4.6 A method for a solitary bee (Osmia sp.) first tier acute contact and oral laboratory test: an update

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Abstract

The recently updated EFSA draft honeybee Guidance document also specifies other hymenopteran pollinators, like solitary bees and bumblebees, as groups to take into consideration when assessing the risk of plant protection products to pollinators. However, no validated test protocol and consequently no extensive data set is available to compare sensitivities of other relevant pollinators to those of honeybees. Within the current project of the ICPPR Non-Apis working group a start was made to develop a first-tier acute contact and oral test for *Osmia* spp. bees.

Based on the honeybee guideline OECD214 and Ladurner et al. (2005) a contact test was designed using dimethoate as test substance, *Osmia bicornis*, *Osmia cornuta* were housed in groups and feed either with a wick-action or open device or a flower petal attractant. First results indicate that reproducible results were obtained using the open and wick-action devices. In these tests, control mortality was never higher than 13 percent. Furthermore, sensitivities of *O. cornuta* and *O. bicornis* appeared to be rather similar with $LD_{50\text{-}96h}$ values ranging from 0.8-1.3 and 0.4-2.3 µg a.s./bee for *O. cornuta* and *O. bicornis*, respectively. Indicating that a validated and workable test guideline is within reach.

Based on the honeybee guideline OECD 213 and the newly developed guideline for bumblebee testing an acute oral test was designed using dimethoate and ring tested in 2017. The first results will be presented during the ICPPR meeting in Valencia.

4.7 Oral toxicity test with solitary bees: Experiences on the acute feeding test

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Introduction

The request for Bumble bee and Solitary bee species in toxicity testing has dramatically increased during the last years due to a growing awareness that results on honey bees may not be completely transferable to other pollinator species. This creates a need for further testing of non-Apis species to cover the risk of exposure of pollinators to plant protection products.

In principle, lower tier oral and contact toxicity tests are designed comparable to the established honey bee acute toxicity tests (OECD 213 & 214, EPPO 170, OCSPP 850.3020), but differ with respect to the biology of the test species (e.g. group vs. individual feeding, light conditions, mode of food presentation).

Oral toxicity tests with the solitary bee species *Osmia bicornis* are tricky, since simple feeding containers are not readily accepted by the bees and a reliable consumption can be very difficult. Therefore, we tested different factors that could influence the consumption of sugar solution.

Material & Methods

Female *Osmia bicornis* not older than 5 days were used for the test. The conditions during the test period were 22±2 °C, a relative humidity of 60±5 % and a 16 hours light/ 8 hours dark cycle. The test unit was a plastic box with a perforated lid for ventilation and the dimensions 18x13.5x12 cm.

As feeders, small plastic lids (Ø 13 mm) with bee attracting color (blue and/or yellow) were used. These lids were covered with a silicone septum (also blue or yellow) with a small hole (~2 mm) in the middle so bees would not sit in or bathe in the feeders. The base was broad enough for the bees not to be able to turn the feeder around or play with it. A very small cup, just big enough to hold 20-30 µL, was inserted into the hole to reduce evaporation and help the bees to find the sugar solution.

The test consisted of different pre-exposure treatments, an exposure and a post-exposure phase. All bees were hatched from cocoons at room temperature, collected twice a day and exposed to the following treatments:

Hatching with no provided food and being placed straight in the fridge until test start.

Hatching with no provided food, then a starvation phase under test conditions for 24 hours, just in a larger container in groups of up to 15 bees. Bees were then placed in the fridge until test start.

Same as 2, but one feeder was provided per 5 bees. Group feeders did not have the small inserted cup but were completely filled with sugar solution (approximately 200 µL).

Bees were stored in the fridge until enough bees had hatched. Then, mating took place in a large flight cage for 24 hours with 1.5 males per female bee and no food provided. Test start directly after mating was finished.

First treatment 3 and then treatement 4.

During the exposure phase, bees were weighed and one female solitary bee was inserted per test unit and left to feed on 20 µL of untreated aqueous sugar solution for 3 hours. Actual consumption was measured by weighing the feeder before and after. During the post-exposure phase, bees were fed *ad libitum* and mortality was assessed at 4, 24 and 48 hours.

Results & Discussion

The amount that each bee consumed was calculated by weighing the feeder before and after exposure and is shown in Figure 1. Average evaporation, measured in separate test vessels without bees, was 4.1 µL, and is subtracted from the consumption rates. Bees were divided into the categories "feeder" and "non-feeder", with feeders being all bees that consumed more than 80% of the average consumption in the group. Mortality rates are shown in Table 1.

Table 1 Mortality and number of "feeders".

Treatment	Number of „feeders"	Number of "non-feeders"	Mortality (All)	Mortality (Feeders)
1- Nothing	8	22	6.7 %	0.0 %
2- Starving	6	24	3.3 %	0.0 %
3- Feeding	15	15	0.0 %	0.0 %
4- Mating	9	21	13 %	22 %
5- Feeding and mating	23	7	6.7 %	0.0 %

The highest number of feeder bees and also the largest consumption rates were seen in those treatments were bees had been offered food beforehand in a group setting. It can be hypothesized that bees learn to feed from each other in this group setting and thus reach higher consumption rates.

Mating itself seemed to have no influence on the feeding behaviour but increased mortality rates, probably as it is a very stressful set-up. The highest amount of feeders/ consumption

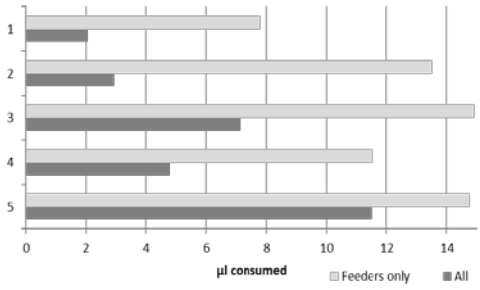

Figure 1 Consumption in each group

was reached in the treatment were group feeding took place and then mating. It is very likely that the consumption did not increase due to the mating process but due to the additional starvation phase after the bees had learned how to feed, as mating itself does not seem to have an effect.

Conclusions

Not only the type of food or feeder offered to Osmia can make a difference in the consumption rates, but the way the bee is treated before the test can have a large influence. This data shows that bees being exposed to a certain type of feeder in a group setting before the experiment will have better consumption rates when that same feeder is used during the experiment.

References

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4.8 Field exposure study: handling three different pollinator species and several matrices of residue analysis

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Introduction

The here presented study was set up to determine residues and ecotoxicologically relevant concentrations (ERCs) of a plant protection product in rapeseed (*Brassica napus*) inflorescences and their respective pollinator food matrices followed by single application after daily bee flight activity. Application was conducted under field conditions and in terms of good agricultural practice on five different trials in Northern-western Switzerland. The maximum mean concentration of residues over time was determined in different matrices collected by honey bee colonies (*Apis mellifera* L. (Hymenoptera: Apidae)), bumble bee colonies (*Bombus terrestris* (Hymenoptera: Apidae)) and solitary bee nesting cavities (*Osmia bicornis* (Hymenoptera: Megachilidae)). Sampling was conducted in a setup that the way of exposure / possible pesticide entry from field to hive could be demonstrated. The presented results and mode of action may be a significant addition and useful approach for creating further input and detailed data needed for the risk assessment on pollinators and their actual, realistic exposure to plant protection products based on the recent EFSA guidance document on the risk assessment of plant protection products for pollinator species (revised version July 2014).

Material & Methods

Content of active ingredient (analysed): 288 g active compound /L

Test species Honey bee (*Apis mellifera carnica*; ecotype: sklenar), 5 to 7 healthy honey bee colonies per field with one hive body including 14 Swiss format frames and containing between 2,350 to 12,300 bees, 4 to 8 frames with brood of all stages and at least 4 frames with stores (honey and pollen).

Bumble bee (*Bombus terrestris*) 8 healthy bumble bee colonies per field with one hive body containing between 48 to 124 bumble bees (manually counted in the lab before the transfer into the field) and a brood nest containing all developmental stages (i.e., eggs, larvae and pupae).

Solitary bee (*Osmia bicornis*) cocoons (in total 40 to 70 female and 40 to 72 male cocoons) were placed in every field at two/three different timepoints.