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Research article

Towards realistic lower-tier toxicological testing: Species, sex and behaviour shape sensitivity to pesticide exposure

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ABSTRACT

Ubiquitous pesticide pollution is a major driver of global wild insect declines and annual managed bee losses. Sensitivity to these chemicals may vary across species, sexes, and ecological contexts. Improving our understanding of the biological and behavioural factors shaping these variations is essential to ensure accuracy in toxicity testing and environmental risk assessment (ERA) schemes. Here, we show clear inter- and intraspecific differences in sensitivity to oral chronic neonicotinoid exposure. Using modified OECD 245 guidelines, we orally exposed female and male honey bees, *Apis mellifera*, bumble bees, *Bombus terrestris*, and solitary bees, *Osmia bicornis* chronically to different thiamethoxam concentrations and monitored mortality and feeding behaviour. When feeding at field-relevant concentrations ($< 40\,ng\,g^{-1}$), individuals remained unaffected across species. However, species-specific sensitivity to thiamethoxam differed: *A. mellifera* was the most vulnerable, followed by *O. bicornis* and *B. terrestris*. Within species, males were generally more sensitive than females (workers), and female honey bees engaged in tending males exhibited greater mortality than idle counterparts—demonstrating that behaviours can modulate sensitivity to chemicals. While reliance on female honey bees in current ERA schemes may align with the precautionary principle for interspecific variation, our findings show that interspecific differences – particularly between sexes and behavioural states – can substantially influence sensitivity. We therefore advocate for the integration of both sexes and behaviourally relevant conditions into future Tier-1 laboratory toxicity testing to improve the accuracy and ecological relevance of lower tier pollinator risk assessments. Improving our knowledge of how pesticide sensitivity varies in relation to sex differences or behaviours is essential to increase the realism of lower tier ERA estimates to ensure more informed evidence-based policy required to protect non-target species.

1. Introduction

Global declines in wild pollinating insect populations (Ghisbain et al., 2023; Michez et al., 2026), coupled with persistently high losses among managed pollinators (Aurell et al., 2024; Gray et al., 2023), have emerged as pressing ecological and food security concerns (Dicks et al., 2021). Among the numerous stressors implicated in these declines and losses (Dicks et al., 2021; Potts et al., 2010), pesticides have been consistently identified as major contributors, owing to their widespread application, and ubiquitous contamination across agricultural and natural landscapes (Brühl et al., 2021). Mounting evidence shows that many commonly applied pesticides exert unintended lethal and/or sublethal effects on non-target organisms, including bees (Iwasaki and Hogendoorn, 2021; Müller, 2018). Yet, despite this recognition, our understanding of the factors that shape variation in insect sensitivity to pesticides remains limited. Identifying the factors that govern sensitivity across species is therefore essential, not only for deepening our mechanistic understanding but also for developing strategies that better

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safeguard both managed and wild insect populations.

Historically, the western honey bee (*Apis mellifera*) has been the predominant model organism for investigating the effects of various environmental and anthropogenic stressors on pollinating insects (Heard et al., 2017). This emphasis stems from its economic importance in agricultural pollination, its ease of management, and its extensively documented biology. However, accumulating evidence suggests that *A. mellifera* workers may not always serve as reliable models for other eusocial and solitary bee species (Fisher et al., 2023), given marked differences in life history, behaviour, sensitivity and exposure among these groups (Franklin and Raine, 2019; Gradish et al., 2019; Sgolastra et al., 2019). Levels of sociality and life history traits are particularly critical, as they can fundamentally influence an organism's ability to endure stress (Gloss et al., 2014; Zuk and Stoehr, 2002). These differences may translate into variation in pesticide sensitivity, as highly eusocial species such as *A. mellifera* benefit from colony-level buffering (Straub et al., 2015), whereas this buffering is reduced or absent in bumble bees and solitary species (Sgolastra, 2025). In addition, variation in body size and detoxification capacity may further modulate sensitivity (Beadle et al., 2019; Thompson, 2016). These considerations underscore the need to expand toxicological research beyond *A. mellifera*, both to capture the breadth of bee diversity and to identify model species that can enhance the field relevance of risk assessments. Recent initiatives to incorporate eusocial bumble bees (*Bombus* spp.) and solitary bee species (e.g., *Osmia* spp.) into ecotoxicological studies are commendable and represent an important step forward (EFSA, 2023; Franke et al., 2021). Yet, despite these advances, comprehensive comparative datasets across species remain scarce, limiting generalizable conclusions about species sensitivity and reflecting the still limited taxonomic breadth of ecotoxicological research, as well as the lack of standardized chronic test guidelines beyond honey bee workers (EFSA, 2023; Fisher et al., 2023; Tissier et al., 2025).

Beyond species-level comparisons, intraspecific variation – especially between males and females – represents a major blind spot in our understanding of how pesticides affect bees. This gap is concerning given the essential role of males in reproduction. Moreover, the limited available evidence suggests that males may be more sensitive to pesticide exposure than females across multiple exposure routes, including larval, topical, and both acute and chronic oral exposure (e.g.,Jütte et al., 2023 ; Linguadoca et al., 2022; Straub et al., 2016; Strobl et al., 2021), although empirical data remain limited. Notably, similar sex-specific differences in susceptibility have been documented for other environmental stressors, such as pathogens and heat stress (Camenzind et al., 2025; Hayes and López-Martínez, 2021). This heightened sensitivity has been partially attributed to sexual size dimorphism, with smaller individuals exhibiting higher mass-specific metabolic rates, leading to greater relative consumption and, consequently, increased exposure (Mobley and Gegear, 2018). Genetic mechanisms may also contribute: in Hymenoptera, haplo-diploid sex determination usually results in diploid females and haploid males (Evans et al., 2004), a system that underlies the haploid-susceptibility hypothesis. According to this hypothesis, the absence of heterozygosity in haploid males could limit immune and detoxification capacity (O'Donnell and Beshers, 2004), though empirical toxicological evidence remains sparse. While physiological and genetic factors may therefore explain part of the variation in sensitivity, they do not act in isolation. Sex- and species-specific behavioural and ecological traits are equally important, as they strongly influence both the likelihood and intensity of pesticide exposure.

Behavioural and physiological complexity further modulates how bees respond to xenobiotic exposure. Energetically demanding activities such as brood care or thermoregulation, along with sex- and species-specific foraging or mating behaviours, shape pollen and nectar consumption – the primary route of oral pesticide exposure – and thereby create variable exposure scenarios (Mobley and Gegear, 2018; Sgolastra et al., 2019). For instance, in contrast to solitary bees and bumble bees, *A. mellifera* males do not forage (Free, 1957), and depend largely on female workers for feeding, resulting in substantially different exposure profiles. These contrasting behaviours highlight not only the complexity of exposure pathways, but also the inadequacy of relying solely on honey bee female workers to infer risk across bee taxa. Importantly, they also highlight that standard laboratory trials, which often neglect behavioural differences (Thompson, 2003), may underestimate or fail to detect certain toxicological effects that only manifest under field conditions where natural behaviours are expressed. This discrepancy underscores the need to incorporate knowledge of behavioural ecology into lower-tier risk assessments to improve their ecological relevance and robustness.

To address these questions, we designed a comparative study to evaluate how species-, sex-, and behaviour-specific traits influence sensitivity to chronic oral toxicity of the neonicotinoid thiamethoxam. This insecticide remains widely used globally (Alsherbeny et al., 2026; Wang et al., 2023) and is frequently detected in pollen and nectar of crops and wild plants, including within the EU where its use is restricted but emergency authorisations and environmental persistence contribute to continued exposure (Epstein et al., 2022; Klingelhöfer et al., 2022; Odemer et al., 2023). We tested three bee species – bumble bees, *Bombus terrestris*, solitary bees, *Osmia bicornis* and western honey bees *Apis mellifera* – with explicit inclusion of both males and females (workers in the social species). These species were selected for their contrasting body sizes, life-histories, degrees of eusociality, and their relevance in ecotoxicological guidelines (EFSA, 2023; Gradish et al., 2019; Sgolastra et al., 2019). To adequately consider the life history of honey bees, male *A. mellifera* (drones) were co-housed with females, enabling us to indirectly assess how male-tending behaviour influences female sensitivity. This design allowed us to test three hypotheses: (i) thiamethoxam sensitivity varies among species, decreasing with larger body size but increasing from solitary to highly eusocial (Fisher et al., 2023); (ii) males are more sensitive than conspecific females (Linguadoca et al., 2022); and (iii) honey bee females engaged in male-tending behaviour are more susceptible than non-tending females (Straub et al., 2020a).

2. Material and methods

2.1. Experimental design

The study was conducted at the Institute of Bee Health, University of Bern, Switzerland. To assess chronic oral toxicity of the neonicotinoid thiamethoxam across three bee species, we followed standard toxicological guidelines (OECD, 2017a). However, due to differences in life history and behaviour (Linguadoca et al., 2022; Sgolastra et al., 2019), modifications were required to assess chronic oral toxicity in male honey bees as well as for both sexes of bumble bees and solitary bees. The modifications were as follows: (1) To exclude potential variation in age-specific sensitivity across species, all adult bees were three days old upon initiation of the experiment. We chose day three because newly emerged bumble bees were three days of age after transport from Biobest Group NV® (Belgium) and *O. bicornis* required time to locate the feeders. (2) In contrast to honey bees, bumble bees and solitary bees were kept individually in plastic cages [100 cm^3] following previous protocols (Straub et al., 2022; Strobl et al., 2021) as suggested by standard guidelines (OECD, 2017b). Individual caging enabled precise dosing and calculations of the exact exposure, which is not feasible in hoarding cages used in the standard chronic oral toxicity testing guideline for *A. mellifera* (OECD, 2017a). (3) Ten honey bee males were kept in plastic cages [250 cm^3] with 20 attending females, as males depend upon females which attend them throughout their lives (Straub et al., 2016, 2020a). Female honey bee cages contained 20 females but no males. (4) To minimize stress, we weighed and exchanged syringe feeders at four specific time-points (days three, six and nine, and 10) rather than daily. A visual representation of the experimental design can be found in the supplementary information (SI Fig. 1)

2.2. Sourcing, housing and treatment allocation

(1) *Bombus terrestris*: To establish known age cohorts, 448 callow (<24 h (Alford, 1975)) *B. terrestris* males and female females ($N_{males} = 224$; $N_{females} = 224$) were randomly collected from 50 different commercial colonies. The bumble bees were three days old upon arrival in Bern. Visual inspections revealed no ectoparasite infestations, clinical symptoms of disease or any other abnormalities. All individuals were placed in individual cages [100 cm^3] and maintained at 28 °C and 60% relative humidity (RH) in complete darkness (Minnameyer et al., 2021). Before being assigned to treatment groups, the body mass of each bee was recorded to the nearest 0.1 mg using an analytic scale (Mettler Toledo AT400, precision 10^{-4} g). Individuals were then randomly and evenly allocated to one of seven treatment groups (Controls or neonicotinoid exposed; $N = 32$ per sex and treatment).

(2) *Osmia bicornis:* Previously sexed male and female cocoons were purchased from Wildbiene + Partner AG (Zürich, Switzerland) and stored at 4 °C and 60% RH (Splitt et al., 2022). In early May 2022, and in accordance with their natural emergence time for central Europe (Splitt et al., 2022), cocoons ($N_{males} = 600$; $N_{females} = 400$) were moved from the fridge to flight cages (BugDorm – Insect rearing cage [47.5 x 47.5 × 47.5 cm], Megaview Science Co. Ltd., Taiwan), where adults were allowed to emerge. To prevent mating, males and females were kept separately at room temperature (RT) (~24 °C). Bee emergence was checked daily. After emergence, each individual's sex was confirmed and all individuals were visually assessed for ectoparasites, clinical disease symptoms or other abnormalities (Bosch, 1992). Individual bees were then removed from the flight cages and their body mass recorded (see earlier). Under laboratory conditions, *O. bicornis* male mortality tends to be higher than in females, especially during the first 72 h, possibly due to difficulties with finding sucrose feeders (Cabrera et al., 2024). Therefore, sample size for male treatments ($N = 46$) was higher than for females ($N = 31$), resulting in a total of 649 bees ($N_{males} = 370$; $N_{females} = 279$), which were placed in individual cages [100 cm^3] containing a small piece of paper for shelter (Strobl et al., 2021). To promote feeding in *O. bicornis*, a petal [3 mm^2] of a commercially purchased organic *Euryops pectinatus* (Asteraceae) was attached to the tip of each syringe (Azpiazu et al., 2019). Caged *O. bicornis* were kept at RT (~24 °C), 50% RH and indirect natural sunlight (Strobl et al., 2021).

(3) *Apis mellifera*: In June 2022, following OECD (OECD, 2017a), brood frames were sampled from three local and queenright *A. mellifera* (L.) colonies which were managed according to best management practices, including an oxalic (2.7%) acid treatment in the previous winter and in early March 2022 against *Varroa destructor* mites. We chose three non-related queens to account for potential genetic variation that may affect pesticide sensitivity (Sandrock et al., 2014) and by treating colonies against mites we aimed to minimize the potential interactive effects of pesticides and pathogens (Straub et al., 2020a). From each colony, a female and male brood frame with capped cells within one day of adult emergence were collected (Straub et al., 2016) and incubated at 34.5 ± 1 °C and 70% RH (Williams et al., 2013). All frames contained honey and beebread for the newly emerged bees to feed on. While the female brood frames were kept without nurse bees, ~50 adult nurse bees from each colony were added to their respective male frame to foster male emergence and ensure feeding (Currie, 1987). Post-emergence, each individual was visually examined for clinical symptoms of disease (e.g., deformed wings) and other physical abnormalities and parasitic mites *V. destructor* (Dainat and Neumann, 2013). Non-infested individuals without abnormalities and/or symptoms were randomly placed in standard hoarding cages [250 cm^3] (OECD, 2017a; Straub et al., 2016).

2.3. Neonicotinoid preparation and exposure

To prepare the feeding solutions, 50 mg of thiamethoxam ((CAS No.: 153,719-23-4), 99% purity, Sigma-Aldrich, UK) were dissolved in five mL of pure acetone (super solution concentration of 1 mg mL^{-1}). Then, appropriate aliquots of the stock solution were diluted in acetone to produce 6 stock solutions 1000 times more concentrated than the desired feeding treatment solution (1.5×10^3, 4.5×10^3, 1×10^4, 2×10^4, 4×10^4, 1×10^6 ng mL^{-1}). Then, 50 µL of each of the stock solutions was added to 50 g of 33% [w/w] sucrose-solution to produce the desired feeding solutions (1.5, 4.5, 10, 20, 40, 100 and 1000 ng g^{-1}). Concentrations were chosen to cover a field-realistic range (i.e., 1.5-40 ng g^{-1} (Mitchell et al., 2017; Thompson et al., 2022)) as well as higher concentrations in line with OECD guidelines (OECD, 2017a), allowing estimation of dose-response relationships. The concentration of acetone in the final feeding solutions was 0.1%. Additionally, a negative control feeding solution (0.1% acetone) and a positive control feeding solution (dimethoate 1′000 ng g^{-1}, 0.1% acetone) were set up. The control solution was prepared by adding 50 µL of acetone to 50 g of 33% [w/w] sucrose solution, while the positive control feeding solution was prepared using the same approach as previously described for thiamethoxam. In brief, a stock solution was prepared at a concentration of 1 mg mL^{-1} in acetone, then 50 µL were added to 50 g of 33% [w/w] sucrose solution. The insecticide dimethoate (CAS-153719-23-4, 99% purity, Sigma-Aldrich) was included as a positive control (reference toxicant) to assess test-system performance following OECD guidance (OECD, 2017a). Bees were orally exposed to their respective treatment for ten days (OECD, 2017a). All stock solutions were kept in closed glass vials at 4 °C to avoid evaporation and potential chemical degradation of the active ingredients. However, degradation of both, the neonicotinoid and dimethoate, are most likely negligible under the given conditions (i.e., indirect or no light and constant temperature, as well as exchanging the feeders at least every three days) (Schläppi et al., 2020). Individuals from all species were fed *ad libitum* with a 33% [w/w] sucrose-solution containing the respective concentration of acetone, thiamethoxam and dimethoate via a five mL syringe (Codan Medical AG, Switzerland) and bees did not receive pollen as recommended by toxicological guidelines (OECD, 2017a). To enable sucrose consumption and exposure measurements, evaporation was accounted for by using five empty cages containing a syringe with sucrose solution for each species (OECD, 2017a). Feeding solutions were prepared freshly, before exchanging syringes.

To confirm that the preparation of the sucrose solutions was performed adequately, a residue analysis using high-performance liquid chromatography (HPLC; Agilent 1290 Infinity II) coupled with mass spectrometry (MS/MS; Agilent 6495C tandem quadrupole) was performed using a pooled subsample across all three experimental runs of the control and the 1.5 ng g^{-1} thiamethoxam sucrose solution. Samples were analysed following established methods (Schaad et al., 2023), and the detected residues (thiamethoxam treatment: 1.54 ng g^{-1}; control: below limit of detection (LOD), 0.02 ng g^{-1}) were within the recommended ranges of the OECD guidelines.

2.4. Consumption, exposure and survival

Survival was recorded daily for all species and dead *A. mellifera* were removed from the hoarding cages. Consumption data were obtained by weighing the mass of the syringe every three days, as well as on the final day (i.e., day 10) or at the point of death of an individual and adjusted for evaporation loss. Total sucrose consumption [g] was divided by the number of days a bee survived to obtain an average daily consumption for all individuals [g bee^{-1} day^{-1}]. In the case of *A. mellifera*, the total consumption [g] was divided by the total number of live bees present in

a cage at any of the assessment timepoints, to determine the daily consumption per bee [g bee^{-1} day^{-1}]. For mixed sex *A. mellifera* cages, the total consumption was divided by the alive individuals and their sex specific mass to determine individual sex-specific consumption. Further, we observed in *O. bicornis*, that there were 101 individuals across both sexes that revealed, after evaporation correction, no consumption within the first three days (i.e., 72 h) - likely due to difficulties recognizing the feeders (Cabrera et al., 2024). To exclude starvation as a potential cofounding factor, we therefore opted for a conservative approach by excluding individuals that died during the first 72 h and did not consume more than 15 mg per day (i.e., 30 mg if a bee died after 48h), as suggested in Azpiazu et al. (2024). Irrespective of whether they survived the first 72 h, if they did not consume $\geq$ 15 mg they were excluded. The excluded individuals were considered to be 'non-feeders', whereas the remaining specimens were considered 'feeders' ($N_{total} = 101$; $N_{males} = 78$, $N_{females} = 23$). Daily consumption and total consumption were then multiplied by the exposure concentration to determine the total [ng bee^{-1}] as well as the daily [ng bee^{-1} day^{-1}] dose. To account for differences in size between species, sexes and individuals, daily consumption and daily dose were divided by the individual's body mass to obtain the weight corrected values of both relative daily consumption [g g^{-1} bee-^{1} day^{-1}] and relative daily dose [ng g^{-1} bee^{-1} day^{-1}].

2.5. Statistical analyses

All analyses were performed using R Statistical Software (R Core Team; V 4.5.1; Austria). Graphs were created using NCSS v. 12 (NCSS, 2024) and R Statistical Software. Results were considered statistically significant at $P < 0.05$, and Bonferroni adjustments were applied when appropriate. Quantile-quantile plots (model residuals) as well as Shapiro-Wilk's test for normality (raw data) were used to determine suitable tests. To determine the best fitting models, backward selections using the Akaike information criterion (AIC) were performed.

2.5.1. Consumption analysis

To investigate factors influencing the probability of *O. bicornis* becoming a non-feeder, a generalized linear model (GLM) with a binomial error distribution was fitted, with feeding status as the response variable and sex and thiamethoxam treatment as explanatory variables: *glm(feeding_status ~ sex + neoncotinoid, family = "binomial")*. The proportion of non-feeders was subsequently compared between sexes using a test of equal proportions with the function *prop.test()*. For relative daily consumption, the suitability of alternative error distributions was evaluated by comparing GLMs using the *compareGLM()* function in the *rcompanion* package (Mangiafico, 2024). To assess treatment effects between species, a gamma error distribution with a log-link function provided the fest fit, and the resulting model included species, survival time, and sex as explanatory variables: *glm(relative_d_cons ~ species + survival time + sex, family = Gamma(link = "log"))*. Following this model, the effects were assessed using Type II analysis-of-deviance tests with the *Anova()* function. Pairwise comparisons of relative daily consumption among species and sexes were performed with using Wilcoxon rank-sum test with the function *pairwise.wilcox.test()*. To assess treatment effects within each species and sex, separate Gamma GLMs with a log-link function were fitted for male and female *B. terrestris*, *O. bicornis*, *A. mellifera*, as well as for the tending *A. mellifera* workers (seven models in total). Treatment was included as a categorical explanatory variable, with the control as the reference level, thereby allowing the effect of each thiamethoxam concentration on relative daily consumption to be assessed relative to the corresponding control, using the following function: *glm(average_daily_consumption ~ treatment, family = Gamma (link = "identity"))*. Post hoc testing, comparing daily consumption of all thiamethoxam concentrations to the negative control, as recommended by testing guidelines (OECD, 2017a), was achieved using the *glth()* function of the *multcomp* package (Hothorn et al., 2008) with the "Dunnett" false discovery rate (FDR) option (Dunnett, 1955).

2.5.2. Survival analysis

To explore correlations between fixed terms (i.e., thiamethoxam concentrations, species and sex) and survival, a parametric accelerated survival time (AFT) model was fitted using the function *survreg(Surv (survival time, death event) ~ Exposure + species + sex + sex:species),* of the package *survival* (Kleinbaum and Klein, 2012; Therneau, 2024). Following Bovier et al. (2024), a two parameter log-logistic dose-response model was applied to determine the 10-day oral Lethal Daily Dosage $(LDD)_{50}$ (expressed in ng g^{-1} bee^{-1} day^{-1}) values with 95% confidence limits by using the R package *drc* (Ritz et al., 2015) and the function *ED()*. Further, LDD_{50} values between species, sexes as well as tending and non-tending *A. mellifera* females were compared for significant differences using the function *EDcomp()*, which calculates and compares relative potencies or the differences between effective doses derived from fitted dose-response curves. Comparison of the cumulative survival rates of treatment groups to the control were performed using the function *pairwise_survdiff()* from the package *survminer* (Kassambara and Kosinski, 2021). The means ± the standard error (SE) or the medians and the interquartile ranges (IQR) are given in the results together with the outcome of the statistical tests. The R-script containing code for all analyses as well as the raw data needed for the analyses are deposited online, see Data availability statement.

3. Results

3.1. Positive control (dimethoate)

Cumulative control survival remaining >85% across all species and sexes (SI Fig. 2a–c). Dimethoate exposure significantly reduced survival relative to controls in both male and female *A. mellifera* and *O. bicornis*, as well as in tending *A. mellifera* workers (all $P < 0.001$; SI Fig. 2a; SI Fig. 3a and b). Complete mortality of *O. bicornis* females and *A. mellifera* non-tending and tending workers occurred by days 10, 8, and 5, respectively, while complete mortality of *O. bicornis* and *A. mellifera* males occurred by days 7 and 6, respectively. In contrast, dimethoate did not significantly affect survival of either male or female *B. terrestris* relative to controls ($\chi^2 = 2$, $P = 0.6$; SI Fig. 2c), with cumulative survival remaining >90% at day 10. Under dimethoate exposure, *B. terrestris* survival was significantly higher than that of *A. mellifera* and *O. bicornis* for both sexes (all $P < 0.001$; SI Fig. 3a and b). Among females, survival also differed significantly between *A. mellifera* and *O. bicornis*, with *A. mellifera* showing lower survival ($P < 0.005$; SI Fig. 3a), whereas no significant difference between these species was observed among males (P = 1.0; SI Fig. 3b).

3.2. Osmia bicornis feeding behaviour

Within the first 72 h, 90 of the 370 *O. bicornis* males (23.7%) and 26 of the 279 *O. bicornis* females (9.3%) consumed less than 15 mg of sucrose solution (SI Fig. 4) and thus considered non-feeders. Consequently, they were excluded from further analyses. Sex significantly correlated with *O. bicornis* feeding behaviour, where males had a 18.9% lower feeding success compared to females ($z = -4.37$, $P < 0.001$). In contrast, thiamethoxam exposure did not significantly correlate with the likelihood of being a non-feeder *O. bicornis* ($z = -0.12$, $P = 0.903$).

3.3. Relative daily consumption

Independent of the bee species and sex and exposure, survival time was significantly positively correlated with relative daily sucrose consumption ($t = 28.26$, $P < 0.001$) and survival correlated positively with increased consumption (SI Fig. 5). In addition, a significant species-specific effect was revealed (*LR* $\chi^2 = 1320.41$, $P < 0.001$), where pairwise comparisons revealed significant differences amongst all tested species, regardless of sex (all *P*'s < 0.001; SI Fig. 5). *Osmia bicornis* (1.70, 1.07-2.19) showed the highest relative daily consumption, followed by

B. terrestris (0.80, 0.57-1.02) and *A. mellifera* (0.15, 0.13-0.24) (median, IQR, [g g^{-1} bee^{-1} day^{-1}]); this resulted in 2.5% and >1000% increase in median relative daily consumption of *O. bicornis* compared to *B. terrestris* and *A. mellifera,* respectively. The relative median daily consumption of *B. terrestris* was 33.3% higher compared to *A. mellifera.* Furthermore, sex revealed a significant effect on the relative daily consumption across all three species ($LR\ \chi^2 = 28.85$, $P < 0.001$), where relative daily consumption was significantly higher in male *B. terrestris* (0.89, 0.63-1.08) and *O. bicornis* (1.99, 1.28-2.38) compared to their female counterparts (both P's < 0.003; SI Fig. 6; median, IQR, [g g^{-1} bee^{-1} day^{-1}]). Subsequently, female *B. terrestris* (0.73, 0.53-0.95) and *O. bicornis* (1.43, 0.99-1.91) consumed 17.98% and 28.14% less than their male counterparts, respectively (median, IQR, [g g^{-1} bee^{-1} day^{-1}]). In contrast, *A. mellifera* males (0.14, 0.13-0.15) showed a significantly lower relative daily consumption compared to their conspecific females (0.25, 0.22-0.26) ($P < 0.001$; SI Fig. 6; median, IQR, [g g^{-1} bee^{-1} day^{-1}]). When comparing the males with the tending females (i.e. caged workers with males) (0.28, 0.26-0.29) and the non-tending females (i.e. workers with no males in their cage), a significant difference in relative daily consumption was revealed amongst all three groups (all P's < 0.01; Fig. 1 a; median, IQR, [g g^{-1} bee^{-1} day^{-1}]) – resulting in tending *A. mellifera* females having the highest relative daily consumption with 12.% more than non-tending females and 100% more than males. Lastly, across all species and sexes, thiamethoxam exposure significantly reduced feeding solution (sucrose) consumption of bees exposed to the highest treatment concentration (1000 ng g^{-1}; Fig. 2 a–g; GLMs, all z's < - 4.16; all P's < 0.001). In *B. terrestris* males, a similar reduction was observed at 100 ng g^{-1} (GLM, $z = -3.26$; $P < 0.01$; Fig. 2 a); resulting in a 24% reduction in sucrose consumption compared to their respective controls. Notably, in *O. bicornis* females, sucrose consumption was significantly increased by 56% at 40 ng g^{-1} compared to their respective controls (GLM; $z = 3.12$; $P < 0.05$; Fig. 2 d).

3.4. Survival analysis

Control male and female survival did not significantly differ across the three species (all P's > 0.07), where cumulative survival was above 85% for all species and sexes. Thiamethoxam exposure revealed a significant dose-dependent effect of thiamethoxam on survival, wherein increasing concentrations negatively correlated with survival ($z = -9.55$, $P < 0.001$). Both, species and sex, significantly influenced survival outcomes following thiamethoxam exposure (both z's ≤ 6.28, P's < 0.001). For *B. terrestris*, only the highest concentration (i.e., 1000 ng g^{-1}) resulted in a significant reduction of survival – all males and females died within five and six days, respectively (both P's < 0.001; Fig. 3 a & b). Likewise, both male and female *O. bicornis* belonging to 1000 ng g^{-1} treatment groups significantly differed from their respective controls (both P's < 0.001; Fig. 3 d & e) – all male and female exposed individuals died within six and two days, respectively. Furthermore, the *O. bicornis* female 100 ng g^{-1} treatment group significantly differed from the controls ($P < 0.001$), which was also the case for the males ($P = 0.001$). For male *A. mellifera*, all thiamethoxam concentrations (i.e., 1.5-1′000 ng g^{-1}) had a significant negative effect on survival (all P's < 0.05; Fig. 3 g). In contrast, for *A. mellifera* females a significant negative effect of thiamethoxam exposure was only observed at and above the 40 ng g^{-1} treatment groups (all P's < 0.001;Fig. 3 h). All male and female *A. mellifera* from the 1000 ng g^{-1} treatment group died with the first 24 h. Lastly, tending female *A. mellifera* from the controls (75; 66.5 - 83.5) revealed a significantly lower survival when compared to non-tending controls (85; 78-92) ($P < 0.001$; cumulative survival [%]; 95% CI). In addition, except for the 20 ng g^{-1} treatment ($P = 0.056$), a significant reduction in survival was observed at and above 10 ng g^{-1} thiamethoxam exposure treatment groups for tending female *A. mellifera* (P's < 0.001; SI Fig. 7).

3.5. Dose-response analysis

Regardless of sex, the relative ten-day LDD_{50} for *B. terrestris* (154.8 ± 16.0) and *O. bicornis* (112.3 ± 14.0) did not significantly differ ($P = 0.09$). However, both revealed a significantly increased LDD_{50} when compared to *A. mellifera* (8.6 ± 0.9); resulting in an 18- and 13-fold lower sensitivity for *B. terrestris* and *O. bicornis* compared to *A. mellifera*, respectively (P's < 0.001; LDD_{50} ± SE [ng g^{-1} bee^{-1} day^{-1}]; SI Fig. 8 a). Interspecific LDD_{50} comparisons for males revealed significant differences between all three species (all P's < 0.036); where the highest LDD_{50} was observed in *B. terrestris* (153.1 ± 21.2), followed by *O. bicornis* (78.5 ± 14.6) and *A. mellifera* males (3.0 ± 0.5) (LDD_{50} ± SE [ng g^{-1} bee^{-1} day^{-1}]; SI Fig. 8 b), resulting in *A. mellifera* males having

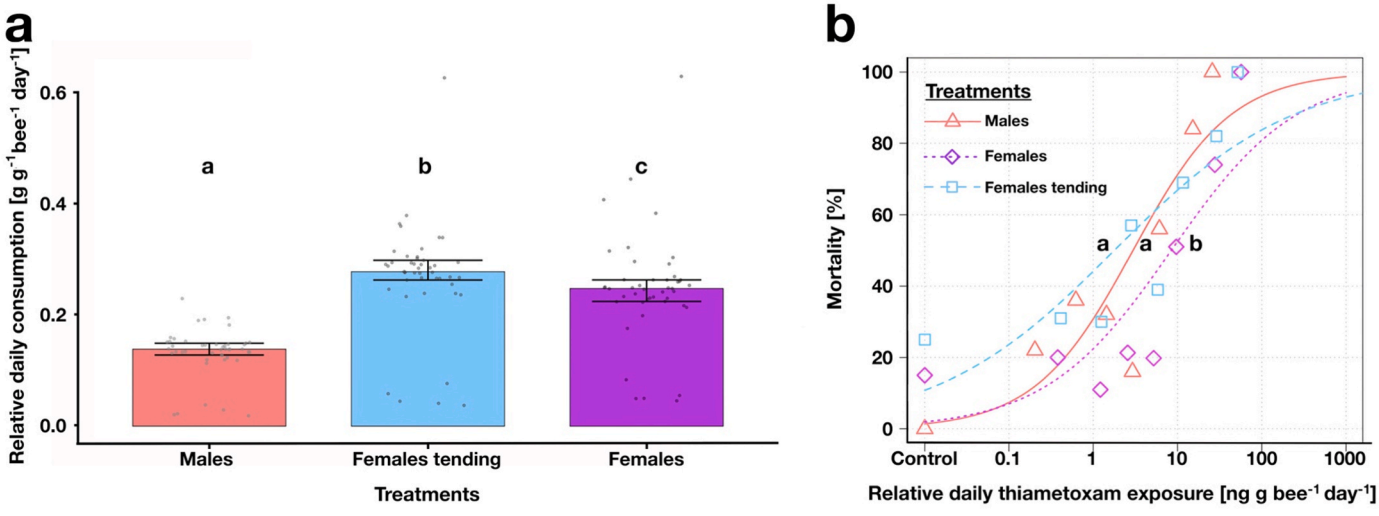


Fig. 1. Impact of male tending on consumption behaviour and thiamethoxam sensitivity of female (worker) western honey bees, *Apis mellifera*. (a) relative daily consumption [g g^{-1} bee^{-1}] of males, females tending to males, and females alone, across all treatment groups. Medians and interquartile ranges (IQR) are displayed for each bar plot - with each individual datapoint overlayed over their respective group. Relative daily consumption differed between tending and non-tending females as well as males of *A. mellifera*, with tending females consuming most, followed by non-tending females and finally males. **(b)** Dose response curves fitted to mortality [%] rates of males, females and tending females. While no significant difference in the Lethal Daily $Dose_{50}$ of tending females and males was observed ($P > 0.05$), both were significantly lower when compared to non-tending females ($P < 0.05$). Medians and IQRs are displayed for the bar plot - with each individual datapoint presented as a dot that overlaps their respective group. In all graphs, significant differences ($P < 0.05$) are indicated by letters (a, b, or c).

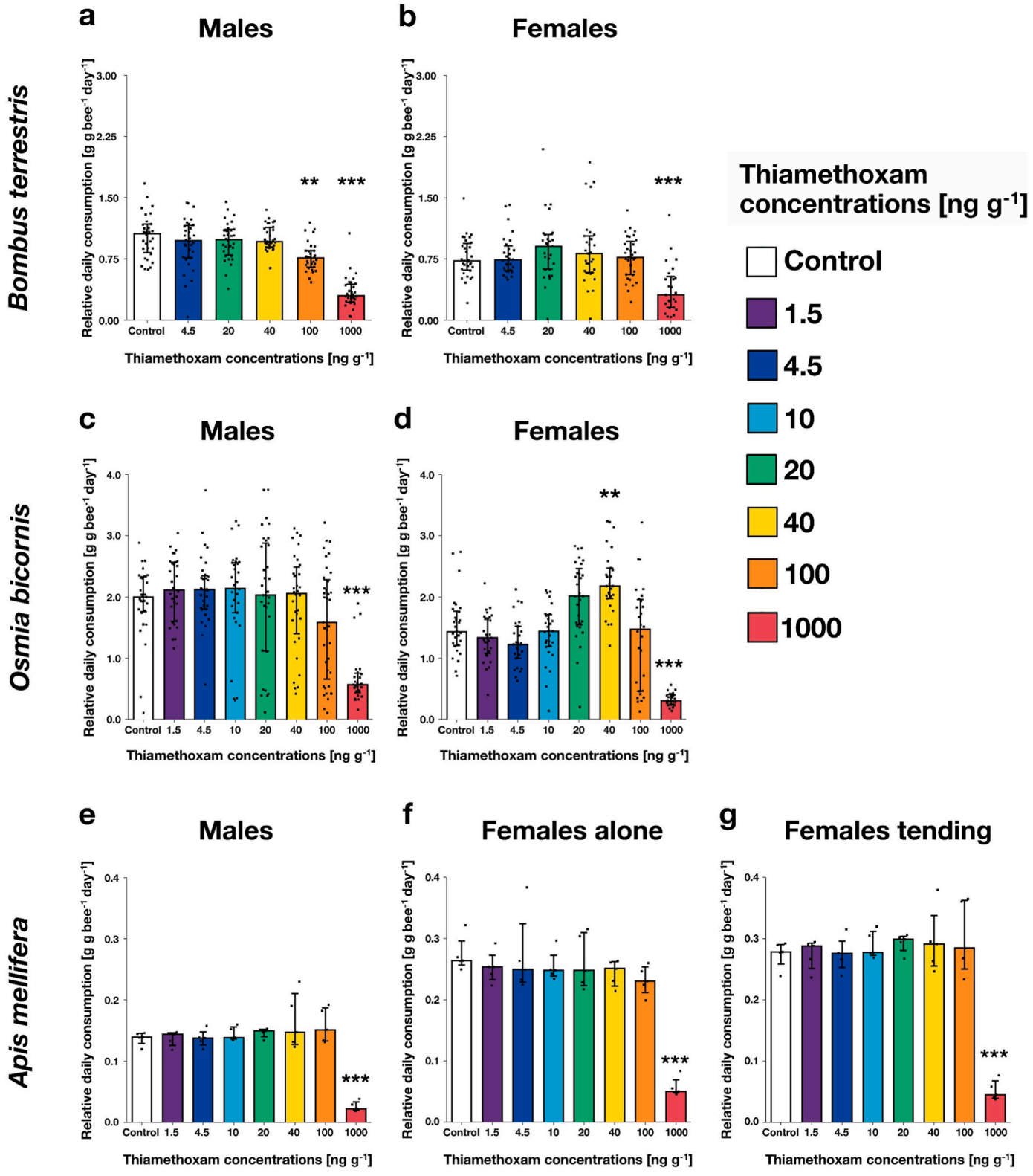


Fig. 2. Effect of thiamethoxam exposure on relative daily consumption of male and female bumble bees, *Bombus terrestris*, solitary bees *Osmia bicornis* and western honey bees, *Apis mellifera*. Bees were exposed to thiamethoxam concentrations ranging from 0 to 1000 ng g^{-1} **(a-g):** Exposure to 1000 ng g^{-1} significantly reduced relative consumption across all species and sexes (all *P*'s < 0.001). In addition, consumption was significantly reduced in *B. terrestris* males at 100 ng g^{-1} **((a)**; $P = 0.00509$), and significantly increased in *O. bicornis* females at 40 ng g^{-1} **((d)**; $P = 0.0011$). No other thiamethoxam concentrations significantly affected relative daily consumption (all *P*'s > 0.05). Bars show median relative daily consumption with interquartile ranges (IQR), and points represent individual observations. Significant differences relative to the control ($P < 0.05$ and $P < 0.001$) are indicated by asterisks (** and ***, respectively).

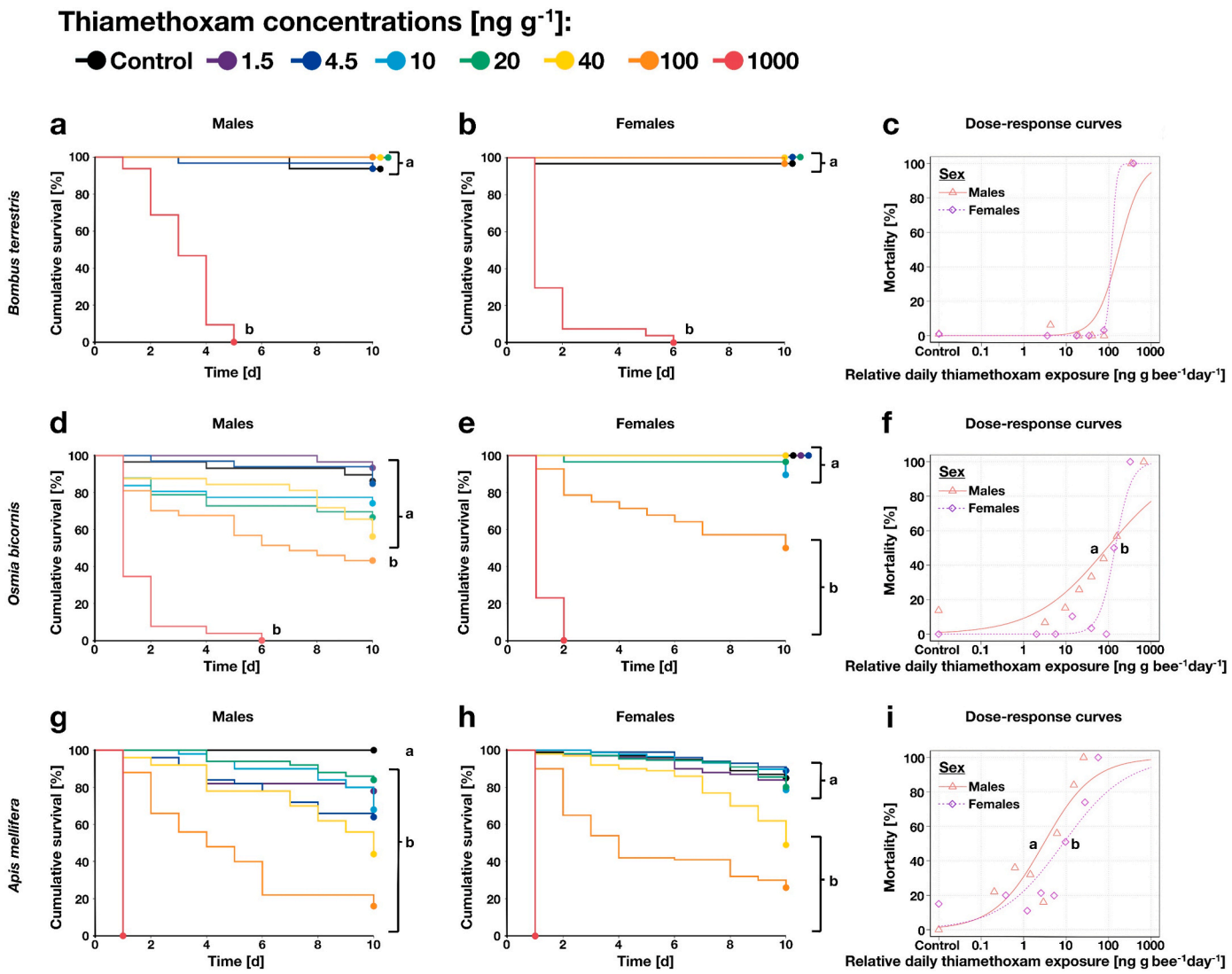


Fig. 3. Kaplan-Meyer survival and dose response curves for thiamethoxam exposed males and females of bumble bees, *Bombus terrestris*, solitary bees *Osmia bicornis* and western honey bees, *Apis mellifera*. Bees were exposed to different concentrations of thiamethoxam ranging from 0 to 1000 ng g^{-1} **(a, b, d, e, g, h)** Compared to the control treatment, exposure to thiamethoxam resulted in increased mortality rates across all species and sexes. ($P < 0.05$) **(c, f, i):** While *A. mellifera* females revealed a significantly higher LDD_{50} compared to males ($P < 0.05$), no significant sex specific difference in the LDD_{50} of *B. terrestris* was observed ($P > 0.05$). In contrast, females of *O. bicornis* revealed a significantly higher LDD_{50} than their male counterparts ($P < 0.05$). In all graphs, significant differences ($P < 0.05$) are indicated by letters (a or b).

a 51-fold and 26-fold increased sensitivity compared to *B. terrestris* and *O. bicornis*, respectively, whereas *O. bicornis* had a twofold increased sensitivity compared to *B. terrestris*. In females, no significant difference in the LDD_{50} between *B. terrestris* (109.9 ± 138.7) and *O. bicornis* (143.6 ± 15.8) was observed ($P > 0.80$). However, both species revealed a significantly higher LDD_{50} compared to *A. mellifera* females (8.6 ± 0.9); resulting in a 13- and 17-fold increase, respectively (P's < 0.001; $LDD_{50} \pm$ SE [ng g^{-1} bee^{-1} day^{-1}]; SI Fig. 8 c). Furthermore, intraspecific sex comparison of the LDD_{50} revealed no significant difference for *B. terrestris* ($P = 0.71$; Fig. 3 c). However, both *O. bicornis* and *A. mellifera* males had a significantly lower LDD_{50} compared to their female counterparts (P's < 0.036), resulting in a 2- and 3-fold increase in sensitivity of males in *O. bicornis* and *A. mellifera*, respectively (P's < 0.03; Fig. 3 f & i). Lastly, while the LDD_{50} for tending *A. mellifera* females (3.0 ± 0.4) did not significantly differ from their male counterparts ($P = 0.97$) a significantly threefold lower LDD_{50} was observed, when compared to the non-tending females ($P < 0.001$; Fig. 1 b; $LDD_{50} \pm$ SE [ng g^{-1} bee^{-1} day^{-1}]).

4. Discussion

The data clearly demonstrate both inter- and intraspecific variation, as well as that behavioural traits strongly influence bee sensitivity to chronic oral thiamethoxam exposure. Specifically, *O. bicornis* and *B. terrestris* were less sensitive than non-tending *A. mellifera*, and males of *O. bicornis* and *A. mellifera* were more sensitive than their conspecific females. These differences likely arise from a combination of body size, genetic background, life-history traits, and detoxification capacity (Beadle et al., 2019; Darveau et al., 2005; O'Donnell and Beshers, 2004; Sgolastra et al., 2019; Straub et al., 2015). Notably, *A. mellifera* female workers engaged in male-tending exhibited elevated sensitivity compared to non-tending females, potentially due to increased consumption and exposure to meet metabolic demands and ingestion of contaminated sucrose prior to regurgitation and provisioning, which together may amplify exposure and constrain detoxification efficiency. Collectively, these results highlight that species-, sex-, and behavioural-specific traits should be accounted for when assessing pesticide risks. While inevitably more elaborate and complex,

incorporating such variation at the earliest stages of environmental risk assessments (e.g., tier 1 tests) would substantially improve the accuracy and ecological realism of lower tier laboratory toxicity evaluations.

Our data reveal species- and sex-specific differences in feeding behaviour, consistent with previous studies (Minnameyer et al., 2021; Strobl et al., 2021). *Osmia bicornis* showed the highest relative chronic sucrose intake, followed by *B. terrestris*, while *A. mellifera* consumed the least. Males of *O. bicornis* and *B. terrestris* consumed more than females, whereas the reverse was observed in *A. mellifera*. Although caging conditions (i.e., individual vs. group hoarding) may have influenced consumption, the patterns are more likely driven by morphological, physiological, and life-history differences. Smaller individuals, such as *O. bicornis* males – the smallest in this study – typically exhibit higher mass-specific metabolic rates (Darveau et al., 2005), explaining their elevated consumption. Since *O. bicornis* overwinter as adults, depleted energy reserves may also increase post-emergence consumption (Raw, 1972). A key life-history distinction is that male *A. mellifera* depend on female attendance for feeding (Free, 1957), necessitating co-housing with females under laboratory conditions. This, in turn, introduced a confounding factor: tending females consumed ~10% more sucrose solution than non-tending females, likely to meet both self-maintenance and caregiving demands. Behavioural ontogeny may further have shaped consumption patterns. *Apis mellifera* males remain hive-bound without actively engaging in colony duties for ~8 days post-emergence, initiating orientation flights around day ten and reaching sexual maturity by day 12 (Jaycox, 1961). In contrast, *O. bicornis* males are sexually mature and immediately active upon emergence (Seidelmann, 1999), and *B. terrestris* males typically initiate foraging and mating-related behaviours by day four (Tasei et al., 1998). These earlier behavioural onsets impose greater energetic demands, reflected in higher consumption amongst *O. bicornis* and *B. terrestris* males compared to *A. mellifera*. Taken together, these findings emphasize the role of species, sex, and behaviour (e.g., tending vs. idle) in shaping exposure scenarios. Given that differences in consumption directly affect vulnerability (Mobley and Gegear, 2018), ignoring task- or context-dependent variation risks misestimating pesticide effects.

Thiamethoxam exposure did not lead to a higher proportion of non-feeding *O. bicornis* individuals. Similarly, across all species, feeding solution (sucrose) intake remained unaffected at field-realistic concentrations ($<40\ ng\ g^{-1}$ (Zioga et al., 2020)), indicating that avoidance-induced starvation was unlikely to influence survival outcomes. However, consistent with previous studies, consumption was significantly reduced at higher concentrations (100 and $1000\ ng\ g^{-1}$) (Minnameyer et al., 2021). This reduction may reflect learned avoidance, impaired feeding behaviour due to neurotoxic effects (Laycock et al., 2014), or simply the effects of acute intoxication and mortality lowering overall consumption. The LDD_{50} values revealed *A. mellifera* to be more susceptible than *O. bicornis* and *B. terrestris*, with the latter two showing 13- and 17-fold higher tolerance, respectively. The LDD_{50} for *A. mellifera* aligns with prior findings (Bovier et al., 2024), while comparable data for *Bombus* and *Osmia* remain limited, particularly for chronic oral exposure, constraining direct interspecific comparisons. Nevertheless, available studies indicate substantial variation among species; for examples, *Osmia cornuta* has been reported to be more sensitive to sulfoxaflor than *B. terrestris*, whereas *Bombus hypnorum* showed lower sensitivity (Dewaele et al., 2024). The observed variation likely reflects a complex interplay of physiological, molecular, and behaviour mechanisms. While normalizing dose by body mass accounts for relative dose, larger species such as *B. terrestris* may still benefit from dose dilution, enhancing tolerance (Jütte et al., 2023). Yet, body size alone cannot explain the pronounced differences we observed.

Species-specific life-history traits and degrees of eusociality provide a more compelling explanation. In highly eusocial species such as *A. mellifera*, workers function analogously to somatic cells, with colony-level buffering mitigating individual losses as long as the germ line remains intact (Straub et al., 2015). By contrast, solitary bees and eusocial species with solitary life stages (e.g., in *B. terrestris* gynes that overwinter alone) lack such buffering and therefore depend on stronger individual defences, likely selecting for enhanced detoxification capacity. Indeed, interspecific variation in detoxification gene expression, particularly in P450 monooxygenases (Beadle et al., 2019), may explain differences in sensitivity. Neonicotinoids are primarily metabolized by P450 enzymes (e.g., CYP genes), whose expression varies across bee taxa and can reduce bioactivity and/or limit receptor binding (Han et al., 2025). Moreover, species-specific differences in nicotinic acetylcholine receptors (nAChRs) subunit composition may further modulate binding affinity and neurotoxicity (Jones and Sattelle, 2010). These findings suggest that *A. mellifera* may represent as a conservative model species, aligning with the precautionary principle in risk assessment for *B. terrestris* and *O. bicornis*. Response to the dimethoate positive control broadly supported this interspecific pattern, with *B. terrestris* again showing the lowest sensitivity, although differences between *A. mellifera* and *O. bicornis* were less consistent. The magnitude of the response also differed between insecticides; notably, *B. terrestris* retained >90% survival at $1000\ ng\ g^{-1}$ dimethoate but experienced complete mortality at the same nominal concentration of thiamethoxam. Although a single dimethoate concentration precludes quantitative comparison of compound-specific toxicity, this contrast highlights that the magnitude of species-specific sensitivity may vary substantially among insecticides, calling for further work to identify the mechanisms underlying these differences.

Beyond interspecific variation, our data show that males of *O. bicornis* and *A. mellifera* are more sensitive than conspecific females, consistent with previous findings (Linguadoca et al., 2022; Mobley and Gegear, 2018). In *A. mellifera*, this is particularly striking given that worker females can metabolize and partially detoxify xenobiotics prior to regurgitating (Jaycox, 1961; Shamjana et al., 2024), effectively acting as a eusocial 'poison shield'. Nevertheless, honey bee males exhibited a threefold higher sensitivity than non-tending females, indicating an intrinsic sensitivity that persists despite reduced direct exposure. While sublethal impairment of tending cannot be fully excluded (Jones and Sattelle, 2010), it is unlikely to account for the observed magnitude of the sex difference. Instead, our results support the haploid-susceptibility hypothesis (O'Donnell and Beshers, 2004), whereby hemizygosity may increase vulnerability to stressors, including pathogens and xenobiotics (Camenzind et al., 2025; Retschnig et al., 2014), potentially through reduced effectiveness and expression of detoxification pathways (Mobley and Gegear, 2018). For *B. terrestris*, the selected dose range did not allow reliable estimation of sex-specific LDD_{50} values. Regardless of the underlying mechanisms, these findings underscore the risk of underestimating toxicological effects when early-stage risk assessments (i.e., Tier 1) only test diploid females. Incorporating males – readably feasible across honey bees, bumble bees, and solitary bees – into modified acute toxicity protocols would improve both accuracy and ecological relevance. In *A. mellifera*, co-housing sexes further enables the inclusion of behaviourally relevant process such as female tending, which may serve as an informative sublethal fitness proxy (Straub et al., 2020a). More broadly, integrating male bees and simple behavioural contexts into existing test protocols represents a practical pathway to enhance the protective value of Tier-1 assessments (e.g., OECD, 2017a, 1998a, 1998b).

Thiamethoxam exposure significantly increased mortality in tending female honey bees compared to non-tending counterparts, underscoring how social context and natural behaviours can heighten pesticide sensitivity. Two non-exclusive mechanisms likely explain this effect. First, the presence of males shifts females from inactivity to active tending, increasing energetic demands, reflected in ~10% higher sucrose consumption. Even under control conditions, tending imposed physiological costs, with higher mortality in tending (25%) versus non-tending (15%) females, while males showed no mortality over 10 days. This increased energetic burden may have amplified thiamethoxam toxicity (Straub et al., 2020a), whereas non-tending females likely

experienced lower oxidative stress and reduced demands on detoxification pathways (e.g., cytochrome P450s, glutathione S-transferases). Similar context-dependent interactions between insecticides and other stressors, such as pathogens and malnutrition, have been reported (Alaux et al., 2010; Siviter et al., 2021). Second, the higher consumption required to sustain individual energetic demands and feeding of males likely increased thiamethoxam exposure and detoxification demands. Together, these factors may have contributed to the threefold lower LDD_{50} in tending compared to non-tending females.

Irrespective of the underlying mechanism, these findings highlight the risk of underestimating pesticide effects in early Tier-1 testing, which largely overlooks behavioural and physiological stressors. While OECD guidelines standardize tests by excluding pollen feeding – creating a conservative, worst-case malnutrition scenario (Barascou et al., 2021; OECD, 2017a) – integrating behaviourally relevant processes would substantially improve ecological realism. Ignoring behaviour-dependent sensitivity may lead to underestimation of real-world risks and failure to trigger higher-tier assessments. Incorporating such refinements into early-tier testing offers a pragmatic pathway to enhance the ecological relevance and protectiveness of pollinator risk assessments without fundamentally altering existing frameworks. Moreover, pesticide exposure occurs within a complex ecological context shaped by interacting stressors such as habitat loss, pathogen exposure and climate change (Siviter et al., 2021; Wagner, 2020). Current ERA schemes largely assess single stressors in isolation, potentially limiting their ability to capture cumulative and context-dependent effects (Fisher et al., 2023; Tissier et al., 2025). Building on our findings, incorporating species-, sex- and behavioural-specific variation alongside additional environmental stressors represents a key next step towards developing more field-realistic risk assessments.

5. Conclusions

The data demonstrate that sensitivity to oral chronic neonicotinoid exposure varies not only among bee species and sexes, but with behavioural context. These findings highlight potential blind spots in current ERA schemes, particularly at early Tier-1 stages where *A. mellifera* females remain the principle surrogate for chronic oral exposure. While such standardized tests provide an important conservative screening framework, they cannot capture the physiological and behavioural complexity that fundamentally shapes xenobiotic sensitivity. Incorporating males and behaviourally relevant contexts into first-tier ecotoxicological testing could therefore improve predictive ecological realism and help identify effects that may otherwise be overlooked, while also enabling the evaluation of sublethal fitness proxies (e.g., male reproductive traits and female tending behaviour) that may signal long-term population consequences (Straub et al., 2020b). Although maintaining males under laboratory conditions poses challenges, the potential gains may outweigh these constraints, particularly given the persistent discrepancies between laboratory and fields studies (Rundlöf et al., 2015; Woodcock et al., 2016). Together with growing evidence from other studies, our results provide a constructive basis for further refinement of OECD testing protocols. The framework to achieve this already exists; integrating behaviourally enriched test systems, even at Tier-1, would strengthen the precautionary principle, increase the ecological realism, and yield more protective thresholds for vulnerable taxa. We welcome the ongoing efforts to develop protocols for solitary and bumble bee species and suggest that additional adjustments for *A. mellifera* – accounting for behavioural activities such as tending-would also be desirable. Together, these findings provide clear guidance for policymakers by identifying feasible refinements to existing Tier-1 protocols that improve the ecological relevance and protective value of pollinator risk assessments. What is needed now is the regulatory momentum to implement these refinements and novel protocols.

CRediT authorship contribution statement

Domenic W. Camenzind: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Andrew F. Brown:** Writing – review & editing, Visualization, Formal analysis. **Selina Bruckner:** Writing – review & editing, Investigation. **Johanna Hehl:** Writing – review & editing, Investigation. **Lukas Jeker:** Writing – review & editing, Investigation, Conceptualization. **Annette Van Oystaeyen:** Writing – review & editing, Resources, Conceptualization. **Flavio Tettamanti:** Writing – review & editing, Investigation. **Verena Strobl:** Writing – review & editing, Investigation. **Geoffrey R. Williams:** Writing – review & editing, Conceptualization. **Peter Neumann:** Writing – review & editing, Conceptualization. **Lars Straub:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Domenic Camenzind reports financial support was provided by Vinetum Foundation. Lars Straub reports a relationship with Federal Office for the Environment that includes: funding grants. Peter Neumann reports a relationship with Federal Office for the Environment that includes: funding grants. Lars Straub reports a relationship with Vinetum Foundation that includes: funding grants. Peter Neumann reports a relationship with Vinetum Foundation that includes: funding grants. Peter Neumann reports a relationship with Agroscope that includes: employment. Lukas Jeker reports a relationship with Agroscope that includes: employment. Geoffrey R. Williams reports a relationship with US Department of Agriculture that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2026.131264.

Data availability

The data has been uploaded to Dryad: https://doi.org/10.5061/dryad.6djh9w1jw

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