Bumble bee acute testing: Why not testing more than one endpoint?

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Introduction

In recent years it became more and more important to add additional pollinator species to the testing scheme of plant protection products. Therefore, ring test groups were established and developed draft guidelines regarding bumble bee oral and contact acute testing. The bumble bee draft guidelines take into consideration the honey bee testing guidelines OECD 213 and 214.

The principle of this method is either to expose adult worker bumble bees to aqueous sugar solution containing the test substance for 2 to 4 hours (oral testing) or to apply drops of an application solution of the test item to the dorsal part of thorax (contact testing). After application of the test substance, the test organisms are provided *ad libitum* with 50 % aqueous sugar solution and observed for the following assessments 24 and 48 hours after application which can be extended to 72 and 96 hours. The endpoint considered is mortality, expressed as LD_{50} and NOED. In addition to the assessment of mortality, the test organisms are checked qualitatively for sub-lethal symptoms of toxicity (affected and moribund condition).

In this presentation, the consumption of food is introduced as an additional sub-lethal endpoint. Measurements of ingested sugar solution 24, 48, 72 and 96 hours after application are expected to give information on possible impacts of test item on feeding behavior.

Methodology

Picture 1: Bumble bee test units

Material and test conditions:

- Test unit: Nicot cages (≈ 16 cm^3)
- Feeders: 2 mL syringes filled with non-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 (150 - 350 mg) with 8 to 24 h acclimatization
- Exposure to treated sugar solution for 2 to 4 h
- Afterwards feeders with non-treated 50 % sugar solution weighed every 24 ± 2 h until 96 h
- Treatments: control (C), solvent or surfactant control (SC), 5 reference doses (Dimethoate) with 30 replicates per treatment
- Doses: expressed as µg Dimethoate / bumble bee

	ORAL (µg Dimethoate / bumble bee)							CONTACT (µg Dimethoate / bumble bee)						
Target doses	C	SC	0.25	0.50	1.0	2.0	4.0	C	SC	1.25	2.5	5.0	10	20
Final doses	-	-	0.26	0.51	1.0	2.0	3.9	-	-	1.22	2.5	5.0	10	20
Mortality (%)	3.3	0.0	0.0	3.3	30	93	93	0.0	0.0	0.0	0.0	0.0	53	97

Results & Discussion

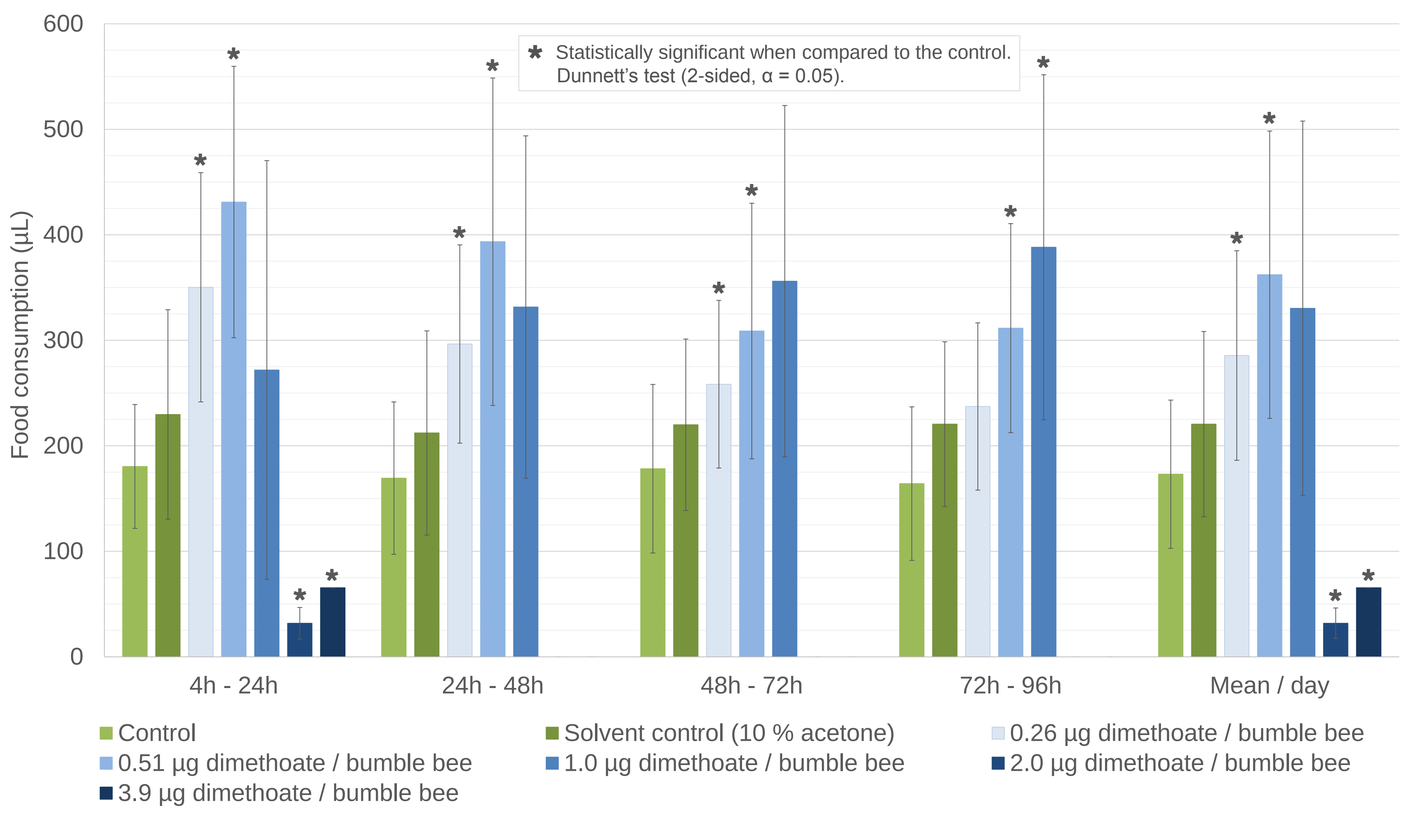

Figure 1: Oral acute test - Consumption of non-treated sugar solution from 4 h to 96 h.
Only the non-treated food consumption of "feeder" bumble bees was taken into account. The individuals are considered as "feeder" when they ate more than 80 % of the mean treated sugar solutions.

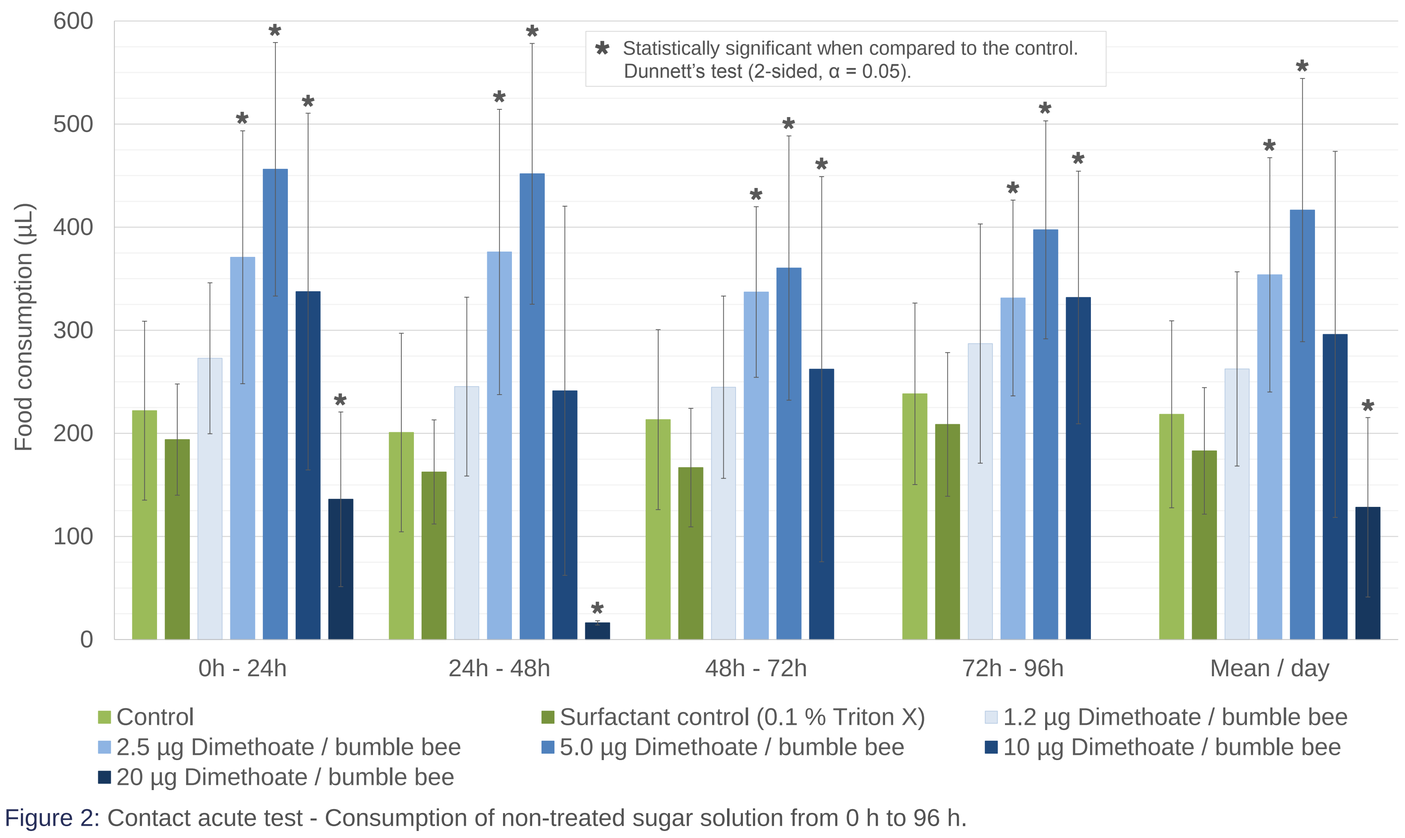

Figure 2: Contact acute test - Consumption of non-treated sugar solution from 0 h to 96 h.

Consumption of non-treated food

- Consumption of non-treated food in the control groups remains stable during the entire post exposure period in the oral test (Figure 1) and the contact test (Figure 2).
- After 24 hours, the consumption of non-treated food in the test item treatments shows a consistent pattern of increase in the three lower test doses of the oral test (Figure 1) and four lower test doses in the contact test (Figure 2). At the later assessment dates the consumption rates in the test item treatments differ.
- In the oral test (Figure 1), the initially increased consumption rates remain increased during the post exposure period. However, a continuous decrease with time was observed at 0.25 and 0.51 µg a.i. / bumble bee, whereas a continuous increase with time is determined at 1.0 µg a.i. / bumble bee (where already significant mortality takes place).
- In the contact test (Figure 1), the initially increased consumption rates remain more or less on the same levels during the post exposure period. However, a small increase between 72 and 96 hours was observed at 1.2, 2.5, 5.0 and 10.0 µg a.i. / bumble bee. Mortality first takes place at 10.0 µg a.i. / bumble bee.
- Our hypothesis for explaining the observed pattern of consumption of non-treated sugar solution during the post exposure period is as follows:

1. The active ingredient as used in this study is supposed to have sub-lethal and lethal effects dependent on dose.

2. The test organisms possess detoxification mechanisms which cost energy:

   - At very low doses, no detoxification is needed ➔ no increase in food consumption.
   - At low doses, energy demanding detoxification is used ➔ increase in food consumption which decreases with time.
   - At higher doses, energy demanding detoxification increases ➔ increase in food consumption which does not decrease with time, first mortality occurs.

Conclusion

Currently, only mortality and sub-lethal effects on activity are considered as endpoints in the bumble bee acute oral and contact toxicity tests. Additionally, the non-treated food consumption following an acute exposure to a test item is worth to be investigated further. Especially the increase in non-treated food consumption after application is suggested to be caused by sub-lethal effects of the test item.

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