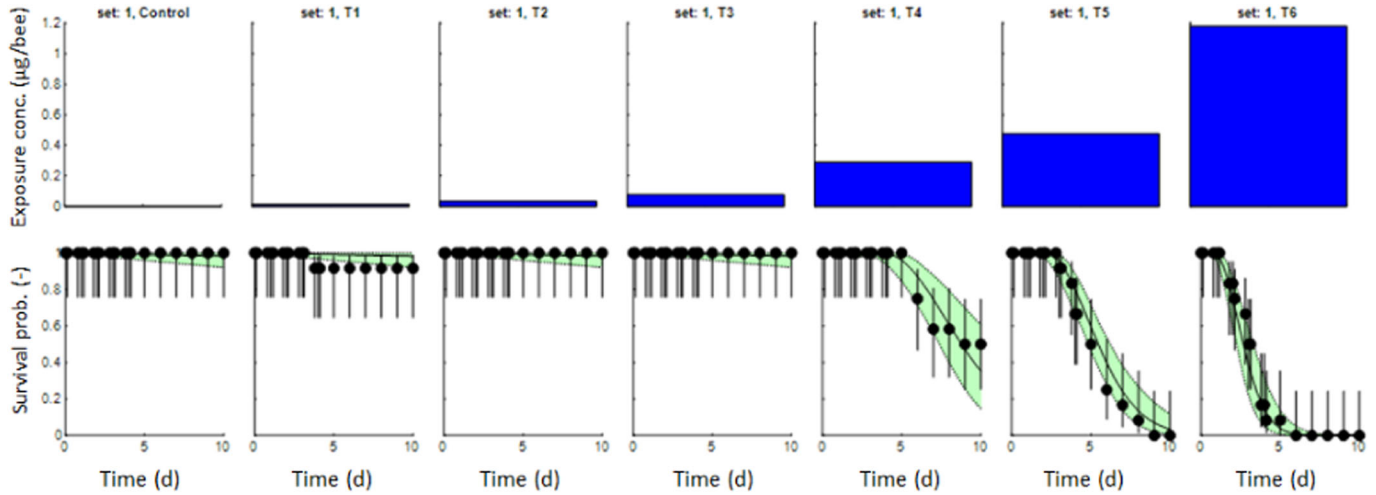

FIGURE 2: Calibration of the stochastic death (SD) model for *Bombus terrestris* exposed to dimethoate. **Top panels**: exposure concentrations for control and treatments T1–T6; **bottom panels**: observations on survival (dots) and model prediction (line) with the 95% confidence interval (green area). Data taken from Heard et al. (2017).

poster presented at SETAC (Cornement et al., 2017), that allows for an extraction of the raw data and a parameter estimate for dimethoate. 3) Dimethoate chronic test data, generated for a ring test, were made available by IBACON.

As an example, Figure 2 shows the calibration of the SD model on the data generated by Heard et al. (2017). The figure shows the general good quality of the observed survival data against the modeled survival data. Table 2 gives a comparison of the results for dimethoate chronic tests. The table shows that the model fits the data, with R^2 values > 0.94, and all calibrations are compliant with EFSA guidelines on TKTD modeling (see the Supporting Information). The three data sets also give comparable parameter values (except for the IT model result for the data generated by Heard et al. [2017]), giving confidence in the repeatability of the test and the quality of the execution of the test by the three laboratories. Apparently, the details of the test have a distinct effect on the rate of effects development, which is reflected in the larger differences in the 48-h LD50s compared with the differences in the effect threshold (mw) and the 10-day LD50.

Additional data for B. terrestris. Chronic test data have been published for 2,4-D, chlothianidin, dimethoate, propiconazole, and tau-fluvalinate (Heard et al., 2017). These data were interpreted with a TKTD model (basically the SD model), and the sensitivitie were compared (Table 3).

Bayer made raw data from acute tests available for deltamethrin, imidacloprid, methiocarb, and tetranilliprole (Supporting Information). The parameters derived from these tests are listed in Table 4.

All results comply with the quality standards put forward in the EFSA guidance document on TKTD modeling; however, parameter estimates can be different with the IT or SD model. Usually, large differences in values for the effect threshold indicate that the parameter values are not very well fixed by the data. In these cases, the SD model data are more plausible.

Other species. The data generated by Heard et al. (2017) for *O. bicornis* have already been mentioned. Very limited additional data could be generated due to lack of a dose–response curve (Uhl et al., 2019; Reid et al., 2020) or lack of raw data (Valdovinos-Núñez et al., 2009). Soares et al. (2015) published a study with acute oral and acute contact data for the effects of imidacloprid on *Scaptotrigona postica*. The acute contact data were extracted from that publication and assessed with the BeeGUTS model. The oral data could not be used because

TABLE 2: Summary of results for dimethoate chronic tests for Bumble bees

	BeeGUTS			Calculated LD50s	
Reference	Model	R^2	mw µg/bee	2-day LD50 µg/bee	10-day LD50 µg/bee
Heard et al. (2017)	SD	0.975	0.11 (0.001–0.20)	1.8 (1.4–2.7)	0.26 (0.19–0.31)
Cornement et al. (2017)	SD	0.995	0.17 (0.15–0.18)	0.90 (0.85–0.96)	0.23 (0.22–0.24)
iBACON	SD	0.982	0.11 (0.084–0.12)	0.22 (0.20–0.27)	0.12 (0.097–0.12)
Heard et al. (2017)	IT	0.945	0.0045 (0.0039–0.13)	1.4 (1.2–1.6)	0.28 (0.24–0.33)
Cornement et al. (2017)	IT	0.989	0.13 (0.095–0.15)	0.85 (0.81–0.89)	0.23 (0.22–0.24)
iBACON	IT	0.973	0.097 (0.085–0.11)	0.24 (0.22–0.26)	0.10 (0.096–0.11)

The data in parentheses represent 95% confidence interval.
BeeGUTS = Bee General Uniform Threshold Model for Survival; iBACON = Institute for Biological Analytics and Consulting; IT = Individual Tolerance; LD50 = median lethal dose; mw = effect threshold; SD = stochastic death.

TABLE 3: Effect threshold in μg/bee for different species

Compound	*A. mellifera* (weight 100 mg)	*B. terrestris* (weight 170 mg)	*O. bicornis* (weight 69 mg)
Dimethoate	0.049	0.079	0.029
Chlothianidin	0.0064	0.0075	0.013
Tau-fluvalinate	8.1	20.4	1.5
Propiconazole	>35	>150	>120
2,4-D	100	>486	345

Data are from Heard et al., 2017.

feeding rates were not available. The results are summarized in Table 5. The quality of the fit is well within the boundaries of the EFSA guideline on TKTD modeling for acceptable results.

Ansell et al. (2021) compared data on imidacloprid, permethrin, and dimethoate for *M. rotundata* and *A. mellifera*. Raw data are available in the Supporting Information on permethrin and imidacloprid. The imidacloprid data were assessed with the BeeGUTS model and gave a best estimate for the effect threshold of 3.9 (0–9.7) ng/bee. The permethrin data were not further assessed because (raw) data on permethrin are not available for other species.

BEE SENSITIVITY COMPARISON

Direct comparison based on the effect threshold

With the data available, four different bee species and seven different compounds were part of the comparison. In Table 6, the available data for each species/compound combination are summarized. The data indicate that the honey bee is consistently among the more sensitive species; see Figure 3, where these results are shown on a relative scale, with the effect threshold of honey bees set to a value of 1.

Weight-corrected comparison

Weight is known to be a contributing factor to bee sensitivity, for example, Devillers et al. (2003), Thompson (2016), and Uhl et al. (2016), so in Table 7 the results are presented on a weight basis. In Figure 4, the weight-corrected results are shown on a relative scale, with the weight-corrected effect threshold of honey bees set to 1.

TABLE 4: Summary of parameter values for the compounds with additional data

Species	Compound	Model	Effect threshold μg/bee	R^2
Bombus terrestris	Deltamethrin	SD	5.1	0.965
B. terrestris	Deltamethrin	IT	3.9	0.957
B. terrestris	Imidacloprid	SD	0.030	0.988
B. terrestris	Imidacloprid	IT	0.0003	0.981
B. terrestris	Methiocarb	SD	0.062	0.906
B. terrestris	Methiocarb	IT	0.061	0.848
B. terrestris	Tetranilliprole	SD	No evaluation	—
B. terrestris	Tetranilliprole	IT	No evaluation	—

IT = Individual Tolerance Model; SD = Stochastic Death Model.

TABLE 5: Imidacloprid parameter estimates for *Scaptotrigona postica*.

Parameter	SD model	IT model
Dominant rate constant (1/day)	>6.0[a]	0.70 (0.18–1.5)
Effect threshold (ng/bee)	<12.1[a] (2.8)	11.6 (3.7–20.2)
Killing rate (1/[ng/bee day])	0.027 (0.19–0.41)	
F values		>3.8[a] (8.0)
Control mortality rate	<0.007[a] (0.0053)	<0.07[a] (0.052)
R^2	0.984	0.994

[a]The parameter-estimating procedure runs into boundaries set in the model as default parameters.
Data are from Soares et al., 2015. Data in parentheses are 95% confidence interval. F = dimensionless distribution parameter in the effect threshold; IT = Individual Tolerance Model; SD = Stochastic Death Model.

Comparison of species sensitivity based on the effect thresholds

In five of eight cases, *Apis mollifera* was overall the most sensitive species, so there are three compounds for which the honey bee is not the most sensitive species (see Figures 3 and 4), but the differences in sensitivity are generally small. *O. bicornis* appears to be the most sensitive species for tau-fluvalinate by approximately a factor of 5 when the standard data are used, and a factor of 4 when the weight-corrected data are used. In addition, *B. terrestris* was slightly more sensitive to methiocarb than the honey bee, and this difference became larger when corrected for weight. *Megachile rotundata* was more sensitive, with an mw value of 3.9 (0–9.7) for imidacloprid, than the honey bee without weight corrections (see the Supporting Information). The best estimate of the mw value for honey bees is 9.8 (3.9–14) ng/bee (Baas et al., 2022). An independent test carried out by the UK Department for Environment, Food, and Agricultural Affairs (DEFRA, 2007) gives an mw value of 4.9 (1.9–7.9) ng/bee (Baas et al., 2022). So overall, the differences are small, and the confidence intervals do overlap, so it is questionable whether this difference is real. However, the comparison is based on the best estimates. For tau-fluvalinate, there is no additional information on confidence intervals, only best estimates are published (Heard et al., 2017), so a further evaluation of these data is not possible.

When the comparisons are corrected for weight, the honey bee is found to be more sensitive for imidacloprid than *M. rotundata*. The honey bee and the bumble bee have a comparable sensitivity toward chlothianidin (0.006 μg/bee for honey bees vs. 0.008 μg/bee for bumble bees), but on a weight basis the bumble bee becomes more sensitive; also here the differences are small and confidence intervals overlap.

Arena and Sgolastra (2014) defined the sensitivity ratio (*R*) which is the *A. mellifera* LD50 divided by the non-*Apis* LD50. For our data set based on the effect threshold, *R* ranges from 0.08 to 5.4 (compare the range reported by Arena & Sgolastra, which was 0.001–2086). The results we present show that the honey bee is consistently among the most sensitive species and when an assessment factor of 6 is used, the honey bee data are protective for the other bee species for which data were available. So the current assessment factor of 10 that is used to extrapolate honey bee sensitivity to protect other bee species is protective for all the species and compounds in this comparison.

TABLE 6: Summary of effect thresholds, calculated with the Stochastic Death model for different species/compound combinations (µg/bee)

Compound	Honey bee	Bumble bee	*Osmia bicornis*	*Osmia cornuta*[a]	*Scaptotrigona postica*	*Megachile rotundata*
Dimethoate	0.014	0.13	0.029	0.029		
Clothianidin	0.006	0.008	0.013			
Deltamethrin	0.60	5.1				
Imidacloprid	0.0098	0.030			0.12	0.0039
Methiocarb	0.070[b]	0.062				
Tau-fluvalinate	8.1	20.4	1.5			
2,4-D	100	>486	345			

[a]Based on Wageningen University and Research ring test data.
[b]Derived from Bayer report 308072 Acute oral test.

Is the honey bee a good predictor for the sensitivity of other species?

Many more (chronic) tests are available for honey bees than for other bee species. The chronic tests are particularly interesting because they are very suitable for the derivation of TKTD parameter values. The acute tests were never designed to be interpreted with a TKTD model, and in most cases when the test is stopped at 48-h, parameter estimates are problematic.

Thus honey bee data can be used to get a preliminary estimate of the sensitivity (i.e., the effect thresholds) of other bee species in a three-step approach: 1) estimate the TKTD parameters for honey bees, preferably based on a chronic test, 2) convert the effect threshold to an effect expressed/gram bee, and 3) take the weight of the bee of interest and calculate the threshold.

With the available data, this approach was used to calculate the effect thresholds for all compounds derived from chronic honey bee tests that were published by Baas et al. (2022); the results are summarized in Table 8.

With the available thresholds derived from the available data, a comparison could be made for a limited number of compounds; the results were normalized for the honey bee and are summarized in Table 9. Unfortunately, the data set is rather limited but the predictions based on the honey bee results are generally conservative.

DISCUSSION

Several comparisons on bee sensitivity for pesticides have been published (Ansell et al., 2021; Arena & Sgolastra, 2014; Devillers et al., 2003; Heard et al., 2017; Thompson, 2016; Uhl et al., 2019) and (Uhl et al., 2016; only dimethoate). Generally, these studies addressed the question of whether the sensitivity of honey bees is a reasonable proxy for other bee species. The general conclusion (based on 2-day LD50 values) of these studies was that the honey bee is a reasonable proxy for the sensitivity of other bee species.

The most extensive comparison was carried out by Arena & Sgolastra (2014). They performed a meta-analysis on available

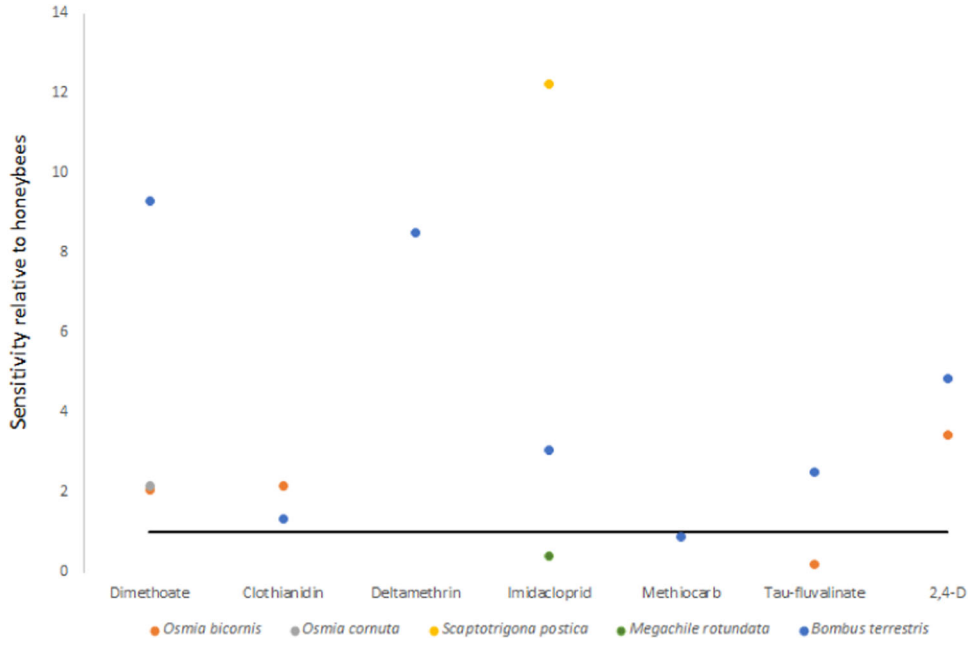

FIGURE 3: Comparison of sensitivity of different bee species based on g/bee normalized for honey bees, shown on a relative scale, with the effect threshold of honey bees set to a value of 1 (effect threshold bee/honeybee).

TABLE 7: Summary of effect thresholds corrected for the weight of the bee for different species/compound combinations (µg/g bee)

Compound	Honey bee	Bumble bee	*Osmia bicornis*	*Osmia cornuta*	*Scaptotrigona postica*	*Megachile rotundata*
Dimethoate	0.14	0.43	0.41	0.41		
Clothianidin	0.06	0.047	0.19			
Deltamethrin	6.0	17				
Imidacloprid	0.098	0.10			0.40	0.30
Methiocarb	0.70	0.21				
Tau-fluvalinate	81	120	22			
2,4-D	1000	>2850	5149			

The weights for the honey bees and bumble bees were taken from Thompson (2016). Honey bee, 100 mg; *Bombus*, 300 mg; *Osmia*, 70 mg (average male and female; Uhl et al., 2016); weight of *S. postica* 0.03 g; weight of *M. rotundata* 13 mg (Ansell et al., 2021).

LD50 data in the open literature, covering 19 species of bees and over 50 pesticides from 150 studies. They concluded that an assessment factor of 10 applied to the honey bee test results would be protective for all bee species in 95% of all cases. Their analysis was purely based on LD50s and was not weight corrected. In contrast, Thompson (2016) re-evaluated their data and corrected for weight, which lowered the assessment factor to 5 (i.e., in 95% of the cases, the honey bee results are protective for all other species with an assessment factor of 5). This dependence on weight was also reported by several other researchers. Devillers and coworkers (2003) found, for instance, a negative correlation of species sensitivity with weight, which was confirmed by Pamminger (2021), Ansell and coworkers (2021), and Thompson (2016).

The differences in relative sensitivities reported by Arena and Sgolastra can be large, as reflected in their range of *R* values (0.001–2086). These reported differences in sensitivity are much larger than the range reported in our study (*R* values between 0.08 and 5.4). Their reported wide range has contributions from several sources, as outlined in the following sections.

When more than one entry was available, the lowest value was taken for further evaluation

This can result in unrealistic differences in species sensitivity, as can be illustrated with dimethoate for an example. Dimethoate is probably the most frequently measured compound in bee testing because it is prescribed to be used as a positive control (OECD, 1998b), and the 48-h LD50 value should be between 0.10 and 0.30 µg/bee to accept the result of an acute contact test. In the original data used by Arena & Sgolastra (2014), four independent entries for acute contact test results for honey bees exposed to dimethoate were available, ranging from 0.0014 to 0.31 µg/bee. The lowest, but not realistic, value 0.0014 µg/bee (originally published by Torchio in 1973), was taken for further evaluation. When this low value is then compared with a realistic 48-h LD50 value for *Osmia lignaria* (1.21 µg/bee), the result is a large assumed difference in sensitivity; in this case the honey bee is estimated to be a factor of 864 times more sensitive than *O. lignaria*. Based on the OECD test results, this would be a factor of approximately 6.

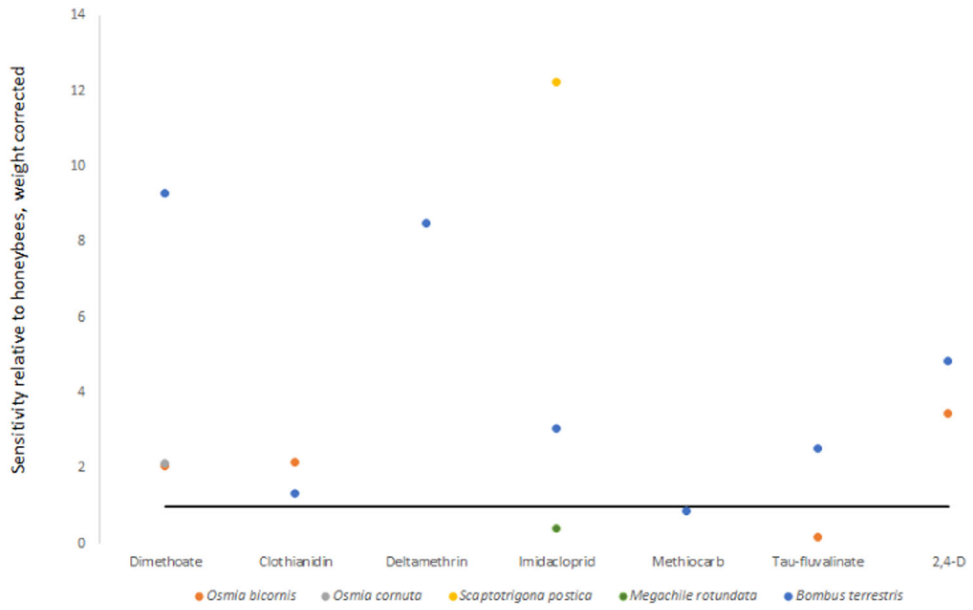

FIGURE 4: Comparison of sensitivity of different bee species based on g/g bee, normalized for honey bees. The weight-corrected results are shown on a relative scale, with the weight-corrected effect threshold of honey bees set to 1.

TABLE 8: Prediction of effect thresholds for different species of bees based on available chronic data for the honey bee (μg/bee)

Compound	Starting value Honey bee	Bumble bee	*Osmia bicornis*	*Osmia cornuta*	*Scaptotrigona postica*	*Megachile rotundata*
Beta-cyfluthrin	9.7	29	6.8	6.8	2.9	1.3
Bromoxynil	29	87	20	20	8.7	3.8
Dimethoate	0.014	0.042	0.0098	0.0098	0.0042	0.0018
Clothianidin	0.006	0.018	0.0042	0.0042	0.0018	0.0008
Deltamethrin	0.60	1.80	0.42	0.42	0.18	0.08
Fenamidone	0.32	0.96	0.22	0.22	0.096	0.042
Imidacloprid	0.0098	0.029	0.0069	0.0069	0.0029	0.0013
Metribuzin	5.1	15	3.6	3.6	1.5	0.66
Thiacloprid	0.82	2.5	0.57	0.57	0.25	0.11

Effect thresholds derived from chronic data for honey bees taken from Baas et al., 2022.

TK effects are likely to have played a role

Only the temporal aspects of the LD50s can lead to different conclusions, as shown in Table 10. Here, the honey bee is the most sensitive species at all time points, but an evaluation based on the 48-h LD50 gives a different interpretation of the differences than an evaluation based on 240-h LD50s. The example shown in Table 10 also shows that the differences become smaller over time, which is a kinetic effect and not an effect based on the intrinsic toxicity of the different species to dimethoate. For the larger *B. terrestris*, the difference in the 48-h LD50s and the 240-h LD50 is larger (7.2) than for the honey bee (4.8) and *O. bicornis* (5.6), suggesting that the standard test duration of 48 h is generally too short to reach the incipient LD50 for larger bees (including *Apis*) but might be sufficient for smaller bees, like *Megachile*. Just the size of the smaller bees implies that uptake and elimination kinetics will be faster, so the incipient LD50 in a test is reached earlier. This effect is enhanced by the experimental procedures in acute contact testing: the pesticide is dissolved in a carrier solvent, and a small drop of the solution is administered to the bee. For *Megachile* bees, there is no guideline, but this droplet size is usually 1 μL, and is also 1 μL for the much larger *Apis* (OECD, 1998b) and 2 μL for *Bombus* (OECD, 2017b; although in practice for *Bombus* a droplet size of 5 μL is frequently used). So, in a *Megachile* test not only is the bee smaller, which leads to faster kinetics, but in addition, a larger relative amount of surface area is covered, also leading to faster kinetics. In practice, this implies that the 48-h exposure period of a *Megachile* bee does not equal the 48-h exposure period of an *Apis* or *Bombus* species. Or, in other words, when the kinetics are removed, the differences in species sensitivity between the species become significantly smaller.

Exposure patterns are species dependent

The comparisons made in the literature do not take into account that the exposure patterns differ for different species. When an LD50 is calculated from an acute test, it is assumed that the exposure is constant in the test. but we have shown in our study that the exposure-related model parameters (expressed in the k_{sr} and k_{ca} values) are species dependent. Therefore, comparing different bee species based on 48-h LD50s has fundamental issues.

Bee sensitivity comparison based on the BeeGUTS approach

The approach we propose for species comparisons overcomes these issues and, in addition, has some other advantages, because it allows us to predict results from exposure that can take place under field-realistic exposures for a variety of different bee species. Here, the differences in the "threshold for effects" parameter allows for a fair and direct comparison of bees' sensitivity.

Finally, we have shown that physiological parameters (like the size of the honey stomach for *Osmia*) can be calculated solely from observation on survival when acute test data are combined with chronic test data.

CONCLUSIONS

A new way of comparing the sensitivities of bees to pesticides was developed based on the BeeGUTS TKTD model. The physiology of the bees and the specifics of the tests are taken into account. Important parameters for the model, like the size of the honey stomach, which plays a key role in the interpretation of an acute oral test, and the contact availability

TABLE 9: Comparison of calculated and actual effect thresholds for different species of bees, normalized for the honey bee (μg/bee)

Compound	Honey bee	Bumble bee	*Osmia bicornis*	*Osmia cornuta*	*Scaptotrigona postica*	*Megachile rotundata*
Dimethoate	1	3	234	234		
Clothianidin	1	2.3	3.1			
Deltamethrin	1	2.8				
Imidacloprid	1	1.0			41	3.1

TABLE 10: LD50s calculated with the Stochastic Death model from the toxicokinetic–toxicodynamic parameters for different bee species for dimethoate for different points in time

	Apis mellifera		*Bombus terrestris*		*Osmia bicornis*	
Time (h)	LD50 (ng/bee)	LD50 normalized to honey bee	LD50 (ng/bee)	LD50 normalized to honey bee	LD50 (ng/bee)	LD50 normalized to honey bee
24	289	1	5300	18.3	1890	6.5
48	101	1	1800	17.8	677	6.7
96	43	1	659	15.3	290	6.7
240	21	1	249	11.9	121	5.8

The data are based on chronic studies (so constant exposure) by DEFRA (*Apis mellifera*) and Heard et al., 2017 (*Bombus terrestris* and *Osmia bicornis*).
LD50 = median lethal dose.

uptake rate, could be estimated for different bee species. Also, direct data on the size of the honey stomach of *O. bicornis* were not available, and the model could be used to estimate its value purely from survival data. The model was calibrated and validated for *B. terrestris* and *O. bicornis* with a combination of chronic and acute data. For *O. cornuta*, *M. rotundata*, and *S. postica* the model could be calibrated and not formally validated.

The threshold for effects is the most important parameter of the BeeGUTS model, because this is a time- and test-independent measure for the intrinsic toxicity of a compound for a bee. Effect thresholds were estimated from acute and chronic data sets for nine different pesticides and five different bee species. These results showed that the honey bee is generally among the more sensitive species, and an assessment factor of 6 on the honey bee threshold for effects is protective for other bee species. This can be reduced to an assessment factor of 4 when the weight of the bees is taken into account.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5871.

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Data Availability Statement—All data in the main text and the Supporting Information are taken from the original references. Raw data from the Bayer reports will be made available on request to Bayer. The model description and parameter estimates are described in detail in the Supporting Information.

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