Title

Sex-specific median lethal dose (LD_{50}) in bumble bee adults orally and topically exposed to two insecticides and one fungicide

Authors

Manon Fievet[1,2*], Luca Dorio[2], Antoine Gekière[2], Herluin Saillard[1], Serena Alabiso[2], Luca Trapanese[2], Laetitia Verdy[1], Bodil Mattsson[2], Denis Michez[2†], Kévin Tougeron[1†]

[†] These authors equally supervised this work.

Affiliations

[1] Ecology of Interactions and Global Change, Research Institute for Biosciences, University of Mons, Mons, Belgium (20 Place du Parc, 7000 Mons, Belgium)

[2] Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium (20 Place du Parc, 7000 Mons, Belgium)

Corresponding author (*)

MF: manon.fievet2@umons.ac.be

ORCID numbers

MF: 0009-0007-5800-9736

LD: 0009-0008-9910-4235

AG: 0000-0001-5337-1305

BM : 0009-0002-7416-0851

DM: 0000-0001-8880-1838

KT: 0000-0003-4897-3787

Funding

MF is supported by a F.R.S.-FNRS PhD grant “FRIA”. AG is supported by a F.R.S.-FNRS PhD grant “Aspirant”. BM is supported by a F.R.S.-FNRS PhD grant “FRIA”. MF, LD, AG, DM and KT are supported by the European Union’s Horizon Europe research and innovation programme under grant agreement No. 101135238 (WildPosh).

Acknowledgments

The authors have no acknowledgments to declare.

Conflict of interest

The authors have no conflict of interest to declare.

Data Availability Statement

Data will be made publicly available upon the publication of the manuscript.

CRediT authorship contribution statement

MF: Conceptualisation, Methodology, Formal analysis, Investigation, Visualisation, Writing – Original draft, Writing – Review & Editing. **LD**: Investigation, Writing – Review & Editing. **AG**: Methodology, Investigation, Writing – Review & Editing. **HS**: Investigation. **SA**: Investigation. **LT**: Investigation. **LV**: Investigation**. BM**: Investigation**. DM**: Resources, Writing – Review & Editing. **KT**: Resources, Writing – Review & Editing.

Sex-specific median lethal dose (LD50) in bumble bee adults orally and topically exposed to two insecticides and one fungicide

Abstract : Wildlife is deeply impacted by human activities. Evidence-based evaluation of the main threats is challenging as it needs to be representative of the diversity of the risk of exposure and the sensitivity of the organisms, specifically for hyper-diverse groups like insects. While bees are a diverse group of >20,000 species, standardised eco-toxicological data for non-*Apis* bees species remain limited, resulting potentially in the underestimation of threats the wild bees are facing. In many cases, pesticide acute data are available for only one exposure route, most often contact median lethal dose 50% (LD50) values, and primarily focus on social workers or solitary females. In this context, we determined the contact and oral LD50 of three active ingredients of two insecticides and one fungicide, namely acetamiprid, cypermethrin and tebuconazole in males and workers of the bee model *Bombus terrestris*. Acetamiprid and tebuconazole were practically non-toxic under both exposure routes, whereas cypermethrin was the most toxic compound, exhibiting higher toxicity via contact exposure. Interestingly, males showed greater sensitivity than females after both oral and topical exposure to cypermethrin, and after oral exposure to acetamiprid. Our findings highlight the need for additional data on reproductives and for the identification of factors that determine their differential sensitivity.

Keywords

Pesticides; *Bombus terrestris*; Sex-specific response; Toxic effects; Dose-response modeling

Introduction

Bees (*Hymenoptera*: *Anthophila*) provide essential ecosystem services by significantly contributing to agricultural productivity, biodiversity conservation, and human health (Klein et al., 2007; Klein et al., 2018; Patel et al., 2021). However, they face significant anthropogenic pressures

such as pesticides use (Brunet & Fragoso, 2024; Dicks et al., 2021; Goulson et al., 2015), which negatively impacts bee populations worldwide as in Europe (Nicholson et al. 2023), and in the United-States (Guzman et al., 2024). Pesticide exposure can lead to lethal or sublethal effects on bees (Tosi et al., 2022), which can ultimately impair the ecological services they provide (Stanley et al., 2015; Tamburini et al., 2021). Notably, a recent review found that such exposure can reduce bee survival by approximately fivefold (Kita et al., 2024).

Among the bee clade, many species of bumble bees have been experiencing a sharp population decline documented since the second half of the 20th century (Cameron & Sadd, 2020), leading to extinction in some regions (i.e. Belgium, Rollin et al. 2020). Pesticides have been shown to contribute to this trend by increasing mortality at label-recommended concentrations (Straw et al., 2021). They also have been reported to impede bumble bees colony initiation (Baron et al., 2017; Kenna et al., 2019), colony performances (Nicholson et al., 2023), foraging behaviour (O'Reilly & Stanley, 2023), and even reproduction (Siviter et al., 2018). Compared with the reference model *Apis mellifera* (Linnaeus, 1758), the honey bee, *Bombus* species exhibit physiological differences, as in detoxification processes (e.g. variations in cytochrome P450 enzymes activities), which may influence their responses to pesticides (Raine & Rundlöf, 2024). In addition, due to their ecology, bumble bees encounter additional exposure pathways (e.g., soils residues), compared to *A. mellifera*, and may therefore experience more intense pesticide exposure throughout their life cycle (Gradish et al., 2019).

Consequently, increasing concerns regarding bumble bee decline, together with the ecological and physiological differences between *Apis* and non-*Apis* bees (Wood et al., 2020), prompted European Food Safety and Authority (EFSA) to include bumble bees in pesticide risk assessment schemes (European Food Safety Authority, 2013). Publication of acute toxicity guidelines was developed

by the Organisation for Economic Co-operation and Development (OECD; test guideline 246, OECD, 2017a; test guideline 247, OECD, 2017b), which led to new standardized measurement of median lethal doses 50% (LD50) values on the European species *Bombus terrestris* (Linnaeus, 1758), the buff-tailed bumblebee (Gradish et al., 2019; Reid et al., 2020). However, most of the studies rely on females and only one study has investigated potential sex-specific differences in acute responses to pesticide exposure (Linguadoca et al., 2022). Even if males do not participate in brood production, the lack of studies on these reproductives is surprising given their critical role in maintaining the genetic diversity and, consequently, population sustainability (Tong et al., 2025). As males are haploid whereas females are diploid, they may differ in their detoxification capacity, as deleterious alleles cannot be masked in haploid individuals, potentially contributing to sex-specific sensitivity (Linguadoca et al., 2022). In addition, information regarding the acute oral and contact toxicity of many commonly used pesticides to bumble bees remains limited for each sex (i.e. workers, males and queens ; Arena & Sgolastra, 2014; Sanchez-Bayo & Goka, 2014; Tosi et al., 2022).

The aim of this study was to assess sex-specific lethal responses to acute exposure of a selection of the most detected and utilized pesticides in Europe. To this end, males and workers of *B. terrestris* were exposed, both orally and topically, to the neonicotinoid acetamiprid, the pyrethroid cypermethrin, and the triazole tebuconazole. *B. terrestris* was selected as the model species because it is a standardized OECD surrogate for non-*Apis* bees, widely used in ecotoxicological testing, and allows the investigation of caste- and sex-specific responses under controlled laboratory conditions. Bioassays were conducted in accordance with the OECD Bumble bee Acute Toxicity Test Guideline n°246 and n°247 respectively for topical and oral exposure (test guideline 246, OECD, 2017a; test guideline 247, OECD, 2017b). We hypothesise that cypermethrin will

demonstrate a greater toxicity than acetamiprid, especially for topical exposure, as pyrethroids are highly lipophilic contact insecticides with efficient cuticular penetration regardless of bee species. We further expect males to exhibit similar or greater sensitivity compared to females, potentially due to detoxification capacity, and physiological resilience to chemical stressors. Lastly, since it is a fungicide, we hypothesise that tebuconazole will show lower acute toxicity for both exposure mode and sex.

Materials & Methods

Selection of pesticides

In Europe, the most frequently found pesticide families in soil and bee matrices include carboxamides, triazoles, glycine derivates, organochlorines, pyrethroids and neonicotinoids (Alaoui et al., 2024; Benito-Murcia et al., 2025; Böhme et al., 2018; Silva et al., 2019). Based on residue data collected within the PoshBee project (Nicholson et al., 2023), and supplemented by findings from other relevant studies, the selection of active ingredients aimed to reflect the most frequently detected and agriculturally relevant compounds across Europe. Cypermethrin and acetamiprid were selected due to their widespread detection, continued authorization in Europe, and evidence of potential synergistic toxicity in bees (Goulson et al., 2015). Finally, the fungicide tebuconazole was chosen because of its mechanistic role in increasing bee sensitivity to pesticide mixtures and its potential to interact with different classes of insecticides (Goulson et al., 2015; Raimets et al., 2018). Overall, the selected compounds were chosen to represent pesticide classes exhibiting contrasting modes of action and toxicity profiles, allowing comparison of sex-specific responses across compounds differing in expected acute toxicity.

Active ingredients and treatments

Analytical standards Pestanal® acetamiprid (99.9% pure), cypermethrin (97.3% pure), tebuconazole (99.5% pure) and dimethoate (98% pure) were purchased from *Sigma Aldrich* (United Kingdom ; see online supplementary material, **Table S1**). The optimal range doses for each pesticide were assessed through range-finder bioassays to determine the nominal treatment doses in the final trial. Alongside the negative (i.e. acetone) and positive (i.e. dimethoate) controls, five doses per pesticide were tested, with intermediate nominal doses following a geometric progression (ratio ≤ 2). For tebuconazole, limit tests were conducted using a single high dose (i.e. 100 µg/bee; test guideline 246, OECD, 2017a; test guideline 247, OECD, 2017b).

To prepare treated solutions, the analytical standards were first solubilized in acetone. Afterwards, oral solutions were prepared by serial dilutions of the acetone-based stock in a 50% w/w aqueous sucrose solution, ensuring an acetone concentration ≤ 5%. Topical solutions were prepared in acetone and mixed with 0.1% Triton X-100 serving as surfactant (test guideline 246, OECD, 2017). All stock and treatment solutions were stored at 4°C in the dark.

Pesticide concentrations in the samples were determined by chromatography coupled with mass spectrometry. Due to the poor ionization of cypermethrin in LC–MS, its quantification was performed using GC–MS after liquid–liquid extraction with dichloromethane. Measured concentrations differed from nominal values, with discrepancies varying among compounds (see online supplementary material, **Table S2**). Cypermethrin concentrations were generally lower than nominal values, particularly at lower doses, whereas acetamiprid and tebuconazole showed closer agreement. These differences likely reflect compound-specific properties such as solubility or stability during preparation and exposure. All reported doses refer to analytically measured concentrations, which better reflect the actual exposure of the bees.

Test organisms

In each bioassay, workers and males of *B. terrestris* were respectively obtained from 5 standard colonies and 10 maculino-systems supplied by *Biobest bvba* (Westerlo, Belgium). Prior to experimentations, colonies and masculino-systems were acclimatised for at least 24 hr with the nectar substitute Biogluc® provided *ad libitum*.

Subsequently, bees were randomly collected under red light, carefully extracted with tweezers, weighted, and individually assigned to a treatment in a Nicot® cage. Each treatment comprised a minimum of 30 bees (i.e., at least 6 individuals per colony), except for the limit tests, which included 60 bees for the unique pesticide dose (**Table 1**). Bumble bees were provided with *ad libitum* 50% w/v aqueous sucrose solution in a 2 mL tipless syringe to acclimatize individually for 24 hr. During acclimatisations and experiments, workers and males were kept under controlled conditions in constant darkness at 26 ± 1°C and 60 ± 10% relative humidity.

Oral toxicity bioassays

Prior to oral exposure, bumble bees were individually starved for 3 hr by replacing the sucrose syringe with an empty one. Then, bumble bees were individually exposed to 40 µL of their respective treatment in a 2mL syringe tip, under white light to stimulate feeding. Four hours after administration, consumption was visually checked. Workers that did not eat the entire solution were considered as non-feeders and were excluded from the test. Remaining bees were supplied with *ad libitum* 50% w/v aqueous sucrose solution in a 2 mL tipless syringe. Mortality was recorded at 4hr, 24hr, 48hr, 72hr and 96hr (test guideline 247, OECD, 2017).

Topical toxicity bioassays

Before topical exposure, bumble bees were individually anesthetized by placing them in a sealed tube on an ice tray for 3 to 5 min maximum, depending on their size. Once asleep, a 2 µL drop of the treatment dose was applied on the dorsal part of their thorax under a chemical hood. After few

seconds to allow cuticle absorption of the drop, bees were returned to their respective Nicot® cages. Individuals were kept at room temperature to facilitate recovery, ensure full awakening and minimize temperature shock before being returned to control conditions. Mortality was recorded similarly to oral bioassays (test guideline 246, OECD, 2017).

Statistical analysis

Dose-response analyses were performed using the *drc* package (Ritz et al., 2015), applying log-logistic regression models. For all sexes, pesticides and exposure routes, dose-response curves were modeled using a three-parameter log-logistic function (LL.3()) within the drm() function (drc package; Ritz et al., 2015). The median lethal dose 50% (LD50), median lethal concentration 50% (LC50) and mass-standardised LD50 (STD50) values were extracted using the ED() function (drc package; Ritz et al., 2015). All reported LD50, LC50 and STD50 values correspond to mortality recorded 96 h after exposure. In accordance with standard OECD acute bee toxicity protocols (test guideline 246, OECD, 2017a; test guideline 247, OECD, 2017b), these toxicity endpoints were based on the dose initially administered at t_0. This approach assumes that the administered dose reflects the effective exposure over the duration of the experiment.

Treatments comparisons within and between sexes were conducted for each exposure mode using a four-parameter log-logistic model (LL.4()), fitted with the drm() function (drc package; Ritz et al., 2015). The median lethal dose 50% and mass-standardised LD50 values were compared using the compParm() function (drc package; Ritz et al., 2015).

For limit tests and to evaluate mortality across pesticide treatments, survival time was analysed using a Bayesian Cox proportional hazards model (brm() function, brms package; Bürkner, 2017) with the cmdstanr backend. This approach was employed because some control groups showed complete separation (i.e., no deaths occurred in the control while deaths occurred in at least one

treatment group, leading to infinite or non-estimable hazard ratios), violating the proportional hazards assumption of conventional Cox models. Treatment was included as a fixed factor, bumble bee mass as a covariate, and colony of origin as a random intercept to account for genetic variability. Posterior hazard ratios and their 95% highest posterior density intervals were extracted using the tidy() function (broom.mixed package; Bolker et Robinson, 2025), and pairwise comparisons among treatments were performed using the emmeans() function (emmeans package; Lenth, 2024).

Dose-response plots were generated using the ggplot() function (ggplot2 package; Wickham et al., 2020). Kaplan-Meier survival curves were visualized for each exposure route and pesticide for workers (see online supplementary material, **Figures S1 and S3**) and males (see online supplementary material, **Figures S2 and S4**) using the ggsurvplot() function (survminer package; Kassambara et al., 2016). All statistical analyses were conducted in R (v.4.4.1; R Core Team, 2024).

Results

Oral exposure

Workers survival

In the worker oral experiment, the estimated LD50 values of cypermethrin (LD50 = 3.94 µg/bee, 95% Confidence Interval (CI) = 2.72–5.16 µg/bee) and acetamiprid (LD50 = 58.67 µg/bee, CI = 52.19–65.14 µg/bee) were significantly different ($p < 0.001$), along with their bee mass-standardised LD50 values ($p < 0.001$, **Figure 1A**; **Table 2**). The estimated lower LD50 value of cypermethrin indicates its higher lethal toxicity compared to acetamiprid in orally exposed workers. Additionally, no mortality was observed under the single dose of tebuconazole during the experiment, and posterior estimates indicated no evidence for increased mortality in

tebuconazole compared to the control (HR = 0.27; CrI: 0.0064–6.94; see online supplementary material, **Figure S1**).

Body mass was negatively associated with mortality in the global model (HR = 0.69, CrI: 0.50–0.96). However, as no mortality occurred in the tebuconazole treatment, this association is unlikely to reflect an effect under tebuconazole exposure and is instead driven by mortality observed in the dimethoate treatment.

Males survival

Males that were orally exposed to cypermethrin exhibited a lower LD50 value (LD50 = 1.99 µg/bee, CI = 1.55–2.45 µg/bee) than those exposed to acetamiprid (LD50 = 61.66 µg/bee, CI = 41.64–81.69 µg/bee; **Table 2**). The LD50 values of the two compounds did not differ significantly ($p = 0.183$), but this result became significant after adjustment for body mass ($p < 0.001$; **Figure 1B**). Concerning tebuconazole, no mortality occurred under the tested dose. Posterior estimates provided no evidence for increased mortality under tebuconazole relative to the negative control (HR = 0.084; CrI: 0.0036–1.01; see online supplementary material, **Figure S2**), supporting its classification as a low-toxicity compound.

Male mass showed no clear influence on mortality in tebuconazole (HR = 1.06, CrI: 0.74–1.51), acetamiprid (HR = 1.10, CrI: 0.89–1.30), and cypermethrin (HR = 1.30, CrI: 0.49–3.71) experiments.

Sex-specific response

In both orally exposed sexes, only tebuconazole was considered as low-lethal (**Table 2**). Cypermethrin was the most lethal of the three pesticides in workers and males. No significant differences were found between LD50 values ($p = 0.096$) for this compound, except for mass-standardised LD_{50} values, which differed significantly between sexes ($p < 0.001$; **Figure 2A**).

Similarly, the LD50 values of workers and males for acetamiprid did not differ significantly (p = 0.731). However, after adjusting for body mass, the mass-standardised LD_{50} of this pesticide showed a moderate evidence of difference between sexes (p = 0.036, **Figure 2B**). The mass-adjusted LD50 in males were lower than in workers for both compounds, indicating males were less resilient than the workers.

Topical exposure

Workers survival

Regarding workers contact exposure, cypermethrin (LD50 = 0.62 µg/bee, CI = 0.55–0.69 µg/bee) was the only highly toxic compound (**Table 3**). In fact, no mortality was observed under the single dose of tebuconazole during the experiment, and posterior estimates indicated little evidence for increased mortality in tebuconazole (HR = 0.21; CrI: 0.0052–4.65; see online supplementary material, **Figure S3**) and in acetamiprid (HR = 1.14, CrI: 0.20–6.92; see online supplementary material, **Figure S3**) compared to the control.

No evidence of an effect of body mass on mortality was detected in tebuconazole (HR = 0.66, CrI: 0.41–1.05), acetamiprid (HR = 0.96, CrI: 0.68–1.32) or cypermethrin (HR = 0.97, CrI: 0.75–1.26) experiments.

Males survival

Cypermethrin (LD50 = 0.46 µg/bee, CI = 0.32–0.57 µg/bee) exhibited a significant mortality (**Table 3**). There was little evidence for increased mortality between acetamiprid (HR = 1.14; CrI: 0.2–6.92; see online supplementary material, **Figure S4**), tebuconazole (HR = 0.22; CrI: 0.005–4.65 see online supplementary material, **Figure S4**) and their respective negative control, indicating that these pesticides exhibited low mortality.

Bumble bee mass mass showed no consistent effect on mortality for tebuconazole (HR = 0.56, 95% CrI: 0.41–1.05), acetamiprid (HR = 0.96, 95% CrI: 0.68–1.32), and cypermethrin (HR = 0.97, CrI: 0.81–1.18) experiments.

Sex-specific response

Among all tested pesticides, only cypermethrin exhibited contact mortality in both sexes (**Table 3**). When comparing this pesticide between sexes, the LD50 values significantly differed ($p < 0.001$). After adjusting for body mass, the bee mass-standardised LD50 values were also statistically different ($p < 0.001$, **Figure 3**). The mass-adjusted LD50 in workers were higher than in males, indicating males were less resilient than the workers.

Discussion

Threatening pesticides to pollinators remain authorized in the European Union, and an additional set has been approved recently (Arena & Sgolastra, 2014; Siviter et al., 2023). This policy context highlights the need for additional data (e.g. first-tier assessments) to evaluate pesticide risks to pollinators that are not classically used as models such as non-*Apis* bees, and thereby reinforce risk assessments and objectify regulatory decisions (Raine & Rundlöf, 2024). To advance the inclusion of a wider range of bee species in regulatory decision-making, it is imperative to account for interspecific and sex differences in sensitivity among the potential surrogate species (i.e. *B. terrestris* and *O. bicornis*), particularly in first-tier measurements.

In our study, the assessed LD50 values are consistent with the active ingredient classification established by the EFSA (Arena et al., 2018; European Food Safety Authority, 2014; Hernandez Jerez et al., 2022). Cypermethrin appeared to be the most lethal of the three compounds tested, showing higher toxicity under acute contact exposure, whereas acetamiprid appeared to be slightly toxic and tebuconazole was practically non-toxic. Furthermore, we found that *B. terrestris* workers

and males were less sensitive to these pesticides than *A. mellifera*. Specifically, *B. terrestris* worker LD50 values to cypermethrin were found to be approximately 22 and 26 times higher than workers of *A. mellifera* by oral and contact respectively (Lewis & Tzilivakis, 2019). Moreover, after adjusting LD50 for body mass, *A. mellifera* workers remain about 10 and 12 times more sensitive than *B. terrestris* workers after oral and contact exposure, indicating that factors beyond body weight influence sensitivity. In that sense, our results suggest that *A. mellifera* remains a conservative surrogate species for first-tier assessment of these compounds.

Furthermore, we provide the first evidence of sex variation in sensitivity to cypermethrin following both oral and contact exposure, and to acetamiprid following oral exposure. Only a few studies have tested the differences in pesticide sensitivity between adult males and workers, or the susceptibility of males alone. To our knowledge, two studies examined the survival rates of males in *A. mellifera* (McAfee et al., 2022; Tong et al., 2025) and one in *B. terrestris* (Linguadoca et al., 2022), although assessing mortality constitutes a primary step in risk assessment. These studies reported a higher sensitivity of males compared to females in both species, except for the fungicide Amistar (azoxystrobin) and the herbicide glyphosate reported as practically non-lethal. Our findings are partly consistent with the outcomes of these previous studies. The similar sensitivity observed topically for acetamiprid and for both routes in tebuconazole likely reflects their relatively low toxicity, due respectively to moderate binding affinity (Casida, 2025; Ito et al., 2024) and the absence of specific target sites (Jing & Behmer, 2025). Furthermore, the low contact toxicity of acetamiprid, compared to its oral toxicity, is consistent with its poor cuticular penetration kinetics, as demonstrated in *A. mellifera* where acetamiprid penetrates the cuticle much less efficiently than nitro-substituted neonicotinoids such as imidacloprid (Zaworra et al., 2019). In contrast, cypermethrin under contact exposure was the most toxic treatment and showed clear

sex differences in both LD50 and STD50. For other treatments, differences emerged only after mass standardisation, indicating that body size can mask underlying physiological differences between sexes.

Several factors may account for the increased sensitivity observed in males relative to workers, such as differences in detoxification processes. Indeed, in insects, neonicotinoid detoxification is mainly mediated by cytochrome P450 monooxygenases (CYP450), while pyrethroid detoxification primarily involves CYP450 and carboxylesterases. (Black et al., 2021; Johnson et al., 2006). Thus, variations in activity of one or both detoxification enzyme families between males and workers, with less activity in males, may increase their sensitivity to these pesticides by reducing the efficiency of their metabolization. No such differences in *Bombus* spp. were found in the literature but sex-related differences in sensitivity to pesticides have been documented in other insect species. For example, a study on the housefly *Musca domestica* showed females exposed to chlorfenapyr exhibited higher overall P450-monooxygenase activity than males, contributing to lower male tolerance to insecticides (Garbaly et al., 2025). Nonetheless, differences in detoxification enzyme activity alone may not fully explain the variation in sensitivity between sexes, as other factors such as sex-specific life-history strategies related to immune investment (McKean & Nunney, 2008; Schwenke et al., 2016) and sex-biased gene expression (Garbaly et al., 2025; Mobley & Gegear, 2018) could also contribute to the greater vulnerability of males.

In addition, the high topical toxicity of cypermethrin is not surprising as pyrethroids are highly lipophilic compounds, making them approximately three times more toxic to bees by contact than oral (Sanchez-Bayo & Goka, 2014). Their lipophilicity facilitates partitioning into and diffusion through the cuticle, enabling efficient transcuticular uptake before reaching inner tissues and binding on their targets. The cuticle size and composition (e.g. lipids, hydrocarbons and chitin)

may therefore influence pesticide infiltration (Ren et al., 2023). Yet, little is known about bees cuticle and potential differences in its structure between species and sexes. However, in other insect species, a study showed that in the mosquito *Anopheles gambiae*, the resistant strain exhibited thicker cuticle layers than the susceptible strain that reduced insecticide penetration (Yahouédo et al., 2017). Also, another study demonstrated that *Drosophila suzukii* exhibited a more permeable cuticle to pesticides than *Drosophila melanogaster*, associated with reduced levels of branched and desaturated cuticular hydrocarbons (Wang et al., 2020). Interestingly, males of both *Drosophila* species showed weaker barrier function than females, suggesting that sex variation in cuticle hydrocarbon composition may contribute to differences in sensitivity to pesticides (Wang et al., 2020). Thus, these studies along with others, showed that the cuticle thickness (Lin et al., 2012; Wang et al., 2025) and composition (Balabanidou et al., 2018; Ren et al., 2023) can contribute to a greater resistance to pesticides in insects. These factors might have contributed, with differences in detoxification processes, to the sex-differences in contact cypermethrin sensitivity in *B. terrestris*.

Finally, to place our toxicity findings in a field-realistic context, we compared our LD50 values against estimated field exposure doses using a Hazard Quotient (HQ) approach (EFSA, 2013). For oral exposure, estimated daily doses were calculated from median nectar residue concentrations reported in the PoshBee dataset (i.e. acetamiprid: 21 µg/L; tebuconazole: 13 µg/L; Nicholson et al., 2023). No nectar residue data for cypermethrin were available in the literature, oral risk for this compound was therefore not assessed. For contact exposure, field deposits were estimated from maximum EU-authorised application rates (EFSA, 2014; EFSA, 2016; EFSA, 2018) following EFSA (2013) guidance. The resulting HQ values were low for acetamiprid and tebuconazole across all castes and routes ($HQ \leq 0.004$ orally; $HQ < 0.021$ by contact), confirming

that field-realistic exposure to these two active ingredients is unlikely to pose a lethal hazard to *B. terrestris* workers or males. However, this result does not extend to cypermethrin via contact exposure. Although HQ values remained below 1 for both workers (HQ = 0.40) and males (HQ = 0.54), they were higher than those obtained for acetamiprid and tebuconazole, and were consistently higher in males than in workers. This indicates that while cypermethrin does not pose an acute lethal risk to *B. terrestris* at current authorised application rates, it represents the compound of greatest concern among the three tested, particularly for males. These findings take on additional relevance when considered alongside broader regulatory trends where the phasing out of most neonicotinoids has been accompanied by a marked increase in pyrethroid use in agricultural fields (Kathage et al., 2018; Reid et al., 2020). Additionally, even if acute lethal risk remains low at current authorised rates, cumulative and repeated exposure scenarios along with potential synergistic effects with other agrochemicals may deserve further attention in future risk assessments.

Conclusion

We showed that the impact of pesticide on bees can be sex-specific. Our study highlights the need for additional first-tier measurements on bee species, focusing not only on workers or solitary females but also on the other reproductives (e.g. males and queens) that contribute to the long-term viability and resilience of populations. We also show that research should further investigate traits and physiological differences that may explain the caste variations in sensitivity. Importantly, *B. terrestris* workers and males were consistently less sensitive than *A. mellifera* to the three tested active ingredients, supporting the continued use of *A. mellifera* as a conservative standard surrogate species for first-tier pesticide risk assessment. In addition, our results demonstrate that among the three tested active ingredients, acetamiprid and tebuconazole posed minimal hazard to

this bumble bee species (HQ < 0.02 for all routes and castes), whereas cypermethrin presented a higher risk through contact exposure (HQ = 0.40 for workers and 0.54 for males), with males being more at risk. These findings emphasise the importance of considering exposure pathways in pesticide hazard evaluation, especially since the ban of most neonicotinoids in Europe has led to the increased use of pyrethroids in agricultural fields (Reid et al., 2020).

References

Alaoui, A., Christ, F., Abrantes, N., Silva, V., González, N., Gai, L., Harkes, P., Navarro, I., Torre, A. de la, Martínez, M. Á., Norgaard, T., Vested, A., Schlünssen, V., Aparicio, V. C., Campos, I., Pasković, I., Pasković, M. P., Glavan, M., Ritsema, C., & Geissen, V. (2024). Assessing pesticide residue occurrence and risks in the environment across Europe and Argentina. *Environmental Pollution*, *363*. https://doi.org/10.1016/j.envpol.2024.125056

Arena, M., Auteri, D., Barmaz, S., Brancato, A., Brocca, D., Bura, L., Carrasco Cabrera, L., Chiusolo, A., Civitella, C., Court Marques, D., Crivellente, F., Ctverackova, L., De Lentdecker, C., Egsmose, M., Erdos, Z., Fait, G., Ferreira, L., Greco, L., ... Villamar-Bouza, L. (2018). Peer review of the pesticide risk assessment of the active substance cypermethrin. *EFSA Journal, 16*(8). https://doi.org/10.2903/j.efsa.2018.5402

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

Balabanidou, V., Grigoraki, L., & Vontas, J. (2018). Insect cuticle: A critical determinant of insecticide resistance. *Current Opinion in Insect Science, 27*, 68–74. https://doi.org/10.1016/j.cois.2018.03.001

Baron, G. L., Jansen, V. A. A., Brown, M. J. F., & Raine, N. E. (2017). Pesticide reduces bumblebee colony initiation and increases probability of population extinction. *Nature Ecology & Evolution, 1*(9), 1308–1316. https://doi.org/10.1038/s41559-017-0260-1

Benito-Murcia, M., García-Vicente, E., Martín, M., Pérez, A., Risco, D., Rodríguez, J. M. A., Soler, F., Pérez-López, M., Míguez-Santiyán, M. P., & Martinez-Morcillo, S. (2025). Pesticide residues in beeswax from Western Spain: Toxicological risk for bee health and beekeeping productivity. *Environmental Toxicology and Pharmacology*, *117*. https://doi.org/10.1016/j.etap.2025.104744

Black, W. C., Snell, T. K., Saavedra-Rodriguez, K., Kading, R. C., & Campbell, C. L. (2021). From global to local—New insights into features of pyrethroid detoxification in vector mosquitoes. *Insects, 12*(4), 276. https://doi.org/10.3390/insects12040276

Böhme, F., Bischoff, G., Zebitz, C. P. W., Rosenkranz, P., & Wallner, K. (2018). Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. *PLoS ONE, 13*(7), e0199995. https://doi.org/10.1371/journal.pone.0199995

Bolker, B., & Robinson, D. (2025). *broom.mixed: Tidying methods for mixed models* (R package version 0.2.9.7). https://github.com/bbolker/broom.mixed

Brunet, J., & Fragoso, F. P. (2024). What are the main reasons for the worldwide decline in pollinator populations? *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *19*(1). https://doi.org/10.1079/CABIREVIEWS.2024.0016

Bürkner, P. C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, *80*. https://doi.org/10.18637/jss.v080.i01

Cameron, S. A., & Sadd, B. M. (2020). Global trends in bumble bee health. *Annual Review of Entomology, 65*, 209–232. https://doi.org/10.1146/ANNUREV-ENTO-011118-111847/1

Casida, J. E. (2018). Neonicotinoids and other insect nicotinic receptor competitive modulators: Progress and prospects. *Annual Review of Entomology, 63*(1), 125–144. https://doi.org/10.1146/annurev-ento-020117-043042

Dicks, L. V., Breeze, T. D., Ngo, H. T., Senapathi, D., An, J., Aizen, M. A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L. A., Gemmill-Herren, B., Howlett, B. G., Imperatriz-Fonseca, V. L., Johnson, S. D., Kovács-Hostyánszki, A., Kwon, Y. J., Lattorff, H. M. G., Lungharwo, T., Seymour, C. L., … Potts, S. G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology & Evolution*, *5*(10), 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

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). https://doi.org/10.2903/j.efsa.2013.3295

European Food Safety Authority. (2014). Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole. *EFSA Journal, 12*(1), 3485. https://doi.org/10.2903/j.efsa.2014.3485

European Food Safety Authority. (2016). Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. *EFSA Journal*, *14*(11), 4610. https://doi.org/10.2903/j.efsa.2016.4610

European Food Safety Authority. (2018). Peer review of the pesticide risk assessment of the active substance cypermethrin. *EFSA Journal*, *16*(7), 5402. https://doi.org/10.2903/j.efsa.2018.5402

Garbaly, V., Krestonoshina, K., Kinareikina, A., Bobreshova, S., Avdeeva, A., Ismagilova, J., Zaitsev, I., & Silivanova, E. (2025). Glutathione S-Transferase (GST) activities and gene expression patterns of different GST classes in *Musca domestica* L. depending on sex and stage of development. *International Journal of Molecular Sciences, 26*(23), 11366. https://doi.org/10.3390/ijms262311366

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229). https://doi.org/10.1126/science.1255957

Gradish, A. E., Van Der Steen, J., Scott-Dupree, C. D., Cabrera, A. R., Cutler, G. C., Goulson, D., Klein, O., Lehmann, D. M., Lückmann, J., O'Neill, B., Raine, N. E., Sharma, B., & Thompson, H. (2019). Comparison of Pesticide Exposure in Honey Bees (Hymenoptera: Apidae) and Bumble Bees (Hymenoptera: Apidae): Implications for Risk Assessments. *Environmental Entomology*, *48*(1), 12–21. https://doi.org/10.1093/EE/NVY168

Guzman, L. M., Elle, E., Morandin, L. A., Cobb, N. S., Chesshire, P. R., McCabe, L. M., Hughes, A., Orr, M., & M'Gonigle, L. K. (2024). Impact of pesticide use on wild bee distributions across the United States.*Nature Sustainability, 7*(10), 1324–1334. https://doi.org/10.1038/s41893-024-01413-8

Hernandez Jerez, A., Adriaanse, P., Berny, P., Coja, T., Duquesne, S., Focks, A., Marinovich, M., Millet, M., Pelkonen, O., Pieper, S., Tiktak, A., Topping, C., Widenfalk, A., Wilks, M., Wolterink, G., Rundlöf, M., Ippolito, A., Linguadoca, A., ... Aldrich, A. (2022). Statement on the active substance acetamiprid. *EFSA Journal, 20*(1). https://doi.org/10.2903/j.efsa.2022.7031

Ito, R., Kamiya, M., Takayama, K., Mori, S., Matsumoto, R., Takebayashi, M., Ojima, H., Fujimura, S., Yamamoto, H., Ohno, M., Ihara, M., Okajima, T., Yamashita, A., Colman, F., Lycett, G. J., Sattelle, D. B., & Matsuda, K. (2024). Unravelling nicotinic receptor and ligand features underlying neonicotinoid knockdown actions on the malaria vector mosquito Anopheles gambiae. *Open Biology*, *14*(7). https://doi.org/10.1098/rsob.240057

Jing, X., & Behmer, S. T. (2025). Insect sterol nutrition: Physiological mechanisms, ecology, and applications. *Annual Review of Entomology, 70*, 45–64. https://doi.org/10.1146/annurev-ento-011019-025017

Johnson, R. M., Wen, Z., Schuler, M. A., & Berenbaum, M. R. (2006). Mediation of pyrethroid insecticide toxicity to honey bees (*Hymenoptera: Apidae*) by cytochrome P450 monooxygenases. *Journal of Economic Entomology, 99*(4), 1046–1050. https://doi.org/10.1603/0022-0493-99.4.1046

Kassambara, A., Kosinski, M., & Biecek, P. (2016). survminer: Drawing Survival Curves using 'ggplot2'. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.survminer

Kathage, J., Castañera, P., Alonso-Prados, J. L., Gómez-Barbero, M., & Rodríguez-Cerezo, E. (2018). The impact of restrictions on neonicotinoid and fipronil insecticides on pest management in maize, oilseed rape and sunflower in eight European Union regions. *Pest Management Science, 74*(1), 88–99. https://doi.org/10.1002/ps.4715

Kenna, D., Cooley, H., Pretelli, I., Ramos Rodrigues, A., Gill, S. D., & Gill, R. J. (2019). Pesticide exposure affects flight dynamics and reduces flight endurance in bumblebees. *Ecology and Evolution*, *9*(10), 5637–5650. https://doi.org/10.1002/ECE3.5143

Kita, C. A., Leal, L. C., & Mello, M. A. R. (2024). Pesticides put our food security at risk by reducing bee survival almost five times. *Apidologie*, *55*(4), 1–14. https://doi.org/10.1007/S13592-024-01087-3

Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C., & Pufal, G. (2018). Relevance of wild and managed bees for human well-being. *Current Opinion in Insect Science*, *26*, 82–88. https://doi.org/10.1016/J.COIS.2018.02.011

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Lenth, R. (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means.*

Lewis, K. A., & Tzilivakis, J. (2019). Wild Bee Toxicity Data for Pesticide Risk Assessments. *Data, 4*(3), 98. https://doi.org/10.3390/DATA4030098

Lin, Y., Jin, T., Zeng, L., & Lu, Y. (2012). Cuticular penetration of β-cypermethrin in insecticide-susceptible and resistant strains of Bactrocera dorsalis. *Pesticide Biochemistry and Physiology*, *103*(3), 189–193. https://doi.org/10.1016/j.pestbp.2012.05.002

Linguadoca, A., Jürison, M., Hellström, S., Straw, E. A., Šima, P., Karise, R., Costa, C., Serra, G., Colombo, R., Paxton, R. J., Mänd, M., & Brown, M. J. F. (2022). Intra-specific variation in sensitivity of *Bombus terrestris* and *Osmia bicornis* to three pesticides. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-22239-4

McAfee, A., Metz, B. N., Milone, J. P., Foster, L. J., & Tarpy, D. R. (2022). Drone honey bees are disproportionately sensitive to abiotic stressors despite expressing high levels of

stress response proteins. *Communications Biology*, *5*(1). https://doi.org/10.1038/s42003-022-03092-7

McKean, K. A., & Nunney, L. (2008). Sexual selection and immune function in Drosophila melanogaster. *Evolution, 62*(2), 386–400. https://doi.org/10.1111/j.1558-5646.2007.00286.x

Mobley, M. W., & Gegear, R. J. (2018). One size does not fit all: Caste and sex differences in the response of bumblebees (*Bombus impatiens*) to chronic oral neonicotinoid exposure. *PLOS ONE, 13*(10), e0200041. https://doi.org/10.1371/journal.pone.0200041

Nicholson, C. C., Knapp, J., Kiljanek, T., Albrecht, M., Chauzat, M.-P., Costa, C., De la Rúa, P., Klein, A.-M., Mänd, M., Potts, S. G., Schweiger, O., Bottero, I., Cini, E., de Miranda, J. R., Di Prisco, G., Dominik, C., Hodge, S., Kaunath, V., Knauer, A., ... Rundlöf, M. (2023). *Pesticide use negatively affects bumble bees across European landscapes*. *Nature, 628*(8007), 355–358. https://doi.org/10.1038/s41586-023-06773-3

Organisation for Economic Co-operation and Development. (2017a). *Test No. 246: Bumblebee, acute contact toxicity test* (OECD Guidelines for the Testing of Chemicals, Section 2). OECD Publishing. https://doi.org/10.1787/9789264284104-en

Organisation for Economic Co-operation and Development. (2017b). *Test No. 247: Bumblebee, acute oral toxicity test* (OECD Guidelines for the Testing of Chemicals, Section 2). OECD Publishing. https://doi.org/10.1787/9789264284128-en

O'Reilly, A. D., & Stanley, D. A. (2023). Non-neonicotinoid pesticides impact bumblebee activity and pollen provisioning. *Journal of Applied Ecology*, *60*(8), 1673–1683. https://doi.org/10.1111/1365-2664.14444

Patel, V., Pauli, N., Biggs, E., Barbour, L., & Boruff, B. (2021). Why bees are critical for achieving sustainable development. *Ambio*, *50*(1), 49–59. https://doi.org/10.1007/S13280-020-01333-9/FIGURES/2

R Core Team. (2024). R: A language and environment for statistical computing (Version 4.4.1) [Software]. R Foundation for Statistical Computing. *Https://Www.R-Project.Org/*.

Raine, N. E., & Rundlöf, M. (2024). Pesticide Exposure and Effects on Non-*Apis* Bees. *Annual Review of Entomology, 69, 551–576.* https://doi.org/10.1146/annurev-ento-040323-020625

Raimets, R., Karise, R., Mänd, M., Kaart, T., Ponting, S., Song, J., & Cresswell, J. E. (2018). Synergistic interactions between a variety of insecticides and an ergosterol-biosynthesis-inhibitor fungicide in dietary exposures of bumble bees (*Bombus terrestris* L.). *Pest Management Science, 74*(3), 541–546. https://doi.org/10.1002/ps.4756

Reid, R. J., Troczka, B. J., Kor, L., Randall, E., Williamson, M. S., Field, L. M., Nauen, R., Bass, C., & Davies, T. G. E. (2020). Assessing the acute toxicity of insecticides to the buff-tailed bumblebee (*Bombus terrestris audax*). *Pesticide Biochemistry and Physiology, 166*, 104562. https://doi.org/10.1016/j.pestbp.2020.104562

Ren, Y., Li, Y., Ju, Y., Zhang, W., & Wang, Y. (2023). Insect cuticle and insecticide development. *Archives of Insect Biochemistry and Physiology, 114*(4), e22057. https://doi.org/10.1002/arch.22057

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-Response Analysis Using R. *PLOS ONE*, *10*(12), e0146021. https://doi.org/10.1371/JOURNAL.PONE.0146021

Rollin, O., Vray, S., Dendoncker, N., Michez, D., Dufrêne, M., & Rasmont, P. (2020). Drastic shifts in the Belgian bumblebee community over the last century. *Biodiversity and Conservation, 29*, 2553–2573. https://doi.org/10.1007/s10531-020-01988-6

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

Schwenke, R. A., Lazzaro, B. P., & Wolfner, M. F. (2016). Reproduction–immunity trade-offs in insects. *Annual Review of Entomology, 61*(1), 239–256. https://doi.org/10.1146/annurev-ento-010715-023924

Silva, V., Mol, H. G. J., Zomer, P., Tienstra, M., Ritsema, C. J., & Geissen, V. (2019). Pesticide residues in European agricultural soils – A hidden reality unfolded. *Science of the Total Environment*, *653*, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441

Siviter, H., Brown, M. J. F., & Leadbeater, E. (2018). Sulfoxaflor exposure reduces bumblebee reproductive success. *Nature, 561*(7721), 109–112. https://doi.org/10.1038/s41586-018-0430-6

Siviter, H., Linguadoca, A., Ippolito, A., & Muth, F. (2023). Pesticide licensing in the EU and protecting pollinators. *Current Biology, 33*(2), R44–R48. https://doi.org/10.1016/j.cub.2022.12.002

Stanley, D. A., Garratt, M. P. D., Wickens, J. B., Wickens, V. J., Potts, S. G., & Raine, N. E. (2015). Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature*, *528*(7583), 548–550. https://doi.org/10.1038/nature16167

Straw, E. A., Carpentier, E. N., & Brown, M. J. F. (2021). Roundup causes high levels of mortality following contact exposure in bumble bees. *Journal of Applied Ecology*, *58*(6), 1167–1176. https://doi.org/10.1111/1365-2664.13867

Tamburini, G., Pereira-Peixoto, M.-H., Borth, J., Lotz, S., Wintermantel, D., Allan, M. J., Dean, R., Schwarz, J. M., Knauer, A., Albrecht, M., & Klein, A.-M. (2021). Fungicide and insecticide exposure adversely impacts bumblebees and pollination services under semi-field conditions. *Environment International*, *157*, 106813. https://doi.org/10.1016/j.envint.2021.106813

Tong, W., Wang, L., Tao, B., Yao, H., Liu, H., Huang, S., Li, J., Xu, X., & Duan, X. (2025). Azoxystrobin and Picoxystrobin Lead to Decreased Fitness of Honey Bee Drones (*Apis mellifera ligustica*). *Agriculture*, *15*(15), 1590. https://doi.org/10.3390/agriculture15151590

Tosi, S., Sfeir, C., Carnesecchi, E., vanEngelsdorp, D., & Chauzat, M.-P. (2022). Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. *Science of The Total Environment*, *844*, 156857. https://doi.org/10.1016/j.scitotenv.2022.156857

Wang, B., Yi, M., Wang, M., Wang, H., Tang, Z., Zhao, H., Wei, P., Liao, X., Xue, W., Pan, L., & Shi, L. (2025). Cuticle thickening mediates insecticide penetration resistance in *Spodoptera litura*. *Journal of Advanced Research, 78*, 163–178. https://doi.org/10.1016/j.jare.2025.02.027

Wang, Y., Farine, J. P., Yang, Y., Yang, J., Tang, W., Gehring, N., Ferveur, J. F., & Moussian, B. (2020). Transcriptional Control of Quality Differences in the Lipid-Based Cuticle Barrier in Drosophila suzukii and Drosophila melanogaster. *Frontiers in Genetics*, *11*. https://doi.org/10.3389/fgene.2020.00887

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., & van den Brand, T. (2020). ggplot2: Create Elegant Data

Visualisations Using the Grammar of Graphics. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.ggplot2

Wood, T. J., Michez, D., Paxton, R. J., Drossart, M., Neumann, P., Gérard, M., Vanderplanck, M., Barraud, A., Martinet, B., Leclercq, N., & Vereecken, N. (2020). Managed honey bees as a radar for wild bee decline? *Apidologie, 51*(6), 1100–1116. https://doi.org/10.1007/s13592-020-00788-9

Yahouédo, G. A., Chandre, F., Rossignol, M., Ginibre, C., Balabanidou, V., Mendez, N. G. A., Pigeon, O., Vontas, J., & Cornelie, S. (2017). Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector Anopheles gambiae. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-11357-z

Figure 1. Oral bee-mass standardised lethal dose 50 % (std LD50) of cypermethrin (blue curve) and acetamiprid (orange curve) in *Bombus terrestris* workers (A) and males (B). Shade areas around the dose-response curves represent the confidence intervals. The points represent the observed survival outcomes for each individual at each dose or mass-standardised dose, where 0 indicates survival and 1 indicates mortality. The horizontal and vertical lines intersecting at 50% mortality show the estimated mass-standardised LD50 for each pesticide.

Alt text: Dose response curves showing oral toxicity of cypermethrin and acetamiprid in *Bombus terrestris* workers and males. The figure shows the estimated mass-standardised LD50 values with confidence intervals.

Figure 2. Oral bee-mass standardised lethal dose 50 % (std LD50) of cypermethrin (A) and acetamiprid (B) in *Bombus terrestris* males (blue curve) and workers (red curve). Shade areas around the dose-response curves represent the confidence intervals. The points represent the observed survival outcomes for each individual at each dose or mass-standardised dose, where 0

indicates survival and 1 indicates mortality. The horizontal and vertical lines intersecting at 50% mortality show the estimated mass-standardised LD50 for each sex.

Alt text: Dose response curves comparing oral toxicity between *Bombus terrestris* workers and males for cypermethrin and acetamiprid. Differences in sensitivity between sexes are illustrated through the estimated mass-standardised LD50 values and confidence intervals.

Figure 3. Contact bee-mass standardised lethal dose 50 % (std LD50) of cypermethrin in *Bombus terrestris* males (blue curve) and workers (red curve). Shade areas around the dose-response curves represent the confidence intervals. The points represent the observed survival outcomes for each individual at each dose or mass-standardised dose, where 0 indicates survival and 1 indicates mortality. The horizontal and vertical lines intersecting at 50% mortality show the estimated mass-standardised LD50 for each sex.

Alt text: Dose response curves comparing contact toxicity of cypermethrin between *Bombus terrestris* workers and males. Differences in sensitivity between sexes are illustrated through the estimated mass-standardised LD50 values and confidence intervals.

Tables

Table 1. Number of exposed *Bombus terrestris* workers and males (excluding individuals that died before treatment in both exposure modes and non-feeders in oral exposure), mean body mass (mg) and measured treatment doses based on preliminary range-finding experiments (µg/bee) for each mode of exposure and pesticide.

Exposure mode	Sex	Pesticide	N	Mean mass (g ± SE)	Measured doses (µg/bee)
Oral	Workers	Cypermethrin	160	0.247 ± 0.063	0.04, 1.6, 4.8, 12, 21
		Acetamiprid	164	0.249 ± 0.058	14, 27, 48, 72, 107
		Tebuconazole	57	0.254 ± 0.059	84
	Males	Cypermethrin	128	0.406 ± 0.058	0.8, 1.2, 2.4, 4.4, 7.6
		Acetamiprid	144	0.423 ± 0.030	44, 55, 86, 121, 178
		Tebuconazole	60	0.406 ± 0.063	84
Contact	Workers	Cypermethrin	149	0.243 ± 0.084	0.08, 0.28, 0.38, 0.6, 1.1
		Acetamiprid	54	0.261 ± 0.072	79
		Tebuconazole	57	0.276 ± 0.057	118
	Males	Cypermethrin	147	0.416 ± 0.070	0.28, 0.34, 0.58, 0.86, 2.42
		Acetamiprid	58	0.396 ± 0.079	79
		Tebuconazole	59	0.398 ± 0.066	118

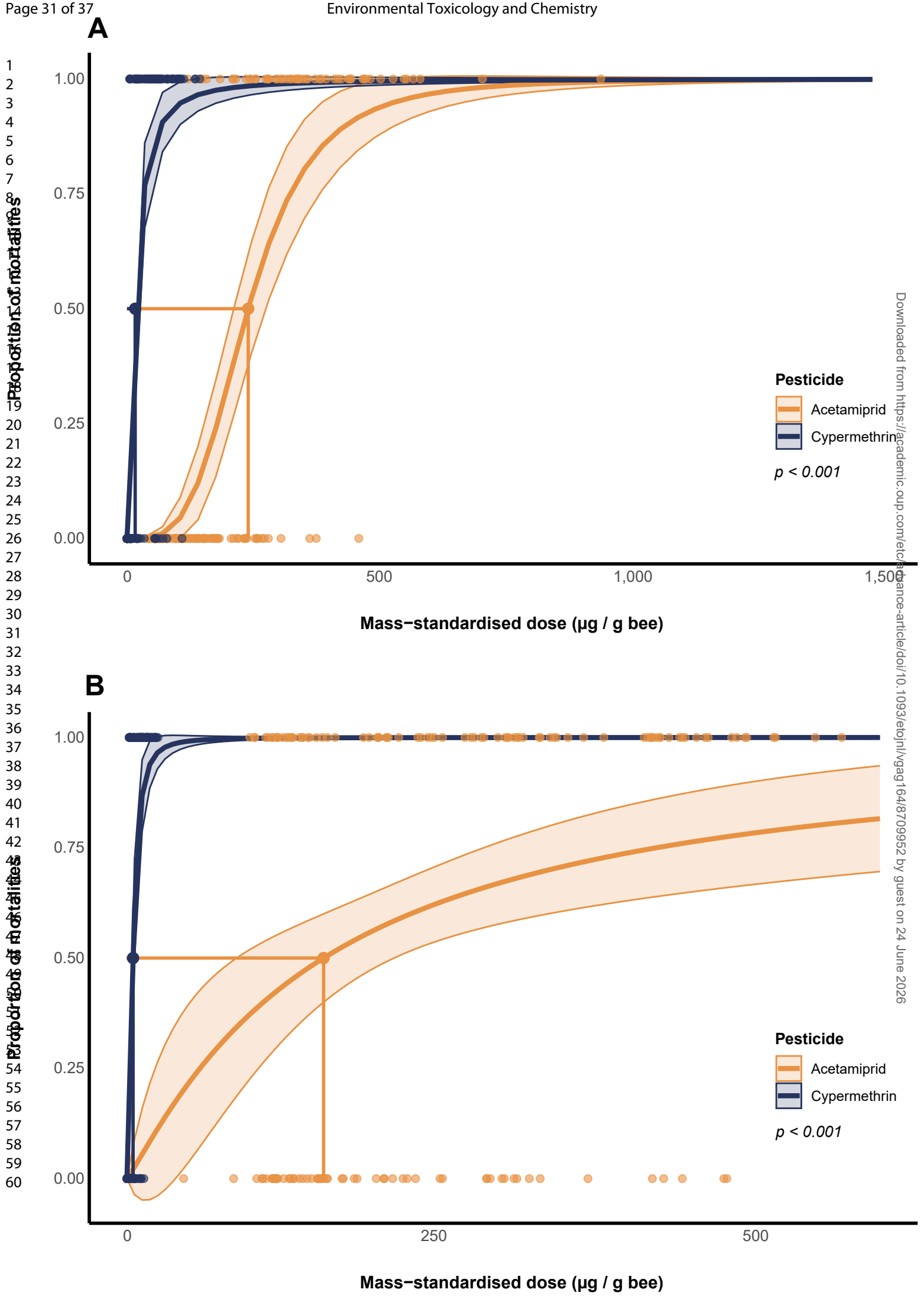
Table 2. *Bombus terrestris* workers lethal dose 50 % (LD50), lethal concentration 50 % (LC50), and bee mass-standardised lethal dose 50 % (STD50) and their confidence intervals [CI] of each exposure mode and pesticide. Confidence intervals are reported for all pesticides except for the limit tests.

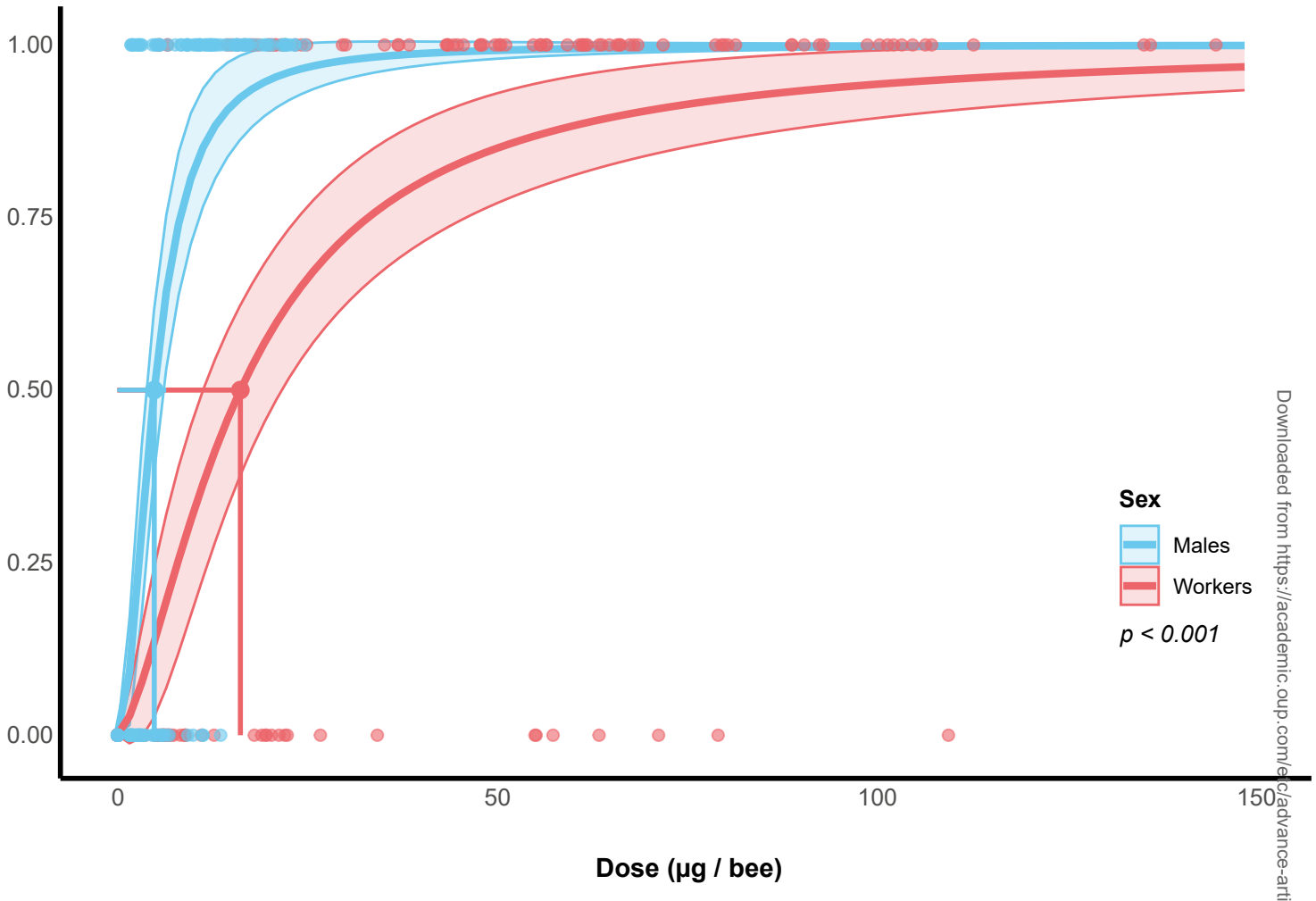
Exposure mode	Pesticide	LD50 [CI] (µg/bee)	LC50 [CI] (µg/bee)	STD50 [CI] (µg/bee)
Oral	Cypermethrin	3.94 [2.72-5.16]	0.1 [0.07-0.13]	16.24 [11.05-21.42]
	Acetamiprid	58.67 [52.19-65.14]	1.46 [1.30-1.63]	241.50 [210.16-272.82]
	Tebuconazole	>84	>2.1	>390.7
Contact	Cypermethrin	0.62 [0.55-0.69]	0.31 [0.27-0.35]	3.02 [2.44-3.06]
	Acetamiprid	>79	>39.5	>367.44
	Tebuconazole	>118	>59	>548.83

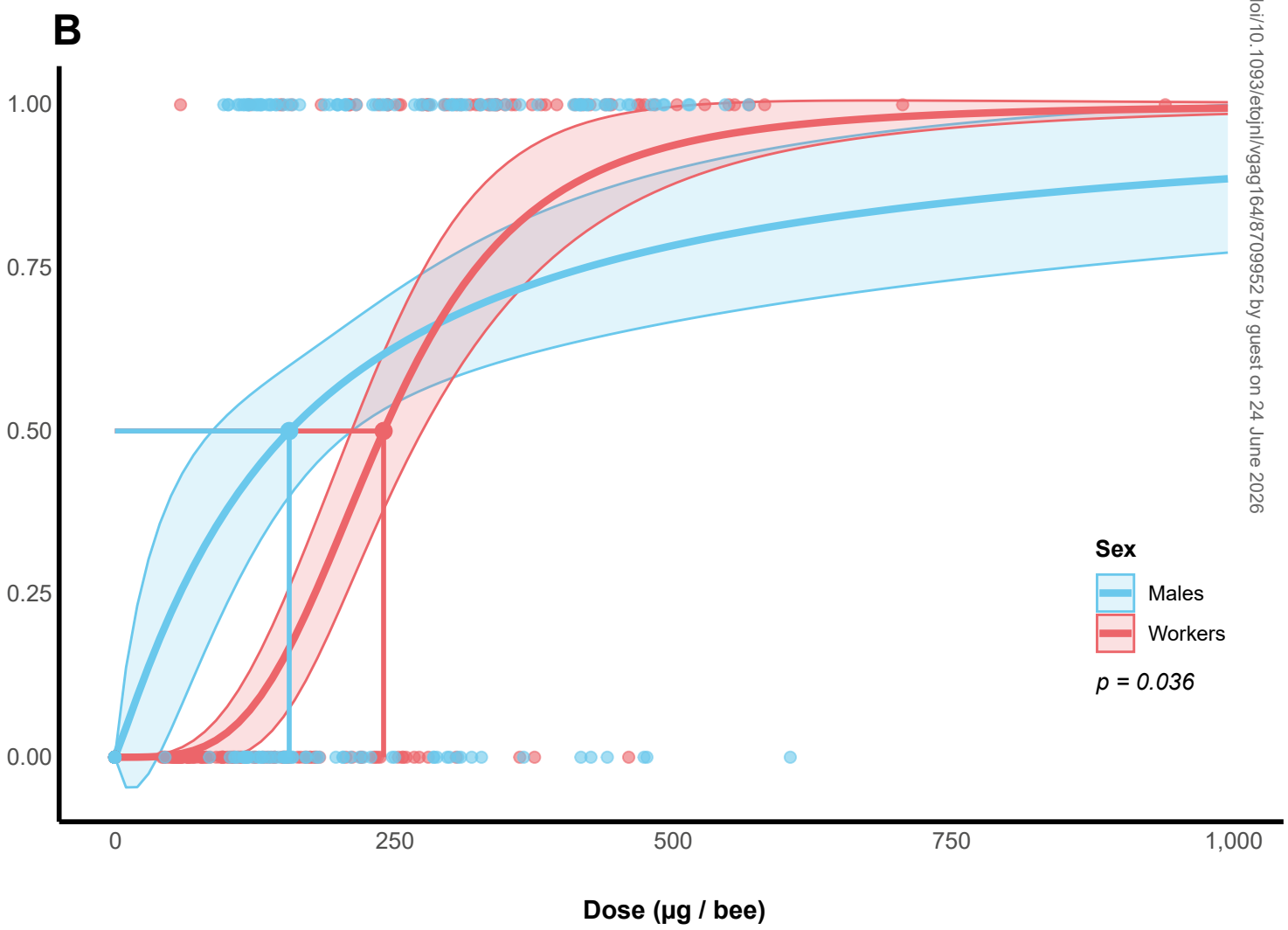
Table 3. *Bombus terrestris* males lethal dose 50 % (LD50), lethal concentration 50 % (LC50), and bee mass-standardised lethal dose 50 % (STD50) and their confidence intervals [CI] of each exposure mode and pesticide. Confidence intervals are reported for all pesticides except for the limit tests.

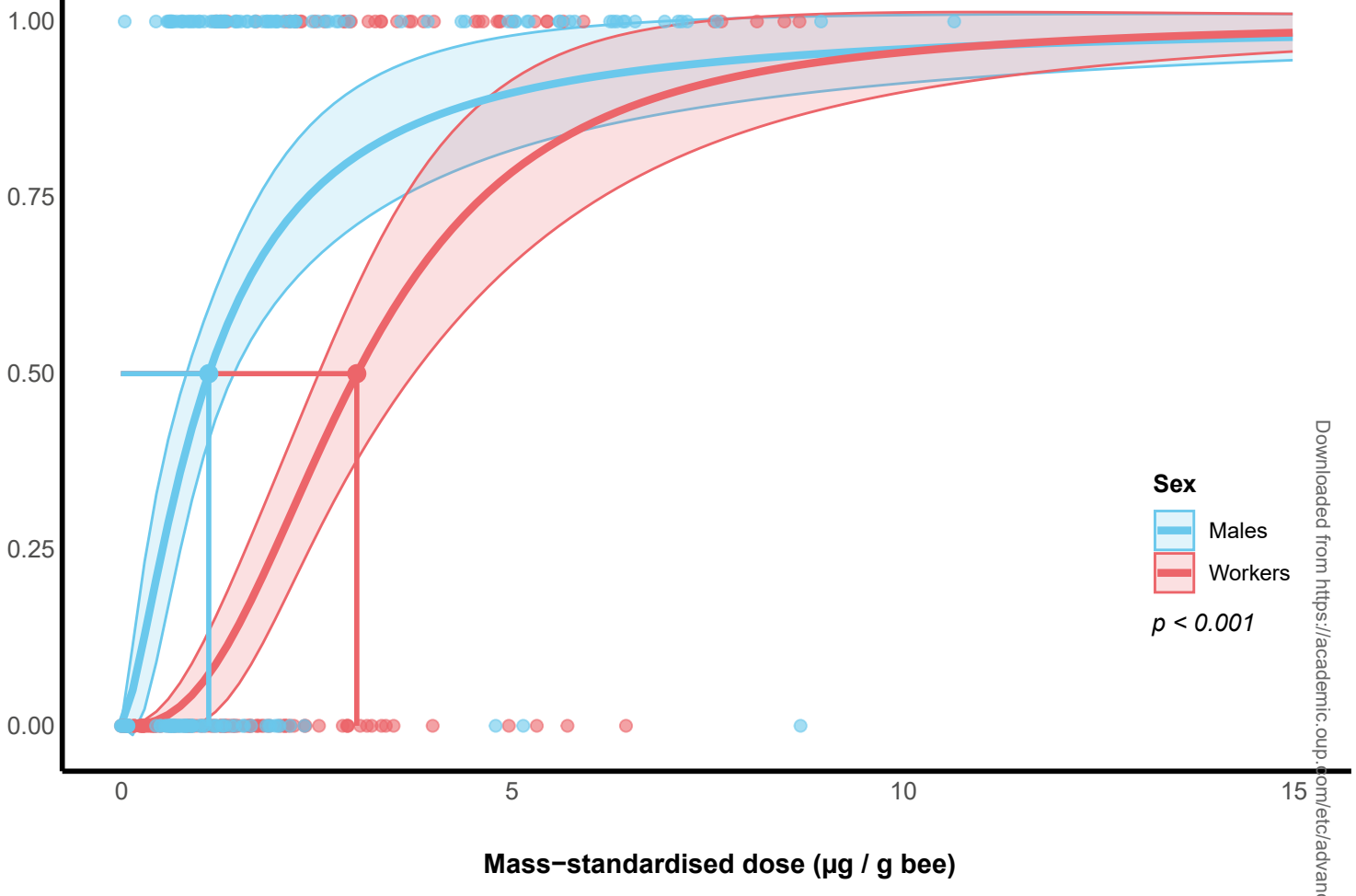
Exposure mode	Pesticide	LD50 [CI] (µg/bee)	LC50 [CI] (µg/bee)	STD50 [CI] (µg/bee)
Oral	Cypermethrin	1.99 [1.55-2.45]	0.05 [0.04-0.06]	4.87 [3.75-5.98]
	Acetamiprid	61.66 [41.64-81.69]	1.54 [1.04-2.04]	156.7 [11.15-213.25]

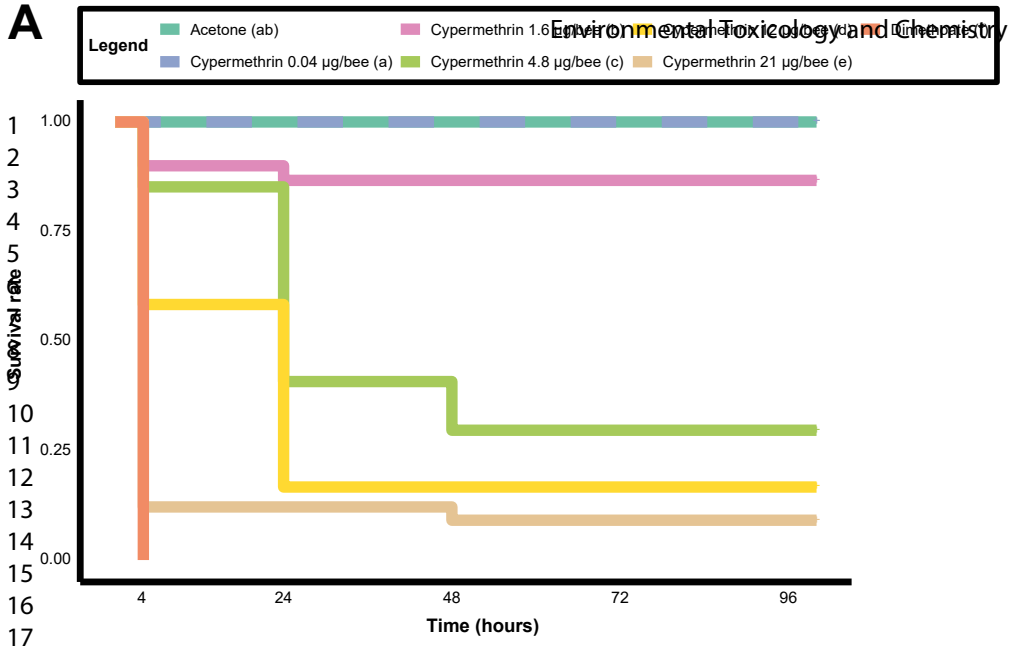
	Tebuconazole	>84	>2.1	246.31
Contact	Cypermethrin	0.46 [0.32-0.57]	0.23 [0.180-291.69]	0.13 [0.84-0.41]
	Acetamiprid	>79	>39.5	>194.10
	Tebuconazole	>118	>59	>144.96

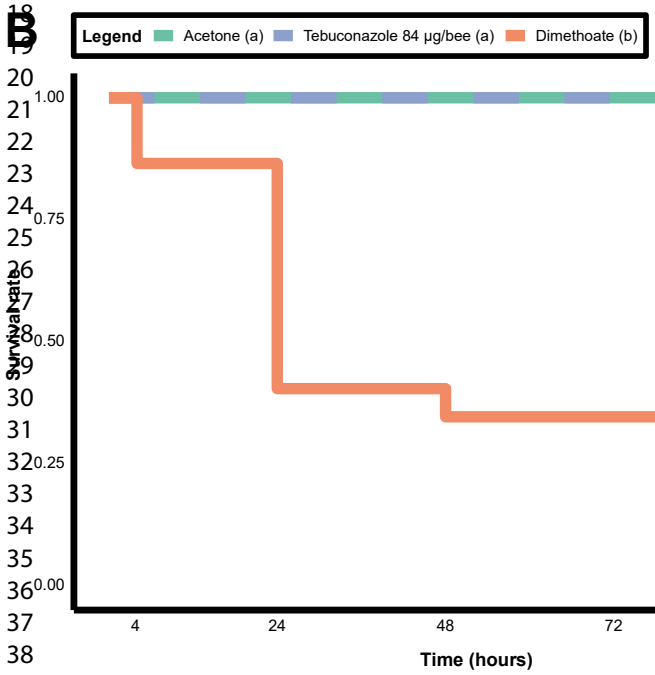


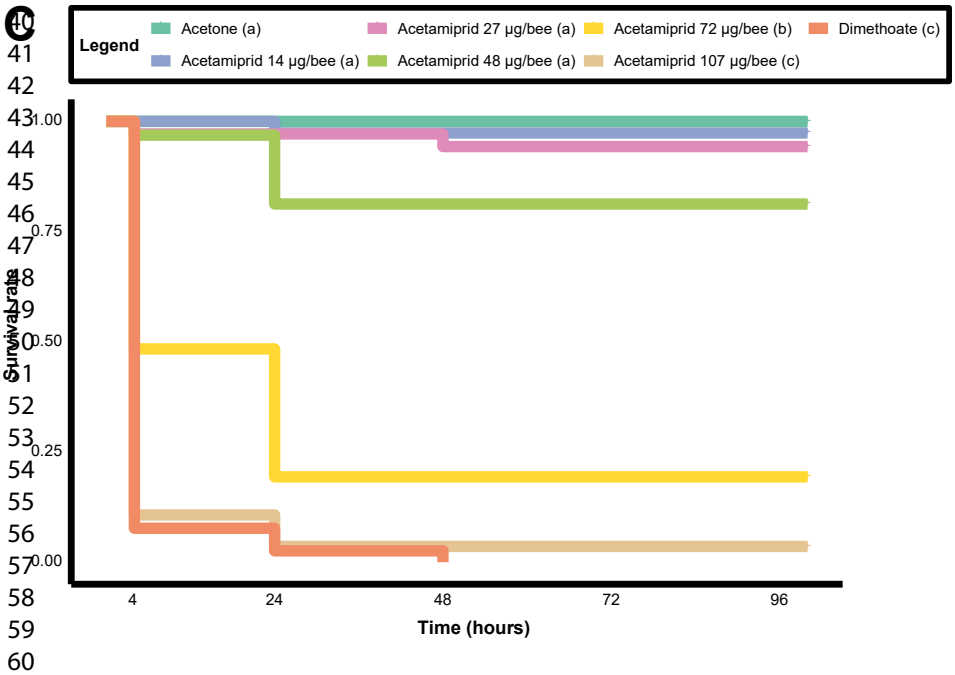


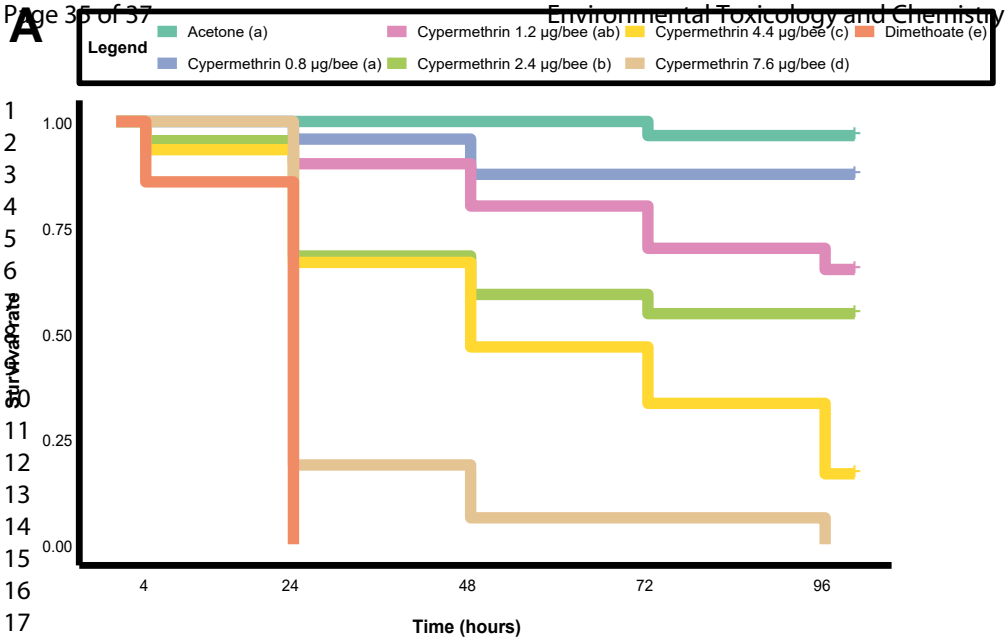


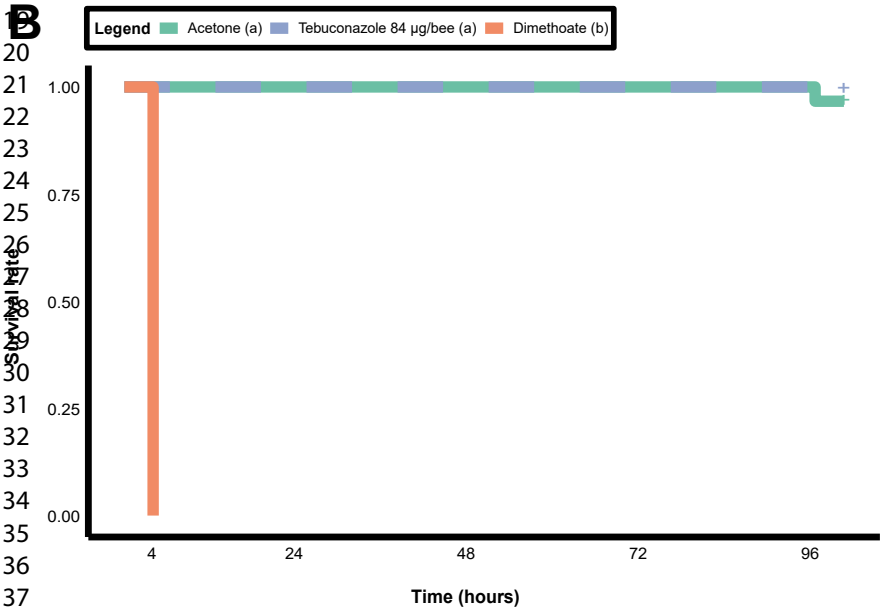


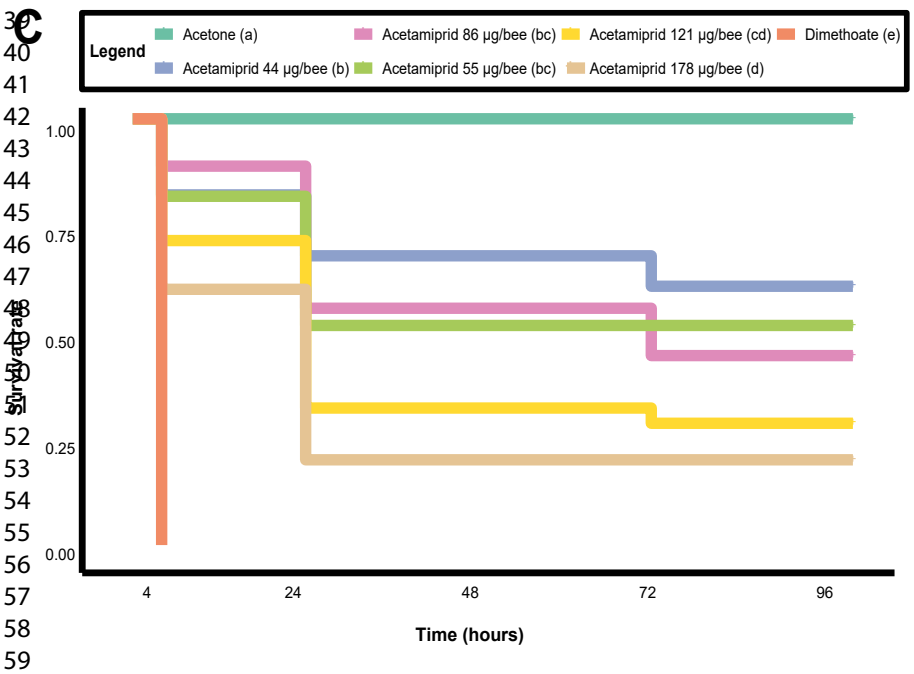




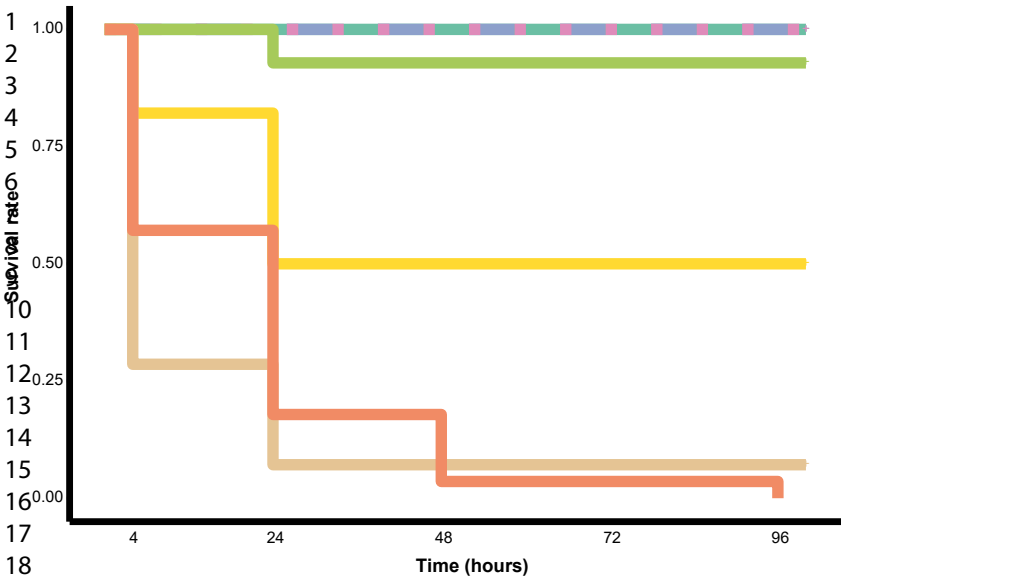




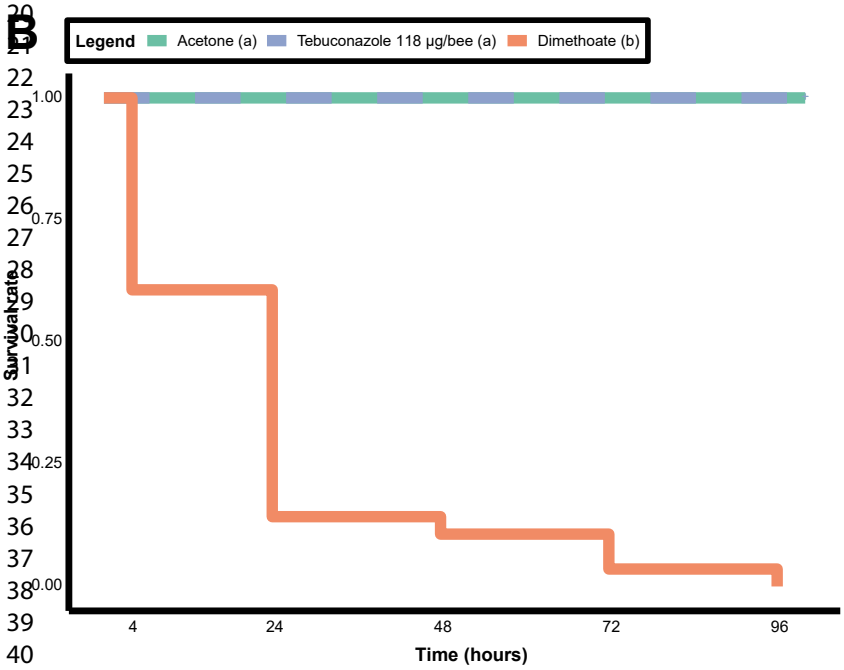




A



B



C