Bulletin of Insectology

Research Article
Bulletin of Insectology 78 2025, 121–130
DOI 10.3897/bull.insectology.168212

Experimental protocol for acute oral toxicity testing in the hoverfly *Eristalinus aeneus* (Scopoli, 1763) (Diptera, Syrphidae)

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Academic editor: Giovanni Burgio ♦ **Received** 8 August 2025 ♦ **Accepted** 28 October 2025 ♦ **Published** 21 November 2025

Abstract

Hoverflies (Syrphidae) play a key role in pollination services across ecosystems but are underrepresented in pesticide toxicity research. Laboratory toxicity testing is a fundamental step toward evaluating pesticide risks, yet established protocols for hoverflies are lacking. This study aims to fill this gap by developing an acute oral toxicity test for *Eristalinus aeneus*, a cosmopolitan hoverfly. A novel oral toxicity test was introduced using a custom-designed test cage with artificial flowers, allowing precise measurement of food consumption and dose assessment. Both individual and group feeding setups were tested, with group feeding proving more feasible, overcoming key limitations of individual feeding, including difficulty in locating food and evaporation of liquid diets. As an additional validation step, an oral toxicity test was conducted with dimethoate — a reference insecticide commonly used in toxicological studies — using three concentrations (0.007, 0.0014, and 0.0007 g/L). The estimated LC_{50} values for *E. aeneus* were ~0.00166 g/L (24 h) and ~0.00137 g/L (48 h), with corresponding LD_{50} values of ~0.56 and ~0.46 µg/g fly. Our results indicate that *E. aeneus* is highly sensitive to dimethoate and slightly more sensitive than *Apis mellifera*. These findings underscore the need to include *E. aeneus* and other syrphids in future toxicity testing and highlight the value of the developed methodology for ecotoxicological studies and conservation efforts.

Key Words

Artificial flowers, dimethoate, laboratory test, pollinators, risk assessment, syrphids

Introduction

Hoverflies (Syrphidae) are a diverse family of Diptera comprising over 6,000 species worldwide (Sommaggio 1999), and among the most important non-bee pollinators, contributing substantially to both wild plant and crop pollination (Klecka et al. 2018; Dunn et al. 2020). In addition to their role as pollinators, some species also contribute to natural pest control by feeding on aphids (Dunn et al. 2020). Like many other pollinating insects, hoverflies are increasingly exposed to various environmental stressors. Although the global decline of pollinators raises concerns for biodiversity and agriculture (Senapathi et al. 2015; Breeze et al. 2016; Potts et al. 2016; Neov et al. 2021), hoverflies remain underrepresented in pollinator research, which has traditionally focused on bees.

Pesticide exposure is considered a key driver of pollinator declines (Botías et al. 2015; Dicks et al. 2021; Birkenbach et al. 2024). While regulatory testing protocols have been well established for honeybees (*Apis mellifera* L. 1758) (OECD 1998a, 1998b), and more recently for bumblebees and solitary bees (OECD 2017a, 2017b; OECD 2024), hoverflies remain largely excluded from such frameworks. This gap persists despite growing evidence of considerable inter- and intraspecific variation in insect sensitivity to pesticides (Hardstone and Scott 2010; Sgolastra et al. 2019; Hellström et al. 2023; Nagloo et al. 2024).

Toxicological research on hoverflies remains limited in both scope and taxonomic coverage. Studies on larval exposure have shown that some insecticides increase mortality and negatively affect reproduction in aphidophagous species (Jansen et al. 2011; Moens et al. 2011), whereas others, such as thiacloprid, thiamethoxam, and acetamiprid, showed no effects on adults at field-realistic doses (Jansen et al. 2011; Basley et al. 2018; Birkenbach et al. 2024). However, standardized methodologies for adult oral toxicity testing are still lacking.

Some recent progress has been made toward addressing this gap. Nagloo et al. (2023) developed an oral toxicity bioassay for *Eristalis tenax* L. 1758 using 3D-printed artificial flowers, demonstrating a much higher tolerance to imidacloprid compared to *A. mellifera*. However, the requirement for specialized equipment limits the broader application of this method. Similarly, Henriques Martins et al. (2024) assessed contact toxicity of an imidacloprid formulation (Confidor®) in *Sphaerophoria rueppellii* (Wiedemann 1820) and *Eristalinus aeneus* (Scopoli 1763), finding that these species were less sensitive than bees.

Given the lack of standardized and easily applicable methods for oral toxicity testing in hoverflies, we aimed to develop a simple and reproducible acute oral toxicity bioassay for *Eristalinus aeneus*. This species was selected due to its wide geographic distribution, high pollination efficiency, and potential for laboratory rearing and commercialization (Campoy et al. 2020). Specifically, our objectives were to: (a) test different feeding designs (with or without artificial flower) to determine which design yields the highest feeding rate under individual feeding conditions, and (b) assess whether group feeding enhances feeding rates compared to individual setups. To validate the method, we tested the toxicity of dimethoate, a standard positive control commonly used to validate experimental design and confirm test sensitivity in toxicity studies (Gough et al. 1994; OECD 1998a, b; OECD 2017a, b; OECD 2024), and estimated the approximate 24- and 48-hour oral LC_{50} and LD_{50} for *E. aeneus* based on a three-dose concentration range. These preliminary values provide a reference for selecting appropriate concentrations of dimethoate as a positive control in future studies assessing the toxicity of other pesticides and allow for eventual comparisons with other species, primarily *A. mellifera* and bumblebees.

Material and methods

Pre-treatment insect rearing

Pupae of *Eristalinus aeneus* were obtained from the University of Alicante (Spain) and randomly placed in perforated commercial plastic boxes (2L, ~30 pupae per box) on a plastic tray (see Suppl. material 1). Each box was supplied with a 50% w/v sucrose solution and water in separate containers. Emerging adults (without separating females and males) were collected daily into new boxes (~10 flies per box) provided with sucrose solution and water. During acclimatization, the flies were kept at room temperature (approximately 22°C) under natural light conditions. Dead or moribund individuals were removed daily, and food was replenished as needed. Experiments began once a sufficient number of adults had emerged (~5 days). *E. aeneus* is also commercially available, making broader application of the method feasible.

Test feeding designs

Commercial plastic boxes (375 mL) with needle-punched perforations were modified for easy manipulation of the food supply. To create cages, the boxes were inverted so that the lid became the bottom. A 10 mm hole was drilled in the centre, sealed with an Eppendorf cap serving as a feeder unit, allowing easy removal or replacement without opening the box (Fig. 1A, Suppl. material 9). Cages contained artificial radially symmetrical flowers, either 2D (white or green base) or 3D (narrow or wide elevated petals on the green base) (Fig. 1B–E). Green bases were introduced to imitate a grassy area (Fig. 1B, D, E). Since hoverflies exhibit colour preferences (Klecka et al. 2018), petals were yellow or white, and the flowers were unscented to prevent potential odor-induced sex bias (Mishra et al. 2025). Their radial symmetry aligned with the floral complexity preference of *E. aeneus* and many other pollinator species (Ladurner et al. 2005b).

Feeding trials

All feeding trials used a 50% w/v sucrose solution as feeding solution. Flies were individually tested on five different feeding designs, four flower designs (Fig. 1B–E) and one feeder without petals (20 flies per design, 100 in total). Group feeding (5 flies per cage) was tested on the 3D flower with wide petals in 20 replicates (100 flies total, Fig. 1E and Fig. 2B, C). Two days before testing, flies were allocated to test cages. Group-fed flies were briefly chilled to immobilize them and marked in four colours (yellow, blue, red, green), with one fly left unmarked per cage for tracking (Fig. 2, Suppl. material 10). Flies were pre-fed on sucrose-soaked cotton wool to familiarize them with the feeding process, followed by an overnight starvation period to standardize hunger levels.

On the test day, caps with 20 µL (individual) or 100 µL (group) sucrose solution were weighed and placed in cages. Flies were monitored until their first feeding (maximum three hours, as proposed by OECD guidelines 1998b, 2017b) and classified as feeders or non-feeders (based on visual confirmation of consumption). After three hours, caps were weighed and replaced with sucrose-soaked cotton wool. Evaporation was controlled using fly-free cages, each with sucrose-filled

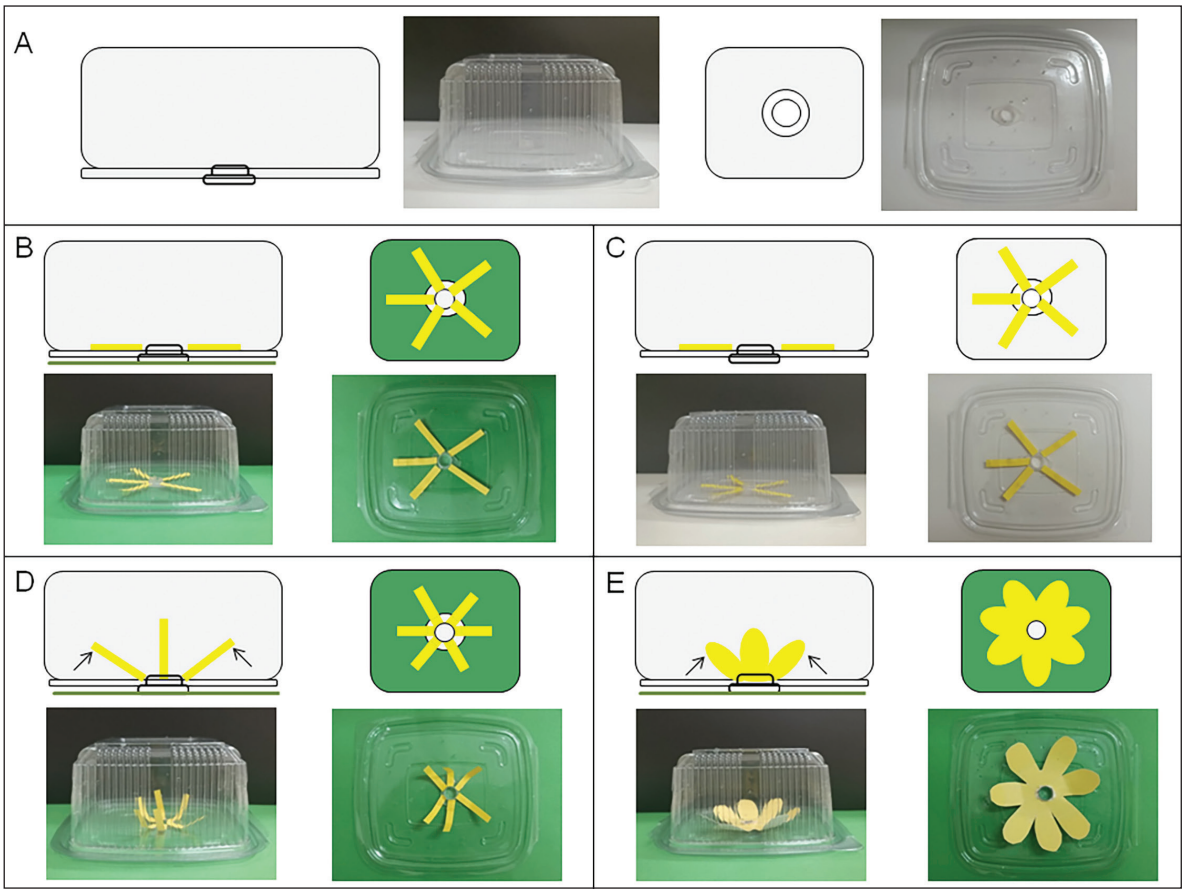

Figure 1. Cages with feeding units and flower setups. Test cages: small, transparent, perforated plastic boxes (375 mL volume) with a 10 mm diameter hole in the centre of the bottom. The hole was sealed with a microtube cap, which serves as a food supply unit and allows easy manipulation without opening the box (**A**). Artificial flower models with flat paper petals arranged around the cap (2D flowers) on a green surface (**B**) and a white surface (**C**), or elevated petals around the cap (3D flowers) with narrow (**D**) or wide (**E**) petals on a green surface.

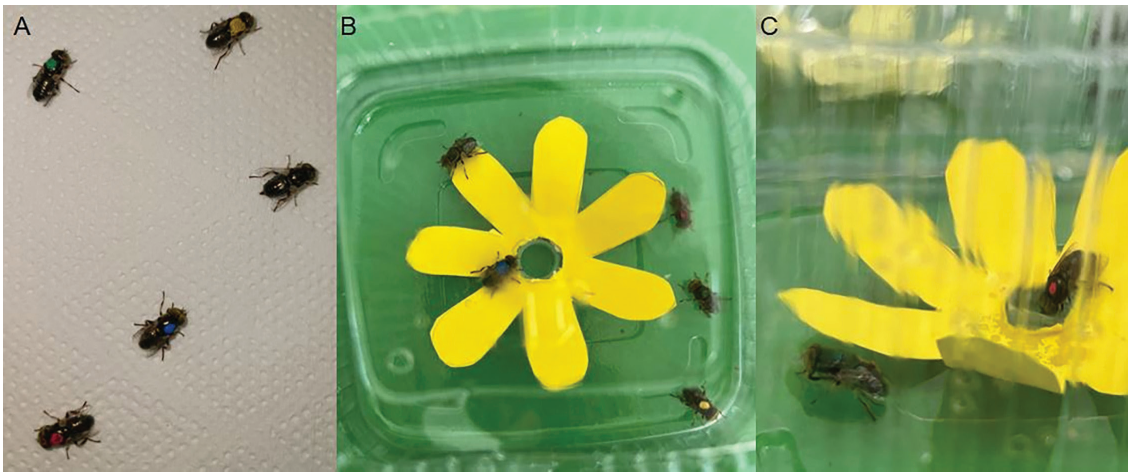

Figure 2. Marked hoverflies in test cage. Five marked individuals of *Eristalinus aeneus* for group feeding: four marked with different colours and one unmarked (**A**), in a test cage with a 3D flower featuring wide petals (**B** and **C**).

caps containing the appropriate volume (twenty caps for 20 µL and twenty caps for 100 µL). Since *E. aeneus* does not exhibit sexual dimorphism in body size or foraging activity (Mishra et al. 2025), both sexes were used in the experiment, ensuring an equal distribution of females and males in the test groups.

Assessment of sensitivity to dimethoate – first setup

Dimethoate (Penstanal®, Sigma-Aldrich) was dissolved in acetone to prepare the stock solution (100 g/L), which was then serially diluted (details in Suppl. material 3).

The treatment solutions were prepared in 50% w/v sucrose, ensuring that the acetone concentration did not exceed 4% v/v (OECD 2017b). Although dimethoate is currently banned in the European Union, it is commonly used as a positive control in toxicological studies to validate experimental design and confirm sensitivity of the test species (Gough et al. 1994; OECD 1998a, b; OECD 2017a, b; OECD 2024).

Based on pilot trials (data not shown), three dimethoate concentrations were tested: D1 (0.007 g/L), D2 (0.0014 g/L), and D3 (0.0007 g/L). Each concentration (100 µL per cage) was tested in a cage containing five marked flies and a 3D flower with wide petals, with two replicates per concentration (10 flies per concentration). The setup was used as a preliminary range-finding test rather than a definitive LD_{50} determination. Additionally, two control cages (C) with five individuals were included, in which flies were fed with 50% w/v sucrose solution with 4% acetone at final concentration. In total, treated and control groups included 40 individuals. To account for evaporation, two fly-free control cages were also included. After three hours, the caps containing the treatment solutions were removed, weighed, and replaced with sucrose-soaked cotton wool. The solution consumed per cage was calculated by subtracting the post-exposure cap weight from the pre-exposure weight, corrected for evaporation. Consumption per fly in group feeding was calculated by dividing total consumption by the number of feeders. Mortality was recorded at 4, 24, and 48 hours post-exposure.

Assessment of sensitivity to dimethoate – second setup

Based on results from the first setup, the second focused on the D2 concentration, which had caused 50% mortality after 24 hours, using a larger sample size to refine the estimates of the median lethal concentration/dose. Five replicate cages, each containing five flies, were exposed to the D2 solution. A control group (C) was also included, consisting of three replicate cages and two fly-free cages as evaporation controls.

Statistical analysis

To determine how feeding success was affected by feeding design, only data from individually caged flies were analysed to avoid confounding with potential effects of group feeding. Feeding success (fed vs. not fed) was modelled using two generalized linear models (GLMs) with binomial error distribution and a logit link, implemented via the glm function in R's "stats" package. The first GLM tested the effect of using petals in general and contained petal presence (yes vs no) as the only predictor. The second GLM tested whether the exact design affected feeding success and contained feeding design as the sole predictor. Pairwise differences among the five feeding designs were tested using estimated marginal means derived with the "emmeans" function and compared using the "pairs" function of the "emmeans" package. *P*-values were adjusted for multiple testing using the false discovery rate (FDR) method.

To assess whether feeding success differed between group and individual feeding, a generalized linear mixed-effects model (GLMM) was applied to the two treatments sharing the same feeding design (3D flower with wide petals). Feeding success (fed vs. not fed) was modeled using a generalized linear mixed-effects model with binomial error distribution and a logit link, implemented via the "glmmTMB" function of the "glmmTMB" package. This model contained feeding design as fixed factor and cage ID as random effect.

For visualization, feeding success in each treatment (design or individual/group feeding) was expressed as the percentage of individuals that fed, and the corresponding 95% confidence intervals were calculated using the "emmeans" function from the "emmeans" package.

To estimate the median lethal dose (LD_{50}) and concentration (LC_{50}) at 24 hours and 48 hours, two-parameter log-logistic dose–response models were fitted using the function "drm" from the "drc" package (Ritz et al. 2015) and data from both setups. The response variable was the proportion of dead individuals out of those that had fed, with the number of feeders included as weights to account for variation in sample size among treatments. A binomial error distribution was assumed. The dose-response relationships and concentration-response relationships were modelled using the log-logistic function specified by *LL2.2*, with the lower limit fixed at zero and both the upper limit and slope estimated from the data. LD_{50} and LC_{50} values and their 95% confidence intervals were derived using the function *ED* from the "drc" package with the argument "interval" = "*fls*", which calculates confidence limits on the log-transformed dose scale and back-transforms them to the original scale. The control group (0 ng/fly; 0 µg/µL) was specified as the reference dose/concentration. For comparison to other species, LD_{50} values were normalized to body mass by dividing by the mean adult body mass of *Eristalinus aeneus* in this experiment (0.043 g).

All analyses were conducted in R (version 4.4.2) (Team RDC 2024), and the R script is provided in Suppl. material 2.

Results and discussion

Toxicological studies on syrphids are scarce, despite their important role as pollinators. Research on acute toxicity has mostly focused on cosmopolitan species, with studies assessing oral toxicity (Nagloo et al. 2023) and contact toxicity (Henriques Martins et al. 2024). As the methodology for oral toxicity testing in syrphids is still in development and given that oral exposure is a critical route of pesticide exposure, we propose a simple and reliable acute oral toxicity test for *Eristalinus aeneus*. Our test was designed to be easily implemented under standard laboratory conditions.

Feeding trials

Individual feeding was tested first, as it allows for precise measurement of treatment solution intake, which is essential for calculating the exact dose of active ingredient consumed. The main methodological challenge in oral toxicity testing is that some species do not readily consume artificial diets in laboratory settings. One approach to overcoming these challenges involves using artificial or natural flowers as feeding platforms (Ladurner et al. 2003, 2005a, 2005b; Miller et al. 2010; Nagloo et al. 2023). Natural flower-based methods have demonstrated higher feeding success rates (Ladurner et al. 2003, 2005a), but their widespread application is hindered by the availability of floral resources. A simplified alternative using a single petal has shown promise for improving feeding consistency in *Osmia* spp. (Azpiazu et al. 2023; Hellström et al. 2023).

In our study, we evaluated artificial flowers with paper petals as a simple and practical approach. The feeding trials included individual feeding in cages with 2D and 3D flower designs (Fig. 1) and cages without petals. Feeding success was measured in terms of the flies' ability to locate the food source and consume the food. Generally, feeding success was higher with artificial flowers than with the petal-free design (76% vs 35%, $P < 0.001$). There was however no significant difference among different artificial flower designs ($P > 0.5$). The highest feeding rate (85%) was observed with the 3D flower with wide downward-sloping petals, followed by the 3D flower with narrower petals and the 2D flower with a green base (both 75%). These three designs yielded a significantly higher feeding rate for individual feeding than the petal-free design (FDR-adjusted Wald test on binomial GLMM, 3D wide petals: $P = 0.026$, other two designs: $P = 0.046$; Fig. 3, Suppl. materials 4, 5). The 2D flower with a white base had also a twice as high observed feeding rate (70%) than the petal-free design (35%), but the difference was not statistically significant ($P = 0.075$) (see Suppl. material 5).

During individual feeding trials, two main challenges emerged. The first issue stemmed from the flies' behaviour: in some cases, they remained stationary for the majority, if not the entirety of the three-hour period, severely limiting their ability to locate food. The second, major challenge was evaporation. In control experiments (Suppl. materials 6, 7), the 100 µL sucrose solution lost approximately 26% of its mass due to evaporation over three hours, whereas the 20 µL solution lost around 50% of its mass as the solvent evaporated and remaining sugar crystallized, which prevented the flies from having access to a liquid food source. For oral toxicity tests, which typically last up to three hours (OECD 1998b; OECD 2017b), the feeding method must ensure efficient food intake throughout the exposure period. Due to these issues, the individual feeding trials were ultimately deemed unsuccessful. Given the challenges of working with wild pollinators, where specimen availability may be limited, optimizing feeding methods is essential for obtaining reliable data.

These issues were successfully resolved by implementing group feeding and feeding success appeared to improve under group feeding conditions. When five flies were provided with a 3D flower with wide petals (Fig. 1E) and a 100 µL sucrose feeder, feeding success increased from 85% under individual feeding to 97% under group feeding ($P = 0.04$; Fig. 3, Suppl. material 4).

Increased interaction among flies led to greater movement and enhanced feeding success. Due to increased fly activity, food was mostly located during the first hour of exposure. Additionally, the larger volume of feeding solutions

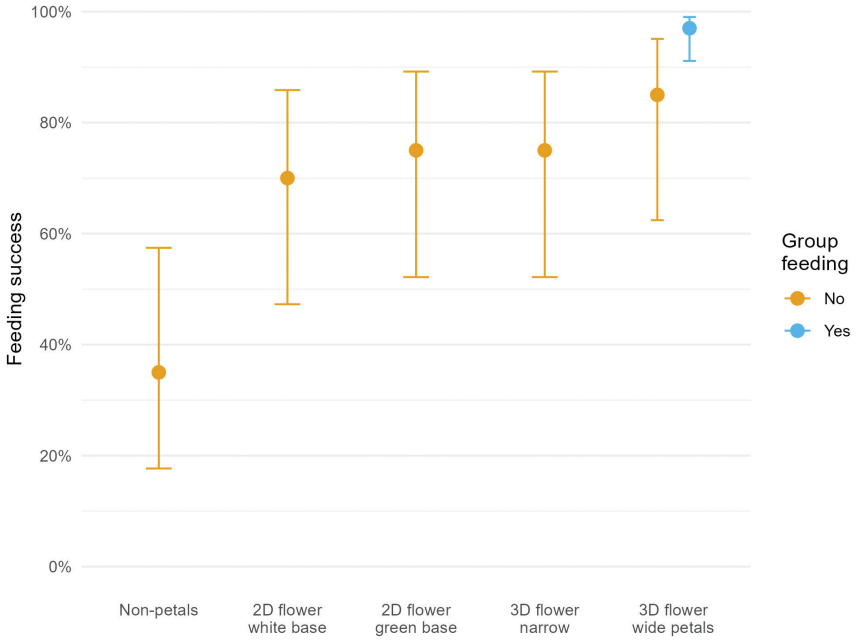

Figure 3. Feeding success of *Eristalinus aeneus* across different cage designs under individual feeding conditions, with one additional group feeding treatment included for comparison. Points represent observed feeding success (percent of flies that fed), with error bars indicating 95% confidence intervals. Colours distinguish between group and individual feeding.

mitigated evaporation, ensuring that the flies had sufficient time to consume the test solution within the exposure period. To facilitate individual tracking of consumption, flies were marked (Fig. 2), allowing for the distinction between feeders and non-feeders. This approach ensured that only flies that consumed from the solution were considered for the treatment. As a result, group feeding with five marked flies per cage and 3D flowers with wide petals was used for all subsequent testing in this study.

While the group feeding method offers several advantages, including potentially higher rates of feeding success, ease of implementation and suitability for laboratories with varying levels of resources, it is important to note its limitations. One key limitation is that consumption per fly is calculated as an average: assuming that all flies consumed the same amount of food. This approach may lead to inaccuracies in estimating individual consumption, as it assumes uniform feeding behaviour and does not account for variability among individuals. However, this limitation is partially mitigated by the use of small groups of only five individuals, which helps to reduce the extent of inter-individual variation. Additionally, the method relies on visual observation for three hours to classify flies as feeders or non-feeders, which may be time consuming. Despite these limitations, the group feeding method remains a practical and reproducible approach for oral toxicity testing in *Eristalinus aeneus*. This method may also be applicable to other Syrphidae species, offering a valuable tool for future ecotoxicological studies. It effectively addresses the challenges associated with individual feeding, such as low feeding success and significant evaporation and/or crystallization of the test solution.

Oral toxicity testing of dimethoate

To test the validity of the oral toxicity testing method, we measured the toxicity of three concentrations of dimethoate (0.007, 0.0014, and 0.0007 g/L, labelled as D1, D2, and D3, respectively) on *Eristalinus aeneus* using two experimental setups (Fig. 4, Suppl. material 8). Three concentrations were chosen as the test aimed to validate

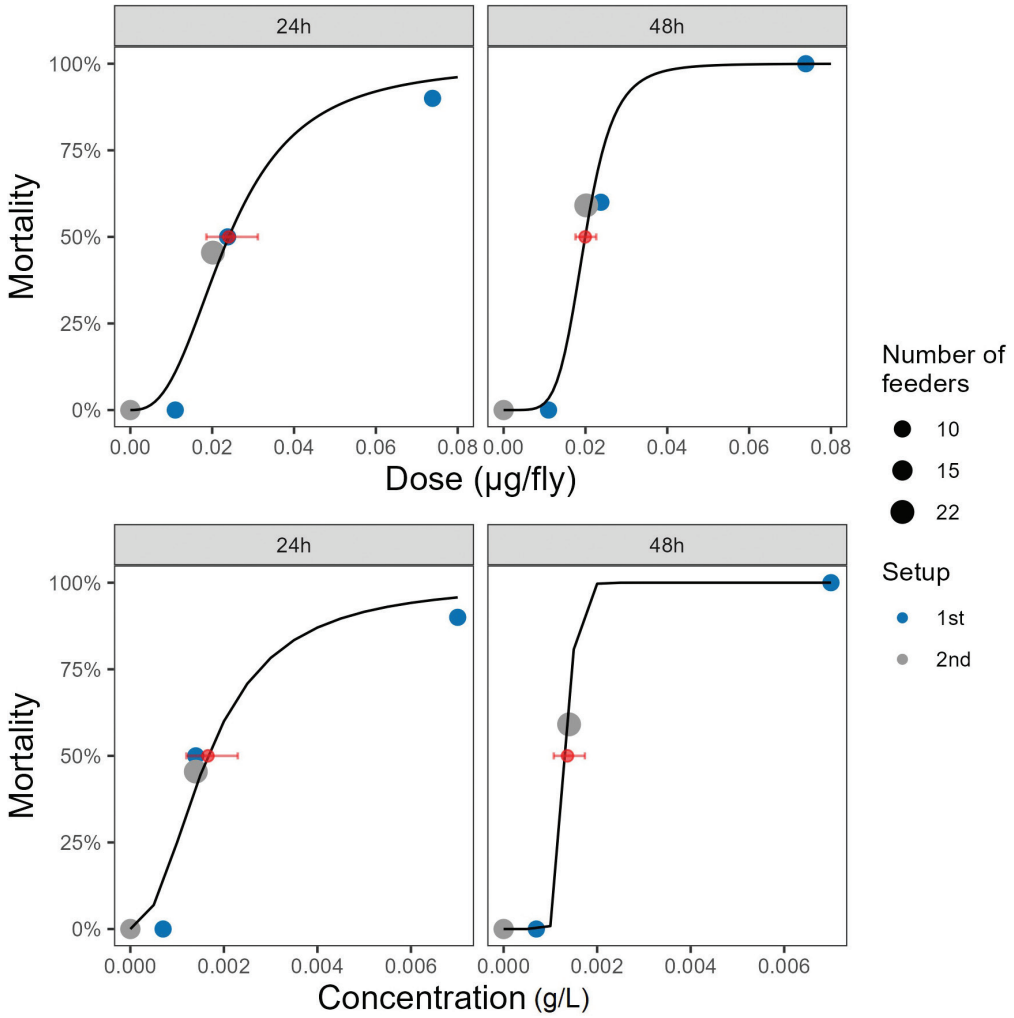

Figure 4. Dose-response curves for acute oral toxicity in *Eristalinus aeneus*-based on mortality after 24 and 48 hours. Points represent observed mortality at each dose level (µg/fly)/concentration (g/L), with point size indicating the number of flies that fed. Black lines show fitted log-logistic models, and red points indicate estimated LD_{50}/LC_{50} values with horizontal red error bars representing 95% confidence intervals.

the experimental design and confirm method sensitivity using a standard positive control, rather than to perform a full regulatory LC_{50} assessment. In the first setup, high mortality was observed at the highest concentration (D1), reaching 100% after 48 hours. Moderate mortality was recorded for D2, with 60% of flies affected after 48 hours, while D3 caused no mortality. Similar mortality patterns and ingested doses were observed in the second setup for D2 concentration. The high success of the feeding approach further validated our methodology as a straightforward assessment of oral toxicity in *Eristalinus aeneus*.

Dimethoate, an organophosphate insecticide, is a commonly used standard in toxicity studies, and estimates of its toxicity for different species are essential for future ecotoxicological research. Despite the fact that standardized procedures were not followed in this study (mortality was assessed for only three concentrations), and considering all limitations of the group feeding approach mentioned above, approximate estimates based on our data suggest that the acute oral LC_{50} for *Eristalinus aeneus* is 0.001659 g/L at 24 h and 0.001366 g/L at 48 h. Corresponding LD_{50} values are 0.56 µg/g fly (0.024 µg/fly) after 24 h, and 0.46 µg/g fly (0.020 µg/fly) after 48 h (Fig. 4). For comparison, the LD_{50} for acute oral toxicity to dimethoate for *Apis mellifera* ranges from 1.1–3.3 µg/g (0.11 to 0.33 µg/bee) after 24 h, with similar values reported for 48 h (Gough et al. 1994). These findings indicate that *E. aeneus* is slightly more sensitive compared to *Apis mellifera*, exhibiting approximately twofold greater sensitivity. These results contrast with previous studies on syrphid species (Nagloo et al. 2023; Henriques Martins et al. 2024), which indicated that syrphids are more robust than *A. mellifera*. As previously mentioned, Nagloo et al. (2023) assessed oral toxicity of imidacloprid in *Eristalis tenax*, while Henriques Martins et al. (2024) focused on contact toxicity of imidacloprid formulations in *E. aeneus*. Since our study assessed the oral toxicity of dimethoate in *E. aeneus*, differences across pesticide classes, exposure types (oral vs. contact), or fly species could be the reason for the observed discrepancies in sensitivity (Nagloo et al. 2024). Further research exploring sensitivity distributions of hoverflies using comparable methodologies are urgently needed to better understand the potential effects of pesticides on this important pollinator group.

In conclusion, this study demonstrates that group feeding in *Eristalinus aeneus* is more effective than individual feeding, with the artificial flower design providing a reliable method for oral toxicity testing. The study design focused on establishing a reproducible and controlled oral exposure setup for hoverflies under laboratory conditions. Given the ecological importance of hoverflies as pollinators, further research is needed to understand their sensitivity distribution and exposure pathways to pesticides. The preliminary toxicity data for dimethoate provide a reference point for selecting its appropriate concentration as a positive control in future studies. The observed higher sensitivity of *E. aeneus* compared to *Apis mellifera* additionally underscores the need to evaluate the effects of commercially used pesticides on hoverflies and other wild pollinators.

Acknowledgements

This work was supported by the European Union's Horizon Europe research and innovation programme under grant agreement No. 101135238 (WildPosh), and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125). The authors would also like to thank the members of Work Package 2 of the WildPosh project for their valuable suggestions, particularly Prof. Dr. Robert Paxton, during the experimental setup and the writing of this paper.

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Supplementary material 1

Holding cage

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić

Data type: pdf

Explanation note: Pupae of *Eristalinus aeneus* in a large perforated (2L) plastic box, provided with a 50% w/v sugar solution (yellow tray) and water (blue tray). The same type of cage was used as a holding cage for newly emerging adults.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl1

Supplementary material 2

R script

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Statistical analysis.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl2

Supplementary material 3

Dimethoate treatment solutions and doses

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Concentrations of acetone-based working and treatment solutions, and targeted ingested doses of dimethoate for acute oral sensitivity testing in *E. aeneus*.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl3

Supplementary material 4

Rate of feeding success

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Rate of feeding success in *Eristalinus aeneus* under different feeding designs in individual and group feeding.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl4

Supplementary material 5

Pairwise comparisons of feeding success

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Pairwise comparisons of feeding success among cage designs under individual feeding conditions.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl5

Supplementary material 6

Control of evaporation of 100 µL

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Control of evaporation of 100 µL of 50% (w/v) sucrose solution over a 3-hour period.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl6

Supplementary material 7

Control of evaporation of 20 µL

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Control of evaporation of 20 µL of 50% (w/v) sucrose solution over a 3-hour period.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl7

Supplementary material 8

Acute oral testing

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: pdf
Explanation note: Acute oral toxicity testing of dimethoate in *Eristalinus aeneus*.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl8

Supplementary material 9

Video-test cage

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: mp4
Explanation note: Preparation of test cage.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl9

Supplementary material 10

Video – marking

Authors: Milica Radenković, Jelena Purać, Nikola Krivokuća, Danijela Kojić, Dimitry Wintermantel, Julia Osterman, Ante Vujić
Data type: mp4
Explanation note: Marking of individuals.

Link: https://doi.org/10.3897/bull.insectology.168212.suppl10