Oral Presentation

Section 1. - Non-Apis bees

A novel approach for acute single dose toxicity testing on a solitary bee, *Osmia bicornis*

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Abstract

Robust laboratory-based guidelines for acute oral toxicity testing in solitary bee species are urgently needed to assess the risks of plant protection products and their active ingredients. Current attempts to develop such an interlaboratory testing system, for instance for the genus *Osmia*, are currently inadequate and face numerous obstalces. A major concerning being inadequate feeding methods. Thus, unlike the acute oral test systems for honey bees (OECD Guideline 213) and for bumblebees (OECD Guideline 247), there is still a lack of a guidance document for solitary bees. Here, we propose a novel testing system for an acute oral toxicity test using the model organism *Osmia bicornis*. To both improve feeding success and ensure that bees ingest the desired amounts of sucrose solution within a short period of time (e.g., within 4 hours), we tested a novel cage design and feeding device and subjected bees to a training period prior to testing to increase feeding success. Compared to Nicot cages, the use of our novel transparent cages that had an increased volume and pipette tips as feeding devices greatly improved acute oral dosing and reduced evaporation of the test substance. Furthermore, control mortality in the control group was low (11.8%), monitoring of bee bahavoiur and handling was simplified which reduced stress on bees as well as decreased labor. Ultimately, our novel method appears a promising approach for testing acute oral toxicity in solitary bees, yet additional studies are required to confirm our findings.

Keywords: Solitary bee; *Osmia bicornis*; acute oral exposure

Introduction

Solid laboratory-based acute oral toxicity test guidelines are urgently required to evaluate of the risks of plant protection products and their active ingredients on solitary bee species, e.g. on the genus *Osmia*. Despite several attemps to develop and ring test such a testing system, the finalization of a robust guidline has yet to be established. In contrast to the acute oral test systems used for honey bees (OECD guideline 213) and for bumble bees (OECD guideline 247), which have both successfully been implemented, the protocols developed for solitary bees are currently inadequate and face numerous obstacles. For instance, the low and highly variable consumption success rates as well as the insufficient consumption of the tested substances reflects to major concerns. In order to improve both the feeding success as well as to ensure that bees consumed desired volumes within a short period

(e.g. up to 4h for acute dosing) of sucrose solution, we tested a novel cage design and feeding device, as well as subjected the bees to a training phase prior to the test to increase feeding performance.

Material and methods

Female and male *Osmia bicornis* cocoons were kept together in a flight cage [150 ♀ & 300 ♂) for three days at RT and indirect natural light, in order to enable hatching and mating. Bees in the flight cage were provided with sucrose solution (30 % w/v) *ad libitum* in a 5 mL disposal syringe. After three days, single females were then transferred into individual cages (round transparent plastic cages (bella plast 100 cm^3) and offered sucrose solution *ad libitum* in a pipet tip 250 µL (Rainin RT-L250WS wide orifice tips) and kept for 48 h. This was considered as "training phase", and thereafter the pipet tip was removed and the bees starved for 18 h. Only bees that have clearly consumed a certain amount of sucrose solution during the training phase (i.e., trained feeders) were used for the subsequent acute oral feeding test. Four treatment groups with each 16 to 17 test bees were established, in which each bee was offered 25 µl in a pipet tip of: 1.) sucrose solution (30 % w/v) (control group), 2.) 0.15 µg dimethoate/bee, 3.) 0.45 µg dimethoate/bee or 4.) 1.35 µg dimethoate/bee in sucrose solution (30 % w/v). The weight of the pipet tips was determined before and after a four-hour exposure phase to assess the ingested amount of feeding solution, hence to calculate the exact intake (dose) of Dimethoate per bee. Additionally, the feeding success was assessed visually after one, two, three and four hours. Calculations for the oral toxicity test were based on ingested doses and the oral LD_{50} and their 95% confidence limits for dimethoate was calculated by Probit analysis.

In parallel we compared the evaporation loss of the Nicot® cups (currently suggested feeding method by other laboratories) and the pipet tip feeding device used here in our experiment (Fig. 3). Therefore, twice ten of each feeder were filled with sucrose solution (30 % w/v) and kept under the same laboratory conditions as the caged test bees. To account for the evaporation, the weight of each feeder was assessd before and after 4 hours.

Results

Our results showed an equal feeding success rate (complete ingestion) of 75 to 88 % of the control group sucrose solution (30 % w/v) and the two lower dimethoate concentrations after 2 hours (Fig. 1). In the group with the highest dose of dimethoate (1.35 µg dimethoate/bee), only 25% of the bees fully consumed the 25 µl of spiked sucrose solution within two hour (Fig. 1). There was no change in feeding success after 2 hours of exposure compared to 3 hours in all treatment groups. Survival rate after 96h oral exposure phase was 88.2%, 75%, 6.2%, and 6.2% in the control group and the treatment groups with 0.15, 0.45, and 1.35 µg dimethoate/bee, respectively (Fig.2).

A statistically significant dose /response was found ($p<0.05$). Base on the calculation the LD_{50}-24h of *O. bicornis* was determined to be 0.140 µg dimethoate/bee (Table 1), which, is within the recommended LD_{50}-24h range given in the OECD 213 guideline for honey bees (oral LD_{50}-24h range 0.10-0.35 µg a.i./bee).

The evaporation observed in nicotine cups after 4h was significantly higher with a mean evaporation of 6.5 % compared to 1.9 % in the pipette tips tested (Fig. 3).

Oral Presentation

Table 1 *Calculation of LD_{50} dose of Dimethoate by Probit analysis in an acute oral feeding test for O. bicornis.*

ToxRatPro Probit-Analysis:		
LD_{50}	**Dimethoate (µg/bee)**	Lower/upper 95%-cl
24h	0.140	(0.001-0.501)
48h	**0.114**	(0.002-0.338)
72h	0.084	(0.002-0.229)
96h	0.084	(0.002-0.229)

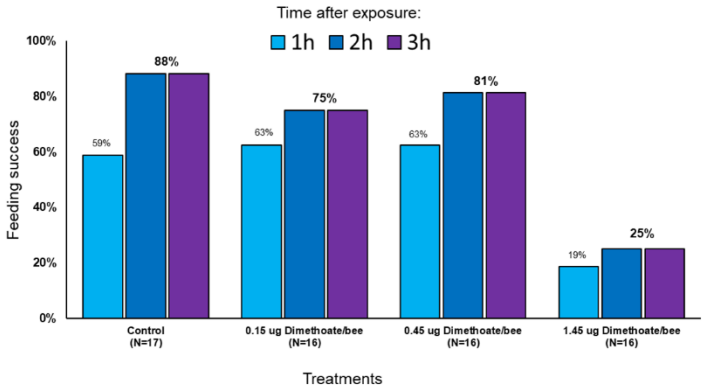

Figure 1 *Feeding success (complet consumption) in treatment groups after 1, 2 and 3 hours.*

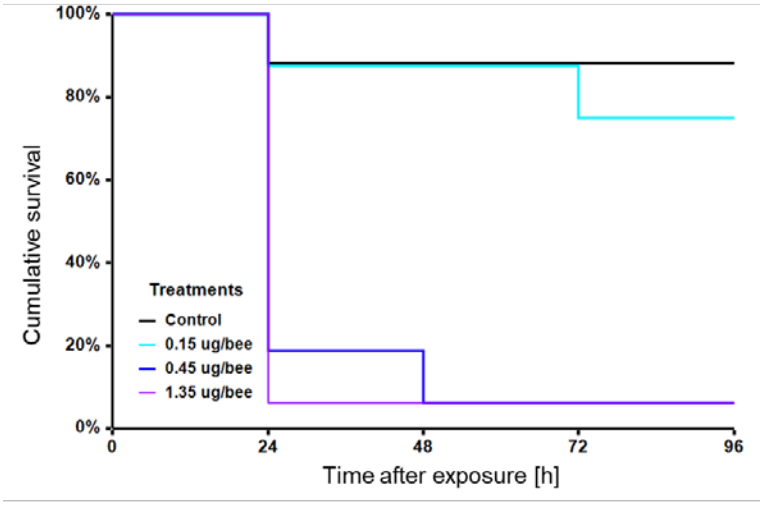

Figure 2 *Cumulative survival (%) 0 - 96 h*

Oral Presentation

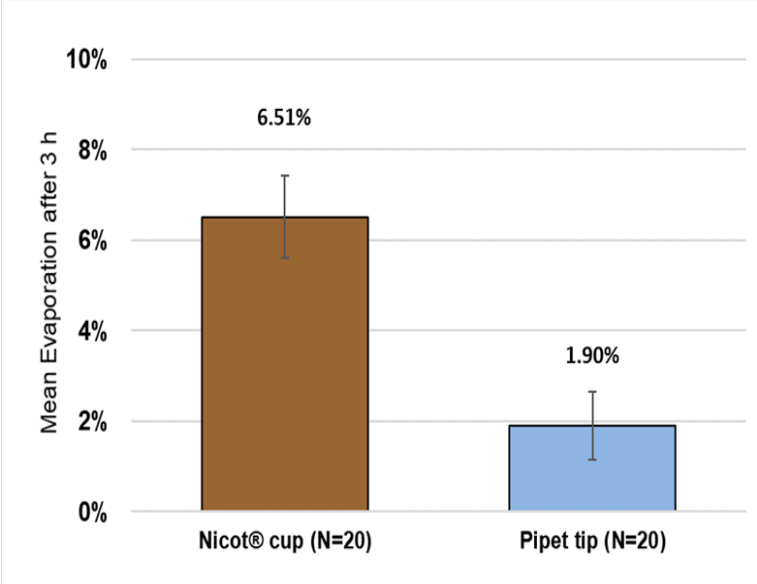

Figure 3 *Evaporation of feeding solution after 4 hours in two diffent feeders: Nicot cups and in pipet tips.*

Discussion

With regard to our preliminary pre-tests and final test, a "training phase" of the bees' feeding system (pipette tip) and selection of bees (successful feeders), as well as a starvation phase prior to the definitive test solution, seems to be the key to ensure high and consistent feeding success rates in our acute oral test. A similar pattern was observed in Knautz et al. (2022), where *O. bicornis* bees were also trained prior to their use in an acute oral test.

In contranst to Nicot cages, the use of transparent cages with larger volumes and pipette tips as a feeder greatly simplified handling and monitoring of the bees. Furthermore, the reduced evaporation of the surcosre solution, which can affect dosage, was several orders of magnitude lower, thus improving the accurarcy (Figure 4).

The preliminary data and results are highly promising, yet further assessments are required to verify our findings. Nevertheless, we would highly encourage and recommend for current and future oral acute toxicity ring tests unsing solitary bee species to consider and apply our new method.

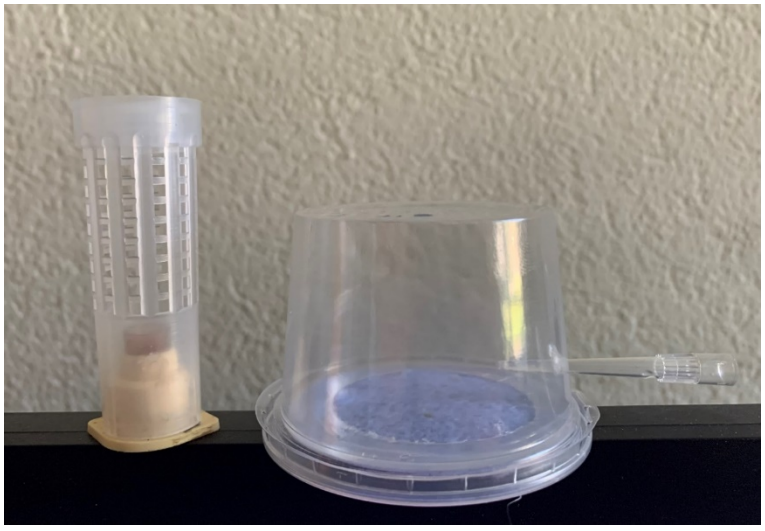

Figure 4 *Left: Assembled Nicot cage with feeder cup, right: clear bell plast cage with pipet tip feeder*

Oral Presentation

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