Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–10, 2023
Received: 28 April 2023 | Revised: 14 June 2023 | Accepted: 22 August 2023

Hazard/Risk Assessment

Sensitivity of the Neotropical Solitary Bee *Centris analis* F. (Hymenoptera, Apidae) to the Reference Insecticide Dimethoate for Pesticide Risk Assessment

Rafaela Tadei,[a,b,]* Vanessa B. Menezes-Oliveira,[c] Claudia I. Silva,[d] Elaine C. Mathias da Silva,[e] and Osmar Malaspina[a]

[a]Institute of Biosciences, São Paulo State University, Rio Claro, Brazil
[b]Department of Environmental Sciences, Federal University of São Carlos, Sorocaba, Brazil
[c]Course Coordination on Environmental Engineering, Federal University of Tocantins, Palmas, Tocantins, Brazil
[d]Consultoria Inteligente em Serviços Ecossistêmicos, Sorocaba, Brazil
[e]Department of Biology, Federal University of São Carlos, Sorocaba, Brazil

Abstract: Currently, only *Apis mellifera* is used in environmental regulation to evaluate the hazard of pesticides to pollinators. The low representativeness of pollinators and bee diversity in this approach may result in insufficient protection for the wild species. This scenario is intensified in tropical environments, where little is known about the effects of pesticides on solitary bees. We aimed to calculate the medium lethal dose (LD50) and medium lethal concentration (LC50) of the insecticide dimethoate in the Neotropical solitary bee *Centris analis*, a cavity-nesting, oil-collecting bee distributed from Brazil to Mexico. Males and females of *C. analis* were exposed orally to dimethoate for 48 h under laboratory conditions. Lethality was assessed every 24 h until 144 h after the beginning of the test. After the LD50 calculation, we compared the value with available LD50 values in the literature of other bee species using the species sensitivity distribution curve. In 48 h of exposure, males showed an LD50 value 1.33 times lower than females (32.78 and 43.84 ng active ingredient/bee, respectively). *Centris analis* was more sensitive to dimethoate than the model species *A. mellifera* and the solitary bee from temperate zones, *Osmia lignaria*. However, on a body weight basis, *C. analis* and *A. mellifera* had similar LD50 values. Ours is the first study that calculated an LD50 for a Neotropical solitary bee. Besides, the results are of crucial importance for a better understanding of the effects of pesticides on the tropical bee fauna and will help to improve the risk assessment of pesticides to bees under tropical conditions, giving attention to wild species, which are commonly neglected. *Environ Toxicol Chem* 2023;00:1–10. © 2023 SETAC

Keywords: Apidae; Ecological risk assessment; Ecotoxicology; Insecticide; LC50; LD50; Oral exposure; Pesticide regulation; Species sensitivity distribution (SSD)

INTRODUCTION

In recent years, critiques and suggestions have been made by several scientists to improve the environmental regulation of pesticide use and hence maximize the protection of native species (Fisher, 2021; More et al., 2021; Topping et al., 2020). For bee populations, the environmental risk of pesticides can be assessed by considering data from the effects posed by the pesticides on bees and information about their exposure to the contaminant. Toxicological endpoints, such as lethal dose (LD), are used in risk-assessment approaches to evaluate the effects of pesticides on bee populations (Cham et al., 2020; European Food Safety Authority [EFSA], 2013). These endpoints may be calculated through laboratory assays, following standard protocols such as those from the Organisation for Economic Co-operation and Development (OECD; OECD, 2013, 2017).

The bee model species used for pesticide evaluation is *Apis mellifera*, a generalist and social bee. During toxicological experiments, the OECD requests a test using dimethoate as a positive control (toxic reference) to validate the assay. Dimethoate is an organophosphorus insecticide that presents robust toxicity data, that is, with a low intraspecific variation of its effects on the exposed bees (Gough et al., 1994).

Although *A. mellifera* is considered a model species for bee species, a sensitivity variation between different bee species

---

This article includes online-only Supporting Information.
* Address correspondence to rafaela.tadei@unesp.br

DOI: 10.1002/etc.5738

can occur during their exposure to the insecticide dimethoate (Uhl et al., 2016) or other pesticides (Arena & Sgolastra, 2014). Hence, to improve environmental risk assessment (ERA) and include pesticide sensitivity interspecific variations, regulators apply a safety factor of 10 to extrapolate the results of the pesticide effects obtained using the model species *A. mellifera* to other bee species. This means that it is assumed that non-*Apis* species are 10 times more sensitive to pesticides than the model species (Arena & Sgolastra, 2014; Cham et al., 2020; EFSA, 2013).

Regarding exposure, to perform an ERA, it is important to recognize the routes from which pesticides may reach bee populations. Bee species have diverse life habits and, therefore, different routes of exposure (Kopit & Pitts-Singer, 2018). However, in Brazil, data from pesticide exposure in the ERA framework only consider *A. mellifera* life habits. This approach may misestimate the exposure of other bee species due to characteristics such as the amount of food consumption, ground-nesting, leaf-cutting behaviors, and other differences among bees' life habits, which may result in different levels of exposure to the pesticides between non-*Apis* species and *A. mellifera* (Cham et al., 2019; Sgolastra et al., 2019).

In Neotropical countries, little is known about the sensitivity of native bees to pesticides (Lourencetti et al., 2023), especially for solitary bees (Lehmann & Camp, 2021). Nevertheless, some studies with Neotropical bees corroborate the greater tolerance of *A. mellifera* to pesticides compared to other bee species (Assis et al., 2022; Miotelo et al., 2021). Thus, the information gap on pesticide effects in Neotropical solitary bees may result in inefficient protection by environmental regulation.

From the recognized gaps, differences in pesticide sensitivities, exposure levels (e.g., nectar and pollen consumption), and exposure routes among bee species have been reported as some of the most important limitations on the current risk assessment of pesticides to pollinators in the world (Assis et al., 2022; Sgolastra et al., 2020; Topping et al., 2021). In the present study, we calculated the LC50 (medium lethal concentration) and LD50 values of dimethoate for males and females of the Neotropical solitary bee *Centris analis* (Fabricius, 1804) as the first step to gather more information regarding pesticide effects on solitary bees, hence helping to improve risk assessment conducted in tropical countries. Because the nutritional conditions of bees can influence their sensitivity to pesticides, we performed a larval food pollen analysis to assess the differences between the individuals used in the present study (Woodard et al., 2019). Besides, a species sensitivity distribution (SSD) curve was produced using LD50 values from different bee species to compare the sensitivity between non-*Apis* species (social and solitary) and *A. mellifera*.

METHODS

Collection of the solitary bee C. analis

The oil-collecting bee *C. analis* is a cavity-nesting, solitary bee (Supporting Information, Figure S1), widely distributed in South America, from Mexico to Brazil (Moure, 2012). This species was selected for our study because it is a multivoltine species with nesting activities in natural and artificial cavities (Moure-Oliveira et al., 2017; Silva et al., 2017) and because there is a preexisting method for the maintenance of adult organisms under laboratory conditions (Tadei et al., 2022).

During summer and spring from 2021 to 2022, nests of *C. analis* were collected from trap-nests allocated in shelters installed in rural areas from São Paulo and Minas Gerais states (Brazil). The shelters were surrounded by fragments of Atlantic forest and Brazilian cerrado. After being taken to the laboratory, the nests were kept under controlled conditions of temperature (28 ± 2 °C) and humidity (65% ± 10%) until the emergence of adults. The use and collection of individuals occurred under the authorization of the Brazilian agency SISBIO (no. 75499-4; in Portuguese, Sistema de Autorização e Informação em Biodiversidade).

After the emergence of the bees, 10 females and 13 males of different nests were used to calculate the bee fresh weight. For the other newly emerged bees, each bee was transferred to a ventilated, transparent plastic cage containing a wooden cube and an artificial flower around the microtube feeder (500 µl), according to the methodology described in Tadei et al. (2022). Acclimatization under laboratory conditions occurred for at least 4 days (Figure 1), where individuals were fed with 35% w/w sucrose solution and kept at 28 ± 2 °C, 75% ± 10% humidity, with the absence of light inside the incubator.

Larval food analysis

To verify the influence of larval nutrition in the individual response of adult bees to the insecticide dimethoate, we evaluated the similarity of diet among bee nests through pollen analysis.

After the emergence of bees, the residual pollen resulting from bee feces or not consumed in the larval phase was separated from the nest and kept in ethanol at 70% for 24 h

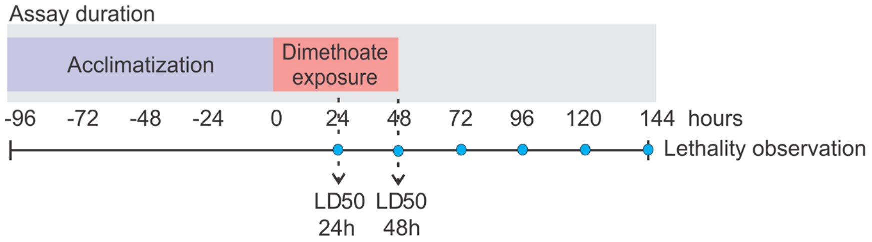

FIGURE 1: Schematic representation of assay steps showing the dimethoate exposure period and times of lethality observation (blue dots). The arrows indicate the time of median lethal dose (LD50) calculation.

(Silva et al., 2010). Next, the ethanol was replaced with glacial acetic acid. After 24 h, the samples were centrifuged at 2000 rpm for 10 min for acid removal and the beginning of the acetolysis process (Erdtman, 1960).

A mixture of acetic anhydride and sulfuric acid (9:1) was added to the pollen samples and kept for 4 min at 100 °C. This solution was removed, and the samples were washed in distilled water. Glycerin 50% was added to the samples for slide mounting and pollen identification in the microscope (Silva et al., 2014). A qualitative analysis was conducted by identifying the plants in the larval food samples per nest.

Pollen grains were identified to the lowest taxonomic level possible using the pollen collection in the RCPol: Online Pollen Catalogs Network as reference (2023). Pollen morphology was verified using scanning electron microscopy. For the quantitative analysis, we counted the first 400 pollen grains in each sample, as suggested by Montero and Tormo (1990), and calculated the percentage of each pollen type in the samples. Then, to evaluate the food composition of bees used in the present study, we calculated the Shannon diversity index (Shannon, 1948), the Pielou equitability index (Pielou, 1966), and the Berger-Parker dominance index (Berger & Parker, 1970). *Centris analis* diet similarity was analyzed using the Bray-Curtis index with the package "vegan" in the R software (Oksanen et al., 2020).

Determination of acute toxicity

To evaluate the toxicity of dimethoate to the bee species *C. analis*, previously acclimatized males and females were exposed orally to the active ingredient (a.i.) dimethoate (Sigma-Aldrich; Chemical Abstracts Service no. 1219794-81-6; ≥98% purity) for 48 h. The lethality of female and male individuals was evaluated in independent experiments. We conducted the experiments using a randomized block design, where each block contained at least one bee of the same sex and age group (from 4 to 7 days old) per concentration. The blocks were repeated until reaching the minimum sample of 7 females and 10 males per concentration. A lower number of females were used because of the sexual rate of 1 female to 1.7 males found in the collected nests.

To calculate LC50 and LD50 values after 24 h of exposure, we used the results obtained from exposure to the dimethoate concentrations 10, 5, 2.5, 1.25, 0.625, and 0 ng a.i./µl. To refine the LC50 and LD50 values in 48 h of exposure, we tested the concentrations 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng a.i./µl. Dimethoate was mixed in 35% w/w sucrose solution according to the concentrations above and offered ad libitum to the bees. After 24 h, the dimethoate solution was renewed. Next, 48 h after the beginning of the test, we replaced the feeder with a new one containing only 35% w/w sucrose solution.

During the experiment, bees were kept at 28 ± 2 °C, at 75% ± 10% relative humidity, and in darkness. Lethality was assessed every 24 h until 144 h, that is, 96 h after the exposure time (Figure 1). To calculate the total dimethoate ingested by each bee, we weighed the feeders at the start of the test and every 24 h. Subsequently, daily food consumption was calculated by the food weight difference minus the evaporation rate. The evaporation rate was calculated by averaging the daily feed difference of five cages without bees kept under the same conditions during the experiments. The food consumed weight was converted to volume using the food density value. Bees without food consumption at 48 h were removed from the assay.

All data were analyzed in the software R Core Team (2022). Survival data were analyzed using the log-rank test from the "Survival" package (Therneau, 2020, 2021). For LC50 and LD50 (nanograms per bee and nanograms per milligram of bee) calculations, we fitted generalized linear models (GLMs) with binomial (logit-link) and quasi-binomial (cauchit-link) distributions. Food consumption data were analyzed using quasi-Poisson and Gaussian models. To assess the goodness of fit of the fitted models, we used the half-normal plots from the "hnp" package (Moral et al., 2017).

SSD curve

We constructed an SSD curve using the toxicological data available in the literature to compare the LD50 value obtained for *C. analis* with other bee species. For this, a literature review was performed using the keywords "dimethoate" AND "bees" AND "LD50" OR "lethal" OR "toxicity" to find LD50 values from oral exposure to dimethoate in the following databases: Web of Science, Capes Periodicals, the ecotoxicological database from the US Environmental Protection Agency (USEPA), and the Pesticide Properties DataBase from the University of Hertfordshire.

The articles were selected based on four parameters: (1) the testing procedure used in the experiments, which should contain an oral exposure to dimethoate under laboratory conditions; (2) a description of the number of concentrations used and the time of food administration, with a maximum time of 24 h of exposure; (3) mortality observation every 24 h; and (4) use of adult individuals from the same sex and similar ages in the assay. Because bees' consumption can change according to the feeding habits of different species, we compared the amount of dimethoate consumed per bee (dose); therefore, studies reporting food consumption conducted with controlled volume or ad libitum food administration were considered.

From these criteria, we obtained the LD50 value from four other species (Table 1) exposed orally to dimethoate. To increase the number of bee species with LD50 values, two observation times from the literature data (24 and 48 h) were used. For species with more than one LD50 value available, we used the geometric mean of the values (Sanchez-Bayo & Goka, 2014; Wheeler et al., 2002). For *C. analis*, only the LD50 obtained at 24 h of exposure was used in the SSD curve, to improve comparisons with the different species used (differences in exposure time are shown in Table 1). The SSD curve was fitted using the USEPA SSD generator program (USEPA, 2005).

To compare the species according to their body weight, a second SSD curve was built using the LD50 in nanograms per milligram of bee (Table 1). In the absence of the LD50 value considering body weight, we calculated the LD50 in nanograms per milligram by dividing the LD50 (nanograms per bee) per their

TABLE 1: Medium lethal dose of adult bees exposed orally to dimethoate under laboratory conditions

Species	Time (hours)	Food administration	LD50 (ng a.i./bee)	LD50 (ng a.i./ mg bee)	Reference
Apis mellifera	24	Ad libitum	80	0.73	Fiedler (1987)
Apis mellifera	24	Controlled volume (10 µl)	150	1.36	Ladurner et al. (2005)
Apis mellifera	24	Controlled volume	170	1.55	Drescher & Geusen-Pfister (1991)
Apis mellifera	24	Ad libitum for 3–4 h	100[a]	0.91	Organisation for Economic Co-operation and Development (1998)
Apis mellifera	24	Controlled volume	177	1.61	Gough et al. (1994)
Apis mellifera	48	Controlled volume (10 µl)	130	1.18	Ladurner et al. (2005)
Apis mellifera	48	Controlled volume (10 µl)	26.7	0.24	Tai et al. (2022)
Apis mellifera	48	Controlled volume	166	1.51	Gough et al. (1994)
Apis mellifera—geometric mean			109.59	1.00	
Bombus terrestris	24	Controlled volume (10 µl)	440	1.96	Marletto et al. (2003)
Leioproctus paahaumaa	48	Controlled volume (10 µl)	56.5	1.09	Tai et al. (2022)
Osmia lignaria	24	Controlled volume (10 µl)	270	2.00	Ladurner et al. (2005)
Osmia lignaria	48	Controlled volume (10 µl)	260	2.08	Ladurner et al. (2005)
Osmia lignaria—geometric mean			265	2.04	

[a] The Organisation for Economic Co-operation and Development guideline provides an LD50 value of 100–350 ng/honeybee. We used the lowest value to calculate the geometric mean.

Time is time in hours of observation of mortality of bees; *Food administration* is a description of the methodology used for oral exposure to the bees.

LD50 = median lethal dose; a.i. = active ingredient.

respective medium fresh body weight: 110 mg for *A. mellifera*, 130 mg for *Osmia lignaria*, and 225 mg for *Bombus terrestris* (Arena & Sgolastra, 2014; Sheffield et al., 2008).

Using a log-normal distribution, the hazardous doses at 5% (HD5) and 50% (HD50) were estimated by the software ETX 2.0 (van Vlaardingen et al., 2004).

RESULTS AND DISCUSSION

Pollen analysis from *C. analis* nests showed that individuals used in the toxicological test had a larval diet with >80% of similarity (Figure 2). The most common pollen identified in the larval diet was from the *Malpighia emarginata* species or Malpighiaceae type (not identified species), representing, respectively, 81.5% and 11.3% of the pollen larval food (Supporting Information, Figures S2 and S3 and Table S1), corroborating the data of Silva et al. (2017). Therefore, as expected because of the high diet similarity, the larval food diversity (Shannon index, $p = 0.05$), equitability (Pielou index, $p = 0.13$), and dominance of plant species (Berger-Parker index, $p = 0.16$) did not influence the mortality of the individuals after dimethoate exposure.

In 24 h, *C. analis* females were more sensitive to dimethoate than males, showing lower LC50 and LD50 values (Table 2). However, in 48 h, males showed an LD50 value 1.33 times lower than females, indicating a higher sensitivity. As expected, for both sexes, the toxicity increased from 24 to 48 h because

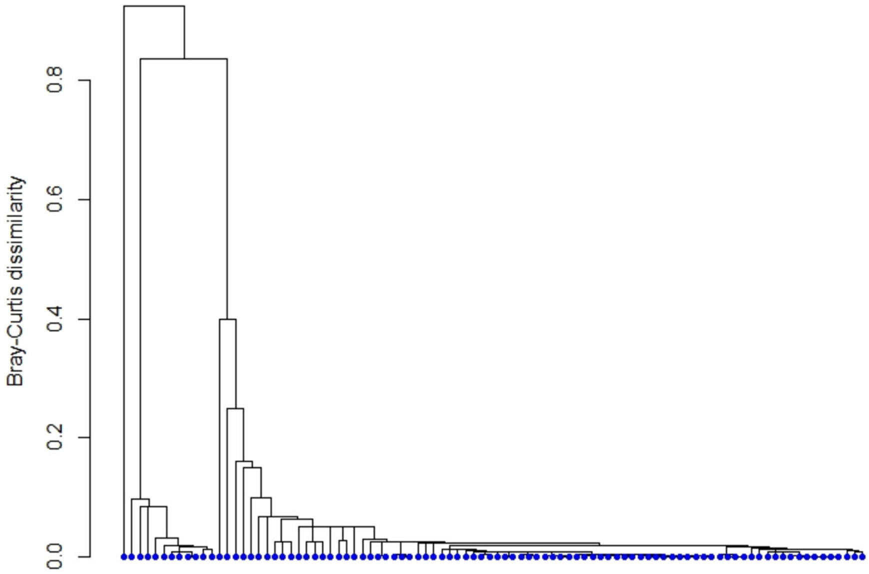

FIGURE 2: Larval diet dissimilarity of *Centris analis* throughout residual pollen analysis from the nests. Blue points represent pollen samples from each nest.

TABLE 2: Medium lethal concentration and medium lethal dose of *Centris analis* continuously exposed orally to the dimethoate

	Time (hours)	Male	Female
LC50 (95% CI; ng a.i./µl)	24	2.09 (1.95–2.23)	1.56 (1.47–1.65)
	48	0.74 (0.68–0.79)	0.63 (0.02–1.24)
LD50 (95% CI; ng a.i./bee)	24	58.34 (58.13–58.84)	52.02 (51.95–52.10)
	48	32.78 (32.54–33.01)	43.84 (43.65–44.04)
LD50 (95% CI; ng a.i./mg bee)	24	0.98 (0.91–1.06)	0.86 (0.82–0.89)
	48	0.68 (0.62–0.74)	0.80 (0.74–0.86)

Time is time of exposure and observation. The dose is expressed as the total dimethoate consumption at the end of the observation time.
LC50 = median lethal concentration; 95% CI = 95% confidence interval; a.i. = active ingredient; LD50 = median lethal dose.

of the increased time of exposure to the insecticide. The same pattern was observed using body weight because the females (61.16 ± 13.46 mg) and males (60.79 ± 9.72 mg) used in the assays had similar weights.

Comparatively, in both concentration ranges, no difference was reported from the log-rank test between the male and female survival curves exposed at the same dimethoate concentration ($p > 0.28$). Hence, for both *C. analis* males and females, dimethoate concentrations >0.6 ng/µl showed lethal effects during the exposure period (24 and 48 h) or 24 h after the exposure (until 72 h from the beginning of the test; Figure 3). No significant survival reduction was observed in the observation times of 96, 120, and 144 h, that is, after 48 h from the end of dimethoate exposure (Figure 3; log-rank test, $\chi^2 = 19.5$, $df = 14$, $p = 0.1$).

In general, *C. analis* food consumption was lower at dimethoate concentrations >1 ng a.i./µl (Figure 4). The reduction in food consumption was more evident after 24 h of exposure and in the first tested concentration range (Figure 4A,B). At 72 h, when all bees received food without pesticide for 24 h, no increase in food consumption was observed in exposed bees compared to the food consumption during dimethoate exposure ($p = 0.25$).

Males exposed to 0.156 and 0.313 ng a.i./µl showed a reduction in food consumption after 24 h (GLM—Gaussian, $p = 0.002$) and 72 h (GLM—quasi-Poisson, $p = 0.01$) from the beginning of the pesticide exposure, even with substitution for a dimethoate-free food (from 48 to 72 h; Figure 4C). Females exposed to concentrations <1.25 ng a.i./µl did not show alteration in food consumption compared to the control group (Figure 4D; $p > 0.1$).

A decrease in the consumption of food containing dimethoate was also reported by Yang et al. (2019) in honeybees (*A. mellifera* and *Apis cerana*) exposed to 1 ng/µl of dimethoate. Dimethoate is a highly toxic organophosphate pesticide to bees (Tai et al., 2022; Waller et al., 1984) that acts in the nervous system through the inhibition of acetylcholinesterase (Christen et al., 2019). Organophosphate pesticides can lead to physiological and motor function alterations (Pashte & Patil Shivshankar, 2018; Williamson et al., 2013), which can explain the inability or difficulty of bees in moving toward the feeder. Because dimethoate fails to cause repellent effects on bees (Danka & Collison, 1987), the reduced consumption of food by exposed bees may have been caused by the bee behavior alterations induced by this insecticide.

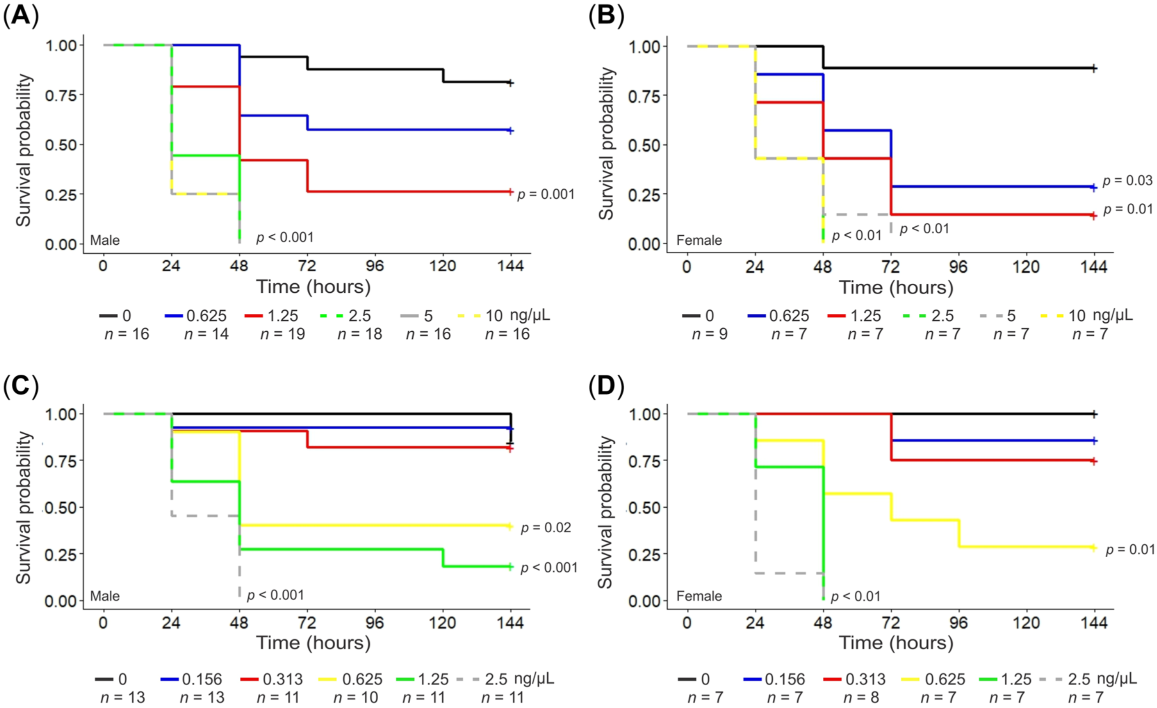

FIGURE 3: Survival probability of adult *Centris analis* exposed orally to dimethoate for 48 h. (**A**, **C**) Survival curve of male individuals from the first and second range of concentrations, respectively; (**B**, **D**) survival curve of female individuals from the first and second range of concentrations, respectively. $p < 0.05$ indicates a difference compared to the control group (0 ng/µl).

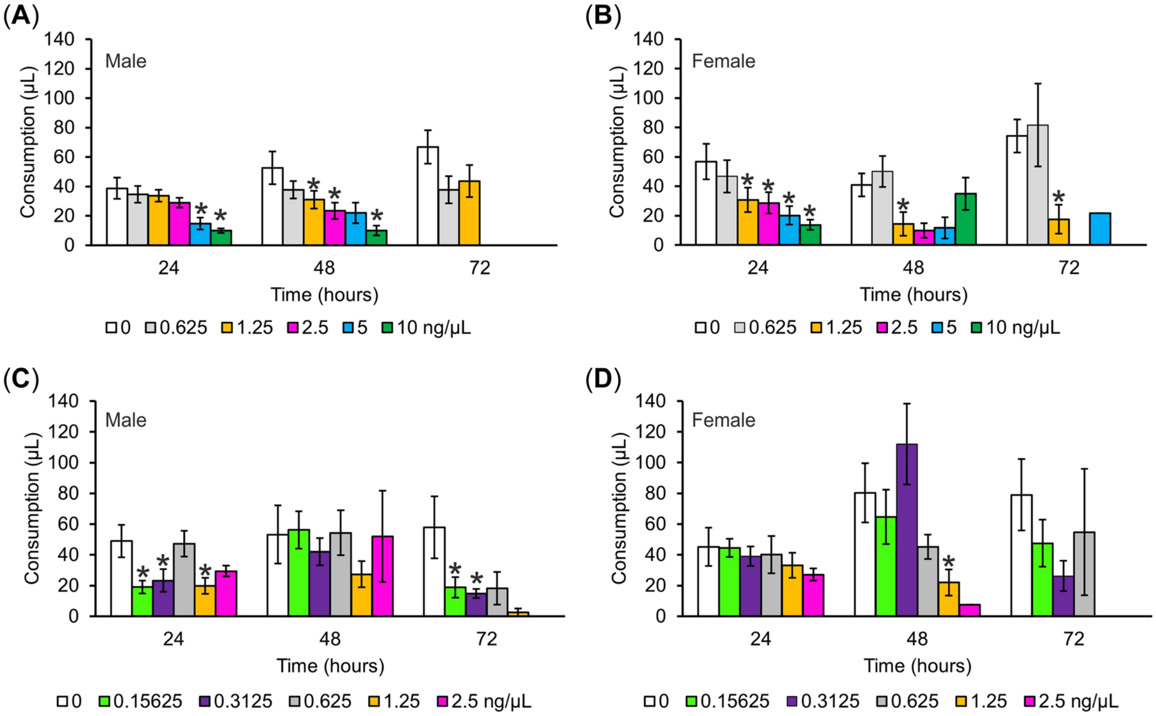

FIGURE 4: Pattern of food consumption of *Centris analis* exposed orally to the insecticide dimethoate for 48 h. Bars show mean values and the respective standard error. For 72 h, all bees were fed with 35% w/w pesticide-free sucrose solution. Consumption was calculated daily according to the feeder weight difference and food density. (**A**, **B**) Individuals exposed to the first range of concentrations; (**C**, **D**) individuals exposed to the second range of concentrations. Asterisks indicate a difference from the control group (0 ng/µl, $p < 0.05$).

Considering the 24-h LD50 on a per bee basis obtained in our toxicological test (Table 2), females of *C. analis* are 2.1-fold more sensitive to oral exposure to dimethoate than *A. mellifera*, 5-fold more sensitive than the solitary bee *O. lignaria* (Ladurner et al., 2005), and 8.5-fold more sensitive than the bumblebee *B. terrestris* (Marletto et al., 2003). *Centris analis* showed a similar LD50 value to the native New Zealand solitary, ground-nesting bee *Leioproctus paahaumaa* (Figure 5A; Tai et al., 2022). However, taking body weight into account, females of *C. analis* were more sensitive than *L. paahaumaa* (Figure 5B) and 1.16-fold more sensitive than *A. mellifera*. Hence, when fitting the SSD curve with the LD50 values available, *C. analis* proved to be the most sensitive bee species based on the effect per individual (Figure 5A) and per weight of the bee (Figure 5B).

The calculated HD5 was 22.38 (3.98–51.64) ng/bee, and the HD50 was 114 (54.14–239.90) ng/bee. Using the body weight of the bees, the HD5 was 0.64 (0.31–0.89) ng/mg bee, and the HD50 was 1.24 (0.91–1.69) ng/mg bee. The species *A. mellifera*, *O. linaria*, and *B. terrestris* showed LD50 values above the HD50 found (Figure 5A). Of these species, *A. mellifera* is a world bee species model, and *Osmia* and *Bombus* genera were recommended by the other studies as potential models for ERA (Eeraerts et al., 2020; EFSA, 2013; Sgolastra et al., 2019). Thus, considering the low sensitivity of these species, their use as representatives of bee diversity must be performed carefully, taking these differences in sensitivities into consideration. Furthermore, few LD50 values were available for other bees' oral exposure to dimethoate, limiting the comparisons with *C. analis*.

Except for *C. analis*, the model species *A. mellifera* showed higher sensitivity to dimethoate when using body weight as a parameter to calculate the LD50 values, in comparison to solitary bees from temperate zones and bumblebees (Figure 5B). Body weight has been used in bee ecotoxicology studies to normalize the differences in size among species, although the sensitivity pattern related to body weight is still not totally clear to all pesticide groups (Lourencetti et al., 2023; Yue et al., 2018).

Although more sensitive than the model species *A. mellifera*, the LD50 of *C. analis* is within the 10-fold safety factor used in the risk assessment to extrapolate the toxicity data of *A. mellifera* to other bee species, at least relative to the standard insecticide dimethoate. Studies with contact exposure to dimethoate showed that the LD50 values for the solitary bee *Megachile rotundata* (Ansell et al., 2021), *Osmia cornifrons* (Biddinger et al., 2013), and the Brazilian social stingless bee *Scaptotrigona postica* (van der Steen et al., 2012) are also within this proposed safety factor, that is, presenting an LD50 greater than the LD50 of *A. mellifera* divided by 10. Corroborating this observation, Uhl et al. (2016) showed that seven European bee species exposed to dimethoate via contact also showed LD50 values higher than the LD50/10 for *A. mellifera*.

However, it is important to note that the susceptibility of bee species can change according to the pesticide, type of exposure, and exposure time (Arena & Sgolastra, 2014; Del Sarto et al., 2014). For example, for the insecticide dimethoate, the solitary bee *L. paahaumaa* showed similar sensitivity to

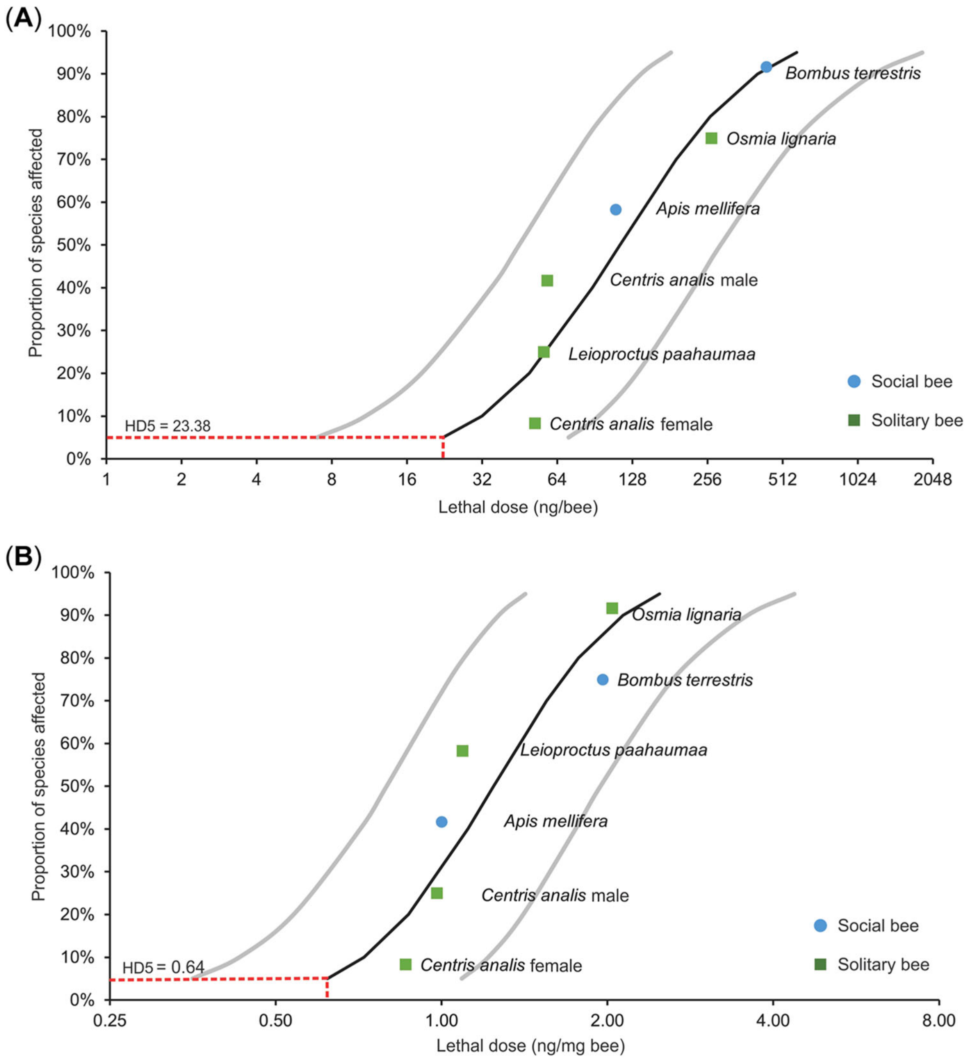

FIGURE 5: Species sensitivity distribution (SSD) curve of bees exposed orally to dimethoate under laboratory conditions. Exposure time ranges from 4 to 24 h. Values refer to the median lethal dose (LD50) value from acute exposure or the geometric mean value for species with more than one LD50 value (Table 1). Gray lines indicate the 95% confidence interval, black line indicates the central log-normal distribution, and red dashed line shows the hazardous dose affecting 5% of species (HD5): (**A**) SSD curve estimated using dose/individual; (**B**) SSD curve estimated using body weight.

A. mellifera. However, based on the LD50 value per bee, when exposed to the insecticide imidacloprid (a systemic neonicotinoid) *L. paahaumaa* proved to be 36-fold more sensitive from oral exposure and 194-fold more sensitive than *A. mellifera* when considering contact exposure (Tai et al., 2022). Those values show that, in this case, the safety factor was not sufficient to protect this species. The same trend was observed in Neotropical stingless bees exposed to the neonicotinoid imidacloprid (Assis et al., 2022). For the first time, the present study calculated an LD50 for a Neotropical solitary bee. Future studies are needed to confirm the Neotropical solitary bee sensitivity observed using other pesticides and to refine the exposure time because in the present study the exposure was conducted continuously for 24 and 48 h.

In addition, the safety factor in risk assessment considers only the toxicity endpoint representing the hazard data. Because different bee species have the same pesticide sensitivity does not necessarily confirm that they will have the same risk of exposure to them. Therefore, the differences in exposure route between *A. mellifera* and solitary bees (Kopit & Pitts-Singer, 2018; Sgolastra et al., 2019) and the vulnerability concept added to population resilience (Schmolke et al., 2021) need to be considered to improve ERA for bee diversity protection.

The risk of pesticides to bee species can change according to the amount of pollen and nectar consumed, nesting habits, sociality level, agricultural landscape, and foraging habits (Kopit & Pitts-Singer, 2018; Sponsler et al., 2019). Knapp et al. (2023), using a trait-based approach, concluded that landscape context modifies pesticide risk for limited (*Osmia bicornis*) and intermediate (*B. terrestris*) foragers but not for extensive foragers (*A. mellifera*), which showed highest exposure risk where there are flowering crops. However, the pesticide risk increases for the non-*Apis* species *B. terrestris* and *O. bicornis* as the intensively managed agricultural area expands because pesticides can drift into surrounding seminatural habitats where these non-*Apis* species collect food resources within their foraging range.

Centris analis prefers pollen from the cultivated plant *Malpighia emarginata*, the acerola fruit tree (Silva et al., 2017), of which Brazil is the world's greatest producer (Farinelli et al., 2021). Currently, 21 pesticides are recommended to be used in the *Malpighia emarginata* crops, including insecticides, fungicides, and acaricides (Ministério de Agricultura, Pecuária e Abastecimento, 2023). Due to the intensive use of oil and pollen from *Malpighia emarginata* flowers to provide larval food for *C. analis*; they are recommended as a manageable bee species to conduct the pollination process in acerola orchards (Magalhães & Freitas, 2013; Oliveira & Schlindwein, 2009). Therefore, it is expected that *C. analis* will forage landscapes with a high agricultural proportion (acerola crops) to collect their pollen and will, consequently, be exposed to different types of pesticides, which should be considered in environmental regulation processes. Combining all the factors—the higher sensitivity of *C. analis* to dimethoate in comparison to *A. mellifera*, the occurrence of *C. analis* in crops treated with pesticides, and considering that non-*Apis* species may have a greater vulnerability to pesticides than *A. mellifera* (Schmolke et al., 2021)—our results highlight the urgent need for more studies using Neotropical solitary bees to improve the risk assessment for bees during pesticide regulation processes.

CONCLUSION

The results obtained in the present study are of great importance for the ERA of pesticides to bees in tropical regions. We bring the first LD50 and LC50 values for a solitary bee, native to tropical countries. The Neotropical solitary bee *C. analis* showed a higher sensitivity to the insecticide dimethoate than the model species *A. mellifera*. However, the LD50 value obtained was within the 10-fold safety factor used in the risk-assessment framework to protect non-*Apis* species. That means that the actual risk-assessment procedure would be enough to cover the difference of pesticide sensitivity of *C. analis* if both species had the same exposure levels to the pesticides. However, it was discussed that the exposure and vulnerability of *C. analis* to pesticides might be higher than for *A. mellifera*. Hence, the variation in sensitivity between types of pesticides, population vulnerabilities, and different routes of exposure needs to be further explored in future studies to improve the ecological risk assessment of pesticides to bees under tropical conditions.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5738.

Acknowledgments—The authors declare no conflicts of interest. The authors thank the São Paulo Research Foundation for the financial support to develop the present study (grants 2017/21097-3 and 2019/27863-5) and P. Decio for assistance in the experiment. They also thank R. Nocelli of the Federal University of São Carlos, Araras, Brazil, for providing the scanning electron microscope for pollen analysis.

Author Contributions Statement—**Rafaela Tadei:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing–original draft; Writing–review & editing. **Vanessa B. Menezes-Oliveira, Claudia I. Silva:** Conceptualization; Data curation; Formal analysis; Methodology; Writing–review & editing. **Elaine C. Mathias da Silva-Zacarin:** Conceptualization; Methodology; Project administration; Supervision; Writing–review & editing. **Osmar Malaspina:** Conceptualization; Project administration; Supervision; Writing–review & editing.

Data Availability Statement—The pollen data are available in the Supporting Information.

REFERENCES

Ansell, G. R., Frewin, A. J., Gradish, A. E., & Scott-Dupree, C. D. (2021). Contact toxicity of three insecticides for use in tier I pesticide risk assessments with *Megachile rotundata* (Hymenoptera: Megachilidae). *PeerJ*, *9*, Article e10744. https://doi.org/10.7717/peerj.10744

Arena, M., & Sgolastra, F. (2014). A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology*, *23*(3), 324–334. https://doi.org/10.1007/s10646-014-1190-1

Assis, J. C., Tadei, R., Menezes-Oliveira, V. B., & Silva-Zacarin, E. C. M. (2022). Are native bees in Brazil at risk from the exposure to the neonicotinoid imidacloprid? *Environmental Research*, *212*, Article 113127. https://doi.org/10.1016/j.envres.2022.113127

Berger, W. H., & Parker, F. L. (1970). Diversity of planktonic Foraminifera in deep-sea sediments. *Science*, *168*(3937), 1345–1347. https://doi.org/10.1126/science.168.3937.1345

Biddinger, D. J., Robertson, J. L., Mullin, C., Frazier, J., Ashcraft, S. A., Rajotte, E. G., Joshi, N. K., & Vaughn, M. (2013). Comparative toxicities and synergism of apple orchard pesticides to *Apis mellifera* (L.) and *Osmia cornifrons* (Radoszkowski). *PLOS ONE*, *8*(9), Article e72587. https://doi.org/10.1371/journal.pone.0072587

Cham, K. O., Nocelli, R. C. F., Borges, L. O., Viana-Silva, F. E. C., Tonelli, C. A. M., Malaspina, O., Menezes, C., Rosa-Fontana, A. S., Blochtein, B., Freitas, B. M., Pires, C. S. S., Oliveira, F. F., Contrera, F. A. L., Torezani, K. R. S., de Fatima Ribeiro, M., Siqueira, M. A. L., & Rocha, M. C. L. S. A. (2019). Pesticide exposure assessment paradigm for stingless bees. *Environmental Entomology*, *48*(1), 36–48. https://doi.org/10.1093/ee/nvy137

Cham, K. O., Rebelo, R. M., Oliveira, R. P., Ferro, A. A., Silva, F. E. C. V., Borges, L. O., Saretto, C. O. S. D., Tonelli, C. A. M., & Macedo, T. C. (2020). *Manual de avaliação de risco ambiental de agrotóxicos para abelhas* (2nd ed.). IBAMA/Diqua.

Christen, V., Joho, Y., Vogel, M., & Fent, K. (2019). Transcriptional and physiological effects of the pyrethroid deltamethrin and the organophosphate dimethoate in the brain of honey bees (*Apis mellifera*). *Environmental Pollution*, *244*, 247–256. https://doi.org/10.1016/j.envpol.2018.10.030

Danka, R. G., & Collison, C. H. (1987). Laboratory evaluation of dimethoate repellence to honey bees. *Journal of Applied Entomology*, *104*(1–5), 211–214. https://doi.org/10.1111/j.1439-0418.1987.tb00518.x

Del Sarto, M. C. L., Oliveira, E. E., Guedes, R. N. C., & Campos, L. A. O. (2014). Differential insecticide susceptibility of the Neotropical stingless bee *Melipona quadrifasciata* and the honey bee *Apis mellifera*. *Apidologie*, *45*(5), 626–636. https://doi.org/10.1007/s13592-014-0281-6

Drescher, W., & Geusen-Pfister, H. (1991). Comparative testing of the oral toxicity of acephate, dimethoate and methomyl to honeybees, bumblebees and syrphidae. *Acta Horticulturae*, *288*, 133–138. https://doi.org/10.17660/ActaHortic.1991.288.16

Eeraerts, M., Pisman, M., Vanderhaegen, R., Meeus, I., & Smagghe, G. (2020). Recommendations for standardized oral toxicity test protocols for larvae of solitary bees, *Osmia* spp. *Apidologie*, *51*(1), 48–60. https://doi.org/10.1007/s13592-019-00704-w

Erdtman, G. (1960). The acetolysis method—A revised description. *Svensk Botanisk Tidskrift*, *54*, 561–564.

European Food Safety Authority. (2013). Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). *EFSA Journal*, *11*(7), Article 3295. https://doi.org/10.2903/j.efsa.2013.3295

Farinelli, D., Portarena, S., da Silva, D. F., Traini, C., da Silva, G. M., da Silva, E. C., da Veiga, J. F., Pollegioni, P., & Villa, F. (2021). Variability of fruit quality among 103 acerola (*Malpighia emarginata* D. C.) phenotypes from the subtropical region of Brazil. *Agriculture*, *11*(11), Article 1078. https://doi.org/10.3390/agriculture11111078

Fiedler, L. (1987). Assessment of chronic toxicity of selected insecticides to honeybees. *Journal of Apicultural Research*, *26*(2), 115–122. https://doi.org/10.1080/00218839.1987.11100747

Fisher, A. (2021). Protect pollinators—Reform pesticide regulations. *Nature*, *595*(7866), 172. https://doi.org/10.1038/d41586-021-01818-x

Gough, H. J., McIndoe, E. C., & Lewis, G. B. (1994). The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.) 1981–1992. *Journal of Apicultural Research*, *33*(2), 119–125. https://doi.org/10.1080/00218839.1994.11100859

Knapp, J. L., Nicholson, C. C., Jonsson, O., de Miranda, J. R., & Rundlöf, M. (2023). Ecological traits interact with landscape context to determine bees' pesticide risk. *Nature Ecology & Evolution*, *7*, 547–556. https://doi.org/10.1038/s41559-023-01990-5

Kopit, A. M., & Pitts-Singer, T. L. (2018). Routes of pesticide exposure in solitary, cavity-nesting bees. *Environmental Entomology*, *47*(3), 499–510. https://doi.org/10.1093/ee/nvy034

Ladurner, E., Bosch, J., Kemp, W. P., & Maini, S. (2005). Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie*, *36*(3), 449–460. https://doi.org/10.1051/apido:2005032

Lehmann, D. M., & Camp, A. A. (2021). A systematic scoping review of the methodological approaches and effects of pesticide exposure on solitary bees. *PLOS ONE*, *16*(5), Article e0251197. https://doi.org/10.1371/journal.pone.0251197

Lourencetti, A. P. S., Azevedo, P., Miotelo, L., Malaspina, O., & Nocelli, R. C. F. (2023). Surrogate species in pesticide risk assessments: Toxicological data of three stingless bee species. *Environmental Pollution*, *318*, Article 120842. https://doi.org/10.1016/j.envpol.2022.120842

Magalhães, C. B., & Freitas, B. M. (2013). Introducing nests of the oil-collecting bee *Centris analis* (Hymenoptera: Apidae: Centridini) for pollination of acerola (*Malpighia emarginata*) increases yield. *Apidologie*, *44*(2), 234–239. https://doi.org/10.1007/s13592-012-0175-4

Marletto, F., Patetta, A., & Manino, A. (2003). Laboratory assessment of pesticide toxicity to bumblebees. *Bulletin of Insectology*, *56*(1), 155–158.

Ministério de Agricultura, Pecuária e Abastecimento. (2023). *AGROFIT: Sistema de agrotóxicos fitossanitários*. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Miotelo, L., Mendes dos Reis, A. L., Malaquias, J. B., Malaspina, O., & Roat, T. C. (2021). *Apis mellifera* and *Melipona scutellaris* exhibit differential sensitivity to thiamethoxam. *Environmental Pollution*, *268*, Article 115770. https://doi.org/10.1016/j.envpol.2020.115770

Montero, I., & Tormo, R. (1990). Análisis polínico de mieles de cuatro zonas montañosas de Extremadura. *Anales de La Asociación de Palinólogos de Lengua Española*, *5*, 71–78.

Moral, R. A., Hinde, J., & Demétrio, C. G. B. (2017). Half-normal plots and overdispersed models in *R*: The hnp package. *Journal of Statistical Software*, *81*(10), 1–23. https://doi.org/10.18637/jss.v081.i10

More, S. J., Auteri, D., Rortais, A., & Pagani, S. (2021). EFSA is working to protect bees and shape the future of environmental risk assessment. *EFSA Journal*, *19*(1), Article e190101. https://doi.org/10.2903/j.efsa.2021.e190101

Moure, J. S. (2012). *Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical region—Online version.* http://moure.cria.org.br/catalogue

Moure-Oliveira, D., Rocha-Filho, L. C., Ferreira-Caliman, M. J., & Garófalo, C. A. (2017). Nesting dynamic and sex allocation of the oil-collecting bee *Centris (Heterocentris) analis* (Fabricius, 1804) (Apidae: Centridini). *Journal of Natural History*, *51*(19–20), 1151–1168. https://doi.org/10.1080/00222933.2017.1324052

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *Vegan: Community ecology package* (Version 2.5-7) [Computer software]. R Foundation for Statistical Computing. https://CRAN.R-project.org/package=vegan

Oliveira, R., & Schlindwein, C. (2009). Searching for a manageable pollinator for acerola orchards: The solitary oil-collecting bee *Centris analis* (Hymenoptera: Apidae: Centridini). *Journal of Economic Entomology*, *102*(1), 265–273. https://doi.org/10.1603/029.102.0136

Organisation for Economic Co-operation and Development. (1998). Test No 213: Honeybees, acute oral toxicity test. *OECD guidelines for the testing of chemicals*.

Organisation for Economic Co-operation and Development. (2013). Test No. 237: Honey bee (*Apis melliferaOECD guidelines for the testing of chemicals*) larval toxicity test, single exposure. https://doi.org/10.1787/9789264203723-en

Organisation for Economic Co-operation and Development. (2017). Test No. 245: Honey bee (*Apis melliferaOECD guidelines for the testing of chemicals* L.), chronic oral toxicity test (10-day feeding). https://doi.org/10.1787/9789264284081-en

Pashte, V., & Patil Shivshankar, C. (2018). Toxicity and poisoning symptoms of selected insecticides to honey bees (*Apis mellifera mellifera* L.). *Archives of Biological Sciences*, *70*(1), 5–12. https://doi.org/10.2298/ABS170131020P

Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, *13*, 131–144. https://doi.org/10.1016/0022-5193(66)90013-0

R Core Team [Computer software]. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

RCPol: Online Pollen Catalogs Network. (2023). https://rcpol.mn.ufrj.br/en/homepage/

Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—A risk assessment. *PLOS ONE*, *9*(4), Article e94482. https://doi.org/10.1371/journal.pone.0094482

Schmolke, A., Galic, N., Feken, M., Thompson, H., Sgolastra, F., Pitts-Singer, T., Elston, C., Pamminger, T., & Hinarejos, S. (2021). Assessment of the vulnerability to pesticide exposures across bee species. *Environmental Toxicology and Chemistry*, *40*(9), 2640–2651. https://doi.org/10.1002/etc.5150

Sgolastra, F., Hinarejos, S., Pitts-Singer, T. L., Boyle, N. K., Joseph, T., Luckmann, J., Raine, N. E., Singh, R., Williams, N. M., & Bosch, J. (2019). Pesticide exposure assessment paradigm for solitary bees. *Environmental Entomology*, *48*(1), 22–35. https://doi.org/10.1093/ee/nvy105

Sgolastra, F., Medrzycki, P., Bortolotti, L., Maini, S., Porrini, C., Simon-Delso, N., & Bosch, J. (2020). Bees and pesticide regulation: Lessons from the neonicotinoid experience. *Biological Conservation*, *241*, Article 108356. https://doi.org/10.1016/j.biocon.2019.108356

Shannon, C. E. A. (1948). Mathematical theory of comunication. *The Bell System Technical Journal*, *27*, 379–423.

Sheffield, C. S., Westby, S. M., Kevan, P. G., & Smith, R. F. (2008). Winter management options for the orchard pollinator *Osmia lignaria* Say (Hymenoptera: Megachilidae) in Nova Scotia. *Journal of the Entomology Society of Ontario*, *139*, 3–18.

Silva, C. I., Ballesteros, P., Palmero, M., Bauermann, S., Evaldt, A., & Oliveira, P. (2010). *Catálogo polínico: Palinologia aplicada em estudos de conservação de abelhas do gênero Xylocopa no triângulo mineiro* (1st ed.). EDUFU.

Silva, C. I., Hirotsu, C. M., de Suza Pacheco Filho, A. J., Queiroz, E. P., & Garófalo, C. A. (2017). Is the maximum reproductive rate of *Centris analis* (Hymenoptera, Apidae, Centridini) associated with floral resource availability? *Arthropod–Plant Interactions*, *11*(3), 389–402. https://doi.org/10.1007/s11829-017-9513-9

Silva, C. I., Imperatriz-Fonseca, V. L., Groppo, M., Bauermann, S. G., Saraiva, A. A., Queiroz, E. P., Evaldit, A. C. P., Aleixo, K. P., Castro, M. M. N., Faria, L. B., Ferreira-Caliman, M. J., Wolff, J. L., Paulino-Neto, H. F., & Garófalo, C. A. (2014). *Catálogo polínico das plantas usadas por abelhas no Campus da USP de Ribeirão Preto*. Holos.

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

Total Environment, *662*, 1012–1027. https://doi.org/10.1016/j.scitotenv.2019.01.016

Tadei, R., Silva, C. I., Decio, P., Silva-Zacarin, E. C. M., & Malaspina, O. (2022). Method for maintaining adult solitary bee *Centris analis* under laboratory conditions. *Methods in Ecology and Evolution*, *13*(3), 619–624. https://doi.org/10.1111/2041-210X.13797

Tai, F. K., Pattemore, D. E., Jochym, M., Beggs, J. R., Northcott, G. L., & Mortensen, A. N. (2022). Honey bee toxicological responses do not accurately predict environmental risk of imidacloprid to a solitary ground-nesting bee species. *Science of the Total Environment*, *839*, Article 156398. https://doi.org/10.1016/j.scitotenv.2022.156398

Therneau, T. M. (2020). A package for survival analysis in R (Version 3.1-12) [Computer software]. R Foundation for Statistical Computing. https://cran.r-project.org/package=survival

Therneau, T. M. (2021). Survival analysis (Version 3.2-13) [Computer software]. R Foundation for Statistical Computing.

Topping, C. J., Aldrich, A., & Berny, P. (2020). Overhaul environmental risk assessment for pesticides. *Science*, *367*(6476), 360–363. https://doi.org/10.1126/science.aay1144

Topping, C. J., Brown, M., Chetcuti, J., de Miranda, J. R., Nazzi, F., Neumann, P., Paxton, R. J., Rundlöf, M., & Stout, J. C. (2021). Holistic environmental risk assessment for bees. *Science*, *371*(6532), 897. https://doi.org/10.1126/science.abg9622

Uhl, P., Franke, L. A., Rehberg, C., Wollmann, C., Stahlschmidt, P., Jeker, L., & Brühl, C. A. (2016). Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment. *Scientific Reports*, *6*(1), Article 34439. https://doi.org/10.1038/srep34439

US Environmental Protection Agency. (2005). Species sensitivity distribution gerator (Version 1.0) [Computer software]. https://www.epa.gov/caddis-vol4/caddis-volume-4-data-analysis-download-software

van der Steen, J., Roessink, I., Kasina, M., Gikungu, M., & Nocelli, R. (2012). Is the European honeybee (*Apis mellifera mellifera*) a good representative for other pollinator species? *Julius-Kühn-Archiv*, *437*, 179. https://doi.org/10.5073/jka.2012.437.047

van Vlaardingen, P., Traas, P., Wintersen, A., & Aldenberg, T. (2004). *ETX 2.0: A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data (Version 2.0) [Computer software]*. National Institute for Public Health and the Environment (RIVM).

Waller, G. D., Erickson, B. J., Harvey, J., & Martin, J. H. (1984). Effects of dimethoate on honey bees (Hymenoptera: Apidae) when applied to flowering lemons. *Journal of Economic Entomology*, *77*(1), 70–74. https://doi.org/10.1093/jee/77.1.70

Wheeler, J., Grist, E. P., Leung, K. M., Morritt, D., & Crane, M. (2002). Species sensitivity distributions: Data and model choice. *Marine Pollution Bulletin*, *45*(1–12), 192–202. https://doi.org/10.1016/S0025-326X(01)00327-7

Williamson, S. M., Moffat, C., Gomersall, M. A. E., Saranzewa, N., Connolly, C. N., & Wright, G. A. (2013). Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. *Frontiers in Physiology*, *4*, Article 13. https://doi.org/10.3389/fphys.2013.00013

Woodard, S. H., Duennes, M. A., Watrous, K. M., & Jha, S. (2019). Diet and nutritional status during early adult life have immediate and persistent effects on queen bumble bees. *Conservation Physiology*, *7*(1), Article coz048. https://doi.org/10.1093/conphys/coz048

Yang, Y., Ma, S., Yan, Z., Liu, F., Diao, Q., & Dai, P. (2019). Effects of three common pesticides on survival, food consumption and midgut bacterial communities of adult workers *Apis cerana* and *Apis mellifera*. *Environmental Pollution*, *249*, 860–867. https://doi.org/10.1016/j.envpol.2019.03.077

Yue, M., Luo, S., Liu, J., & Wu, J. (2018). *Apis cerana* is less sensitive to most neonicotinoids, despite of their smaller body mass. *Journal of Economic Entomology*, *111*(1), 39–42. https://doi.org/10.1093/jee/tox342