First experiences and first steps towards a chronic bumble bee (*Bombus terrestris*) test design

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Introduction

With the European Food Safety Authority (EFSA) Guidance Document on the risk assessment of plant protection products on bees (2013), a number of changes and impacts on assessing the potential risk for pollinators has moved into focus. The approach presented here is intended to help fulfilling the multiple amount of new requests within the document stated above. The main goal of the presented data is to develop chronic bumble bee testing towards a robust, feasible and reliable test design. Based on the presented data, the setup of an international ring test phase may be accommodated.

Methodology

Picture 1: Test unit with one bumble bee

Material and test conditions:

- Test unit: plastic box (≈ 496 cm^3), absorbing paper, transparent lid with holes, one bumble bee per test unit
- Feeders: 2 mL syringes filled with 1 mL of treated sugar solution
- Test conditions: 25 ± 2°C, 60 ± 10% humidity, constant darkness (artificial light during assessments)

How to proceed:

- Average size bumble bee workers (weight 150 - 350 mg)
- 8 to 24 h acclimatization
- Exposure to treated sugar solution for 10 days
- Feeders renewed every 24 ± 2 h, weighed before and after feeding period

Reference test:

- Treatments: control, 10 % acetone control, 5 reference doses (Dimethoate)
- Replicates: 30/ treatment + 5 evaporation control replicates (test unit with feeder but no bumble bee)
- Target volume: 250 µL / bumble bee / day
- Doses: Target → 0.050 – 0.10 – 0.20 – 0.40 – 0.80 µg a.i./bumble bee/day
  Final → 0.055 – 0.12 – 0.23 – 0.40 – 0.73 µg a.i./bumble bee/day

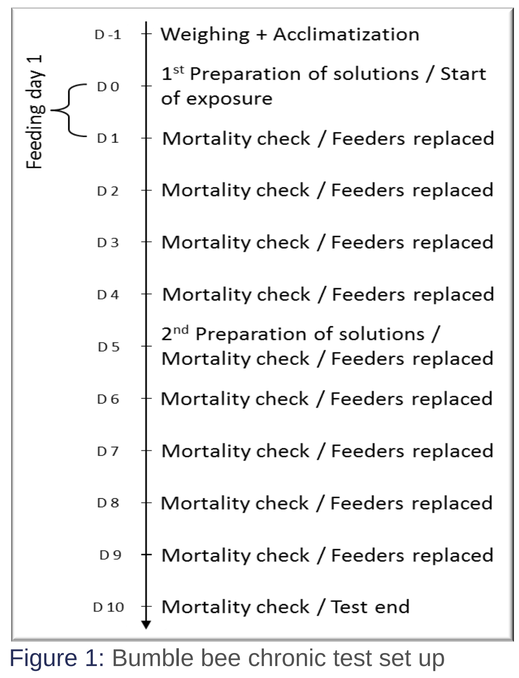

Figure 1: Bumble bee chronic test set up

Results & Discussion

Evaporation

The evaporation of feeding solution was considered in this experiment. The mean daily evaporated volume represents 45 ± 6 µL/day while the mean daily food consumption amounts to 293 µL/day resulting in 15.4 % loss due to evaporation. This suggests that the evaporation should be taken into consideration in the bumble bee chronic test design. As a result, the food consumption was corrected for evaporation and the results analyzed with corrected values.

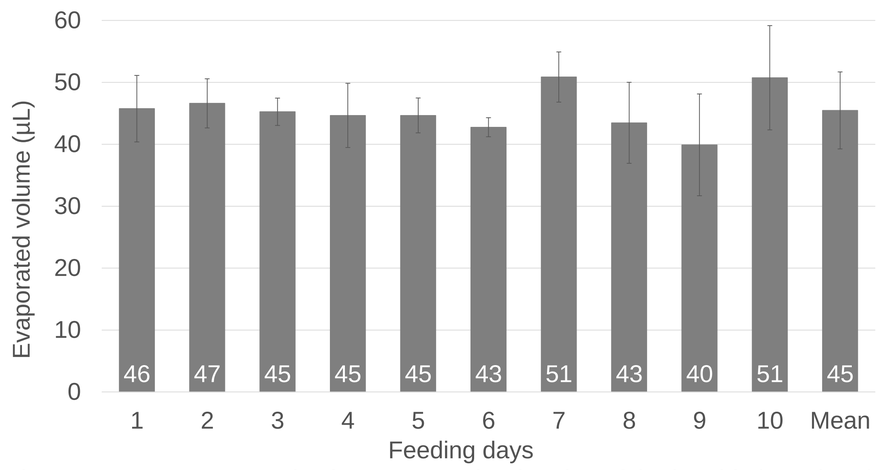

Figure 2: Mean evaporated volume across the duration of the bumble bee chronic test

Mortality and sub-lethal effects

In this test, the untreated control, the 10 % acetone control and the 2 lowest test item treatments (0.055 and 0.12 µg Dimethoate/bumble bee/day) showed no sub-lethal effects while the 3 highest test item doses (0.23, 0.40 and 0.73 µg Dimethoate/bumble bee/day) presented sub-lethal effects from day 5 to 10, 2 to 6, and 1 to 3, respectively. The bumble bees presenting sub-lethal effects looked affected (bumble bees are still upright and attempting to walk but displaying signs of reduced coordination).

In this test, the untreated control as well as the 10 % acetone control showed no mortality while the test item (Dimethoate) shows a clear dose response at the end of the 10 days of exposure.

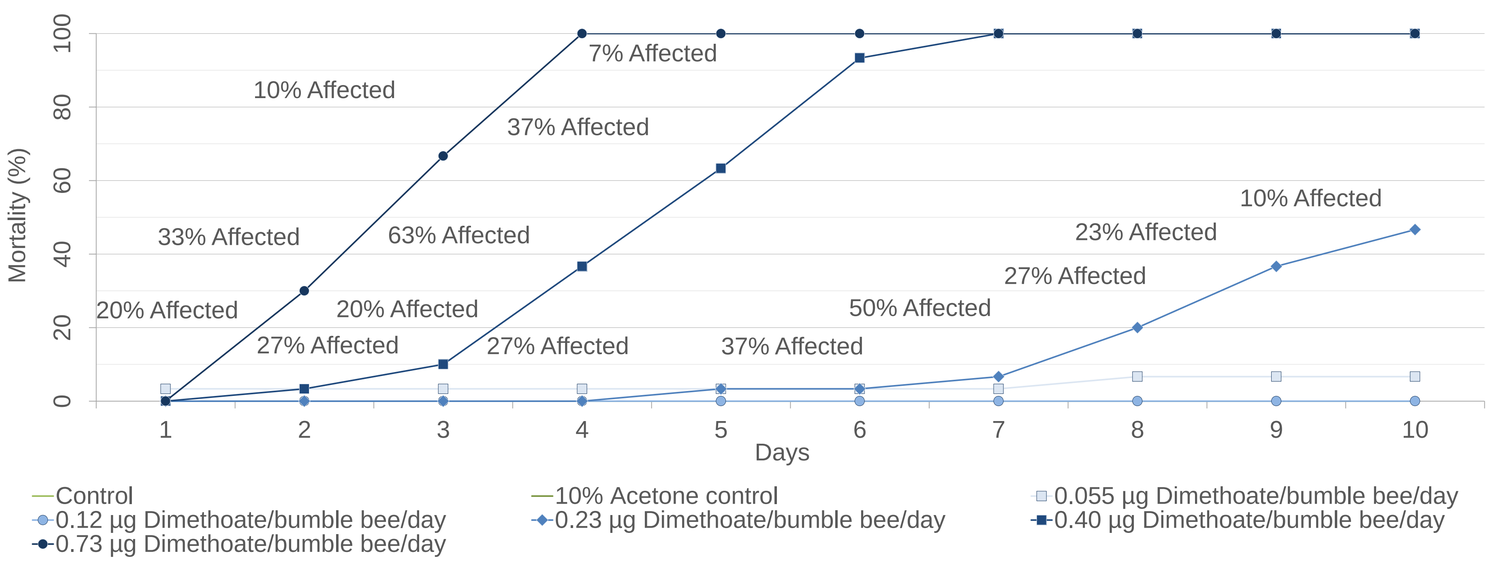

Figure 3: Mortality and sub-lethal effects after chronic oral exposure to different Dimethoate doses
Affected: bumble bees are still upright and attempting to walk but displaying signs of reduced coordination

Food consumption

Food consumption in the controls appears to be higher on feeding day 1 to 3 and then to stabilize around a food consumption of 210 µL/day. This decrease in food consumption may be caused by a lesser need for food since the bumble bees are separated from their colony and may show reduced activity in the test units.

The food consumption in the test item treatments appears to be higher than in the controls from the beginning of the exposure until the food consumption decreases due to sub-lethal effects and mortality. The dose-dependent decrease in food consumption could be further investigated in order to determine whether and at which time during the exposure the substance has an attractive or repellent effect toward bumble bees.

The results also suggest a possible link between the time when the food consumption starts decreasing and the observation of the first sub lethal effects. Indeed, the food consumption in the treatment group with a dose of 0.23 µg a.i./bumble bee/day decreases for 3 days before the display of sub-lethal effects starts on day 5.

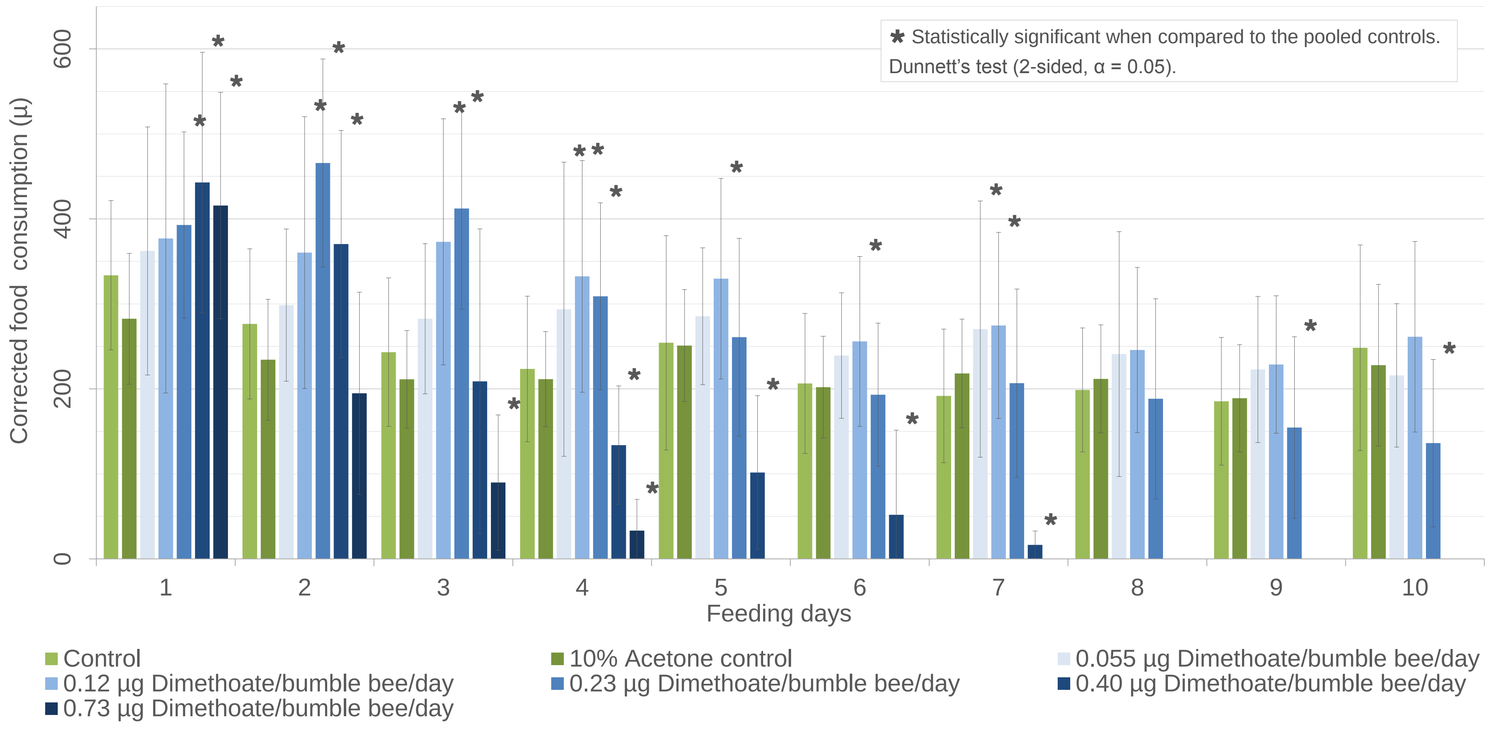

Figure 4: Mean daily food consumption

Conclusion

- Need to take into account evaporation
- Endpoints to consider for the test design:
  - ➡ Sub-lethal effects
  - ➡ Mortality
  - ➡ Food consumption: Determination of
    - ⇨ The real dose ingested
    - ⇨ The impact on feeding rate (attractive / repellent substance)
    - ⇨ A possible link between feeding rate & sub lethal effects

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