Oral Toxicity Test with Solitary Bees: Long-term feeding behavior

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Introduction and Methods

As the current version of the ICPPR working document on the Oral Acute Toxicity Test with Solitary Bees (ICPPR, 2018) is being prepared to become an OECD guideline, there are still practical issues with the test, most notably that all labs experience a high number of bees which do not feed on the provided solution in the given time.

Three small-scale experiments were performed to determine, firstly, if consumption becomes more robust over longer time and secondly, if bees possibly have a problem with the sugar solution and if providing different kinds of foods would increase consumption.

Test 1:

- 60 bees were provided 50 % (w/v) sugar solution over 10 days
- **Background**: Considering if a chronic test with solitary bees would be possible, but also assessing if prolonging exposure in the acute test or performing a pre-exposure phase in the acute test would lead to a better consumption by the bees

Test 2:

- 20 bees each were provided a solution with 20 %, 30 %, 40 % or 50 % (w/v) sugar
- Exposure for 72 hours
- **Background:** Sugar uptake rates in *Bombus impatiens* are highest at 27 % and decrease at higher or lower sugar concentrations (Nardone et. al, 2013)

Test 3:

- 50 bees were provided only 50 % (w/v) sugar solution
- 20 bees were provided 50 % (w/v) sugar solution and additionally a feeder with fresh pollen
- 20 bees were provided a feeder with a paste mixed of half sugar solution and half pollen
- Exposure for 72 hours
- **Background**: Adult females of *Osmia californica* require dietary pollen to reproduce, and pollen feeding paces the onset of solitary bees' reproductive output (Cane et. al, 2016). It was hypothesized that this could possibly also increase feeding behavior

Material and test conditions:

- One bee and one feeding device (Blue cup with a fill volume of ~200 µL) per cage
- After hatching, bees were collected in the fridge and then directly placed into the test units
- Feeding cups were weighed to assess consumption
- Mortality and consumption were assessed after 4 and 24 hours and thereafter every 24 hours
- Consumption values were corrected for evaporation
- A bee was considered to be a "feeder" if consumption was >12 µL in the first 3-4 hours and >25 µL in one 24 hour period (After subtracting the evaporation)

Results & Discussion

Different sugar concentrations

Consumption of the different sugar concentrations did not differ significantly up to 48 hours (ANOVA, $p>0.05$, Figure 1). At 72 hours, consumption at the lower sugar concentrations was significantly increased (LSD test, $p<0.05$). This is probably due to the bees having to take up more of the lower concentrated solution to get the same amount of energy.
Conclusion: Lower sugar concentrations do not increase sugar solution consumption in an acute (48 hour) test. However, mortality was not higher in the group of bees feeding on lower sugar concentrations, meaning lower sugar concentrations could be used, for example when testing substances which have a very low solubility.
Another approach could be to test different combinations of glucose, fructose and sucrose to see if that will impact consumption.

Additional pollen feeding

The amount of consumed sugar solution did not differ significantly between bees fed only sugar and the ones additionally fed pollen or the pollen/sugar mix up to 48 hours (ANOVA, $p>0.05$, Figure 1). There was an overall trend that the bees feeding only on sugar consumed more of the sugar solution and the difference was significant at 72 hours.
Conclusion: The pollen covers a part of the nutritional need of the bees. Additional pollen feeding does not help increase sugar consumption.

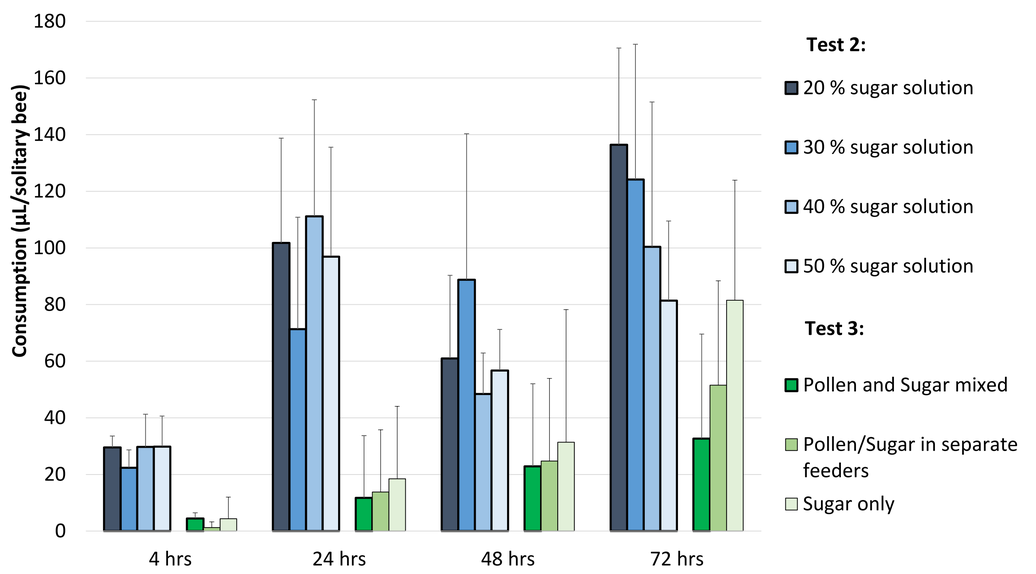

Figure 1: Results of test 2 and 3. Consumption rates are shown.

Mortality

The bees seem to be extremely robust to starvation, some bees did not start feeding until 24 or even 48 hours. However, assessment of feeding rates was complicated due to highly variable evaporation. This is also the reason why some non-feeders survived very prolonged periods (Figure 2).

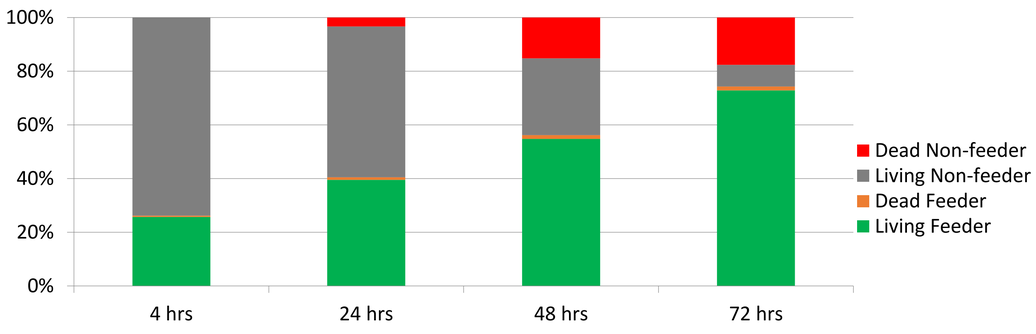

Figure 2: Mortality of feeders and non-feeders (210 bees from all 3 trails)

Long term feeding behavior

Only about a quarter of all bees (in all 3 trials) had consumed sugar solution over the first 3-4 hours. In the first three days most bees either start consuming sugar solution or die (probably of starvation, see Figure 2). Once the bees have started to consume the sugar solution, they mostly kept feeding on a daily basis (See Figure 3 and 4).
Conclusion: A pre-exposure phase makes sense as the number of feeders can be increased and the number of spare bees needed for an acute test is reduced. This trial also shows that a chronic approach is feasible.

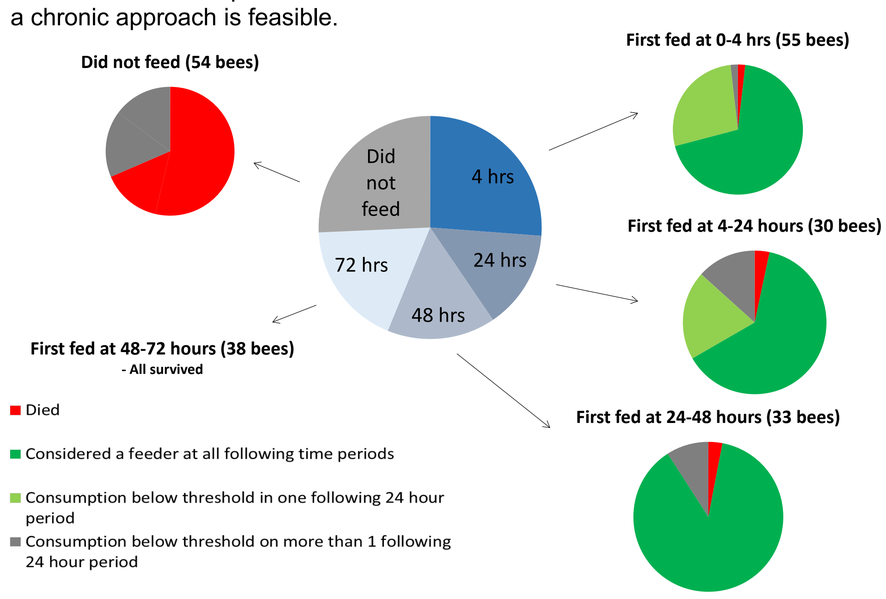

Figure 3: Time point when bees first fed an amount above the threshold (>25 µL/24 hours or 12 µL/4 hours). Results for the first 72 hours of all 3 trails are combined, 210 bees were assessed.

Average Consumption and Evaporation

Evaporation is very high and variable (average 23.5 µL/day, ranging from 12.5 to 40.5 µL/day). This leads to difficulties in determining the actual consumption. A consumption of about 2x the average evaporation was chosen as the threshold to determine whether a bee is a "feeder", which leads to a lot of bees that fed only small amounts being considered "non-feeders". This is especially visible in the "did not eat on one day "bees (Figure 3). Some bees that were feeders after 4 hours would not have been feeders after 24 hours.
Conclusion: The average amount of sugar solution that bees consume in one day (corrected for evaporation) is approximately 70 µL (Figure 4).

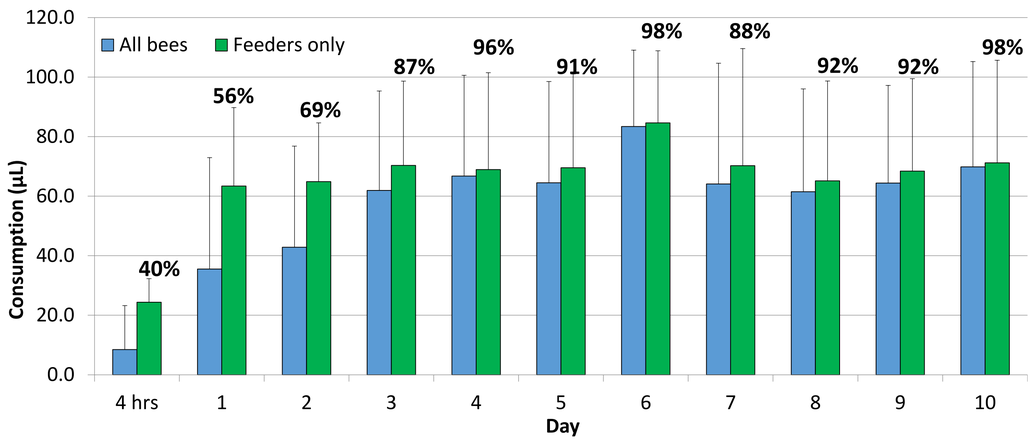

Figure 4: Results of test 1. Consumption rates are shown. Feeders are all bees that consume above the threshold (>25 µL after evaporation) on the specific day. % numbers show the % of surviving bees that were feeders.

Conclusions

- Overall, once bees start to feed they will continue to feed over the following days. This is an important first step if a chronic test should be considered.
- Even in the acute test, if there would be a pre-exposure phase in which the bees can "learn" to feed, there would be a higher number of feeders and the overall number of bees required for one test would be smaller.
- Consumption is variable and high evaporation is still a big problem, as it is difficult to see whether the missing volume is due to evaporation or consumption of the bees. Overall, bees seem to consume an average amount of about 70 µL/day.
- There are bees that starve to death rather than feed on the provided food. This does not make the test robust, and the reason why some bees do not feed needs to be investigated further.
- Feeding additional pollen or different sugar concentrations does not increase consumption. Lower sugar concentrations were taken up just as well and did not increase mortality, so they could be used for test items with a low solubility.

Literature

- ICPPR workgroup non-Apis bees. Solitary bee Acute Toxicity Test, version from 2016 (Contact) and 2018 (Oral).
- Nardone, Dey and Kevan 2013. The effect of sugar solution type, sugar concentration and viscosity on the imbibition and energy intake rate of bumblebees. Journal of Insect Physiology 59 (2013) 919–933
- Cane, J. H., 2016. Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. Journal of Insect Physiology 95 (2016) 105–109

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