

# Non-uniform distribution of treated sucrose solution via trophallaxis by honeybees affects homing success variability and mortality

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## Introduction

Based on our observations and a recently published article<sup>1</sup> food sharing in a group via trophallaxis might lead to a non-uniform distribution of pesticide spiked sucrose solution between caged honeybees. This can cause high variability in the **homing success rate** or **mortality** among group members and treatment replicates. In order to improve the oral food distribution of tested sucrose solution we compared two feeding schemes with **two** or **ten** bees per cage (20 µL/bee) and evaluated the impact on homing success rate and mortality.

## Method

**RFID Homing flight ring-test:** According to the homing flight ring-test protocol, bees were exposed orally to different sub-lethal concentrations of thiamethoxam (0, 0.11, 0.33 or 1 ng/bee). For each treatment scheme (two and ten bees/cage) three runs were conducted between June and July 2017 in Liebefeld, Switzerland (fig.1;2). In all groups homing flight success was assessed after 24h.

**Acute Toxicity Test:** According to the TG OECD 213, bees were exposed orally to different concentrations of dimethoate (0, 0.033, 0.07, 0.1, 0.13, and 0.35 µg/bee). As above, oral treatment scheme was performed three times for both groups (two and ten bees/cage). Mortality was always assessed after 24h (fig. 3;4).



Fig. 1: group feeding with 2 bees (tagged with RFID chip) per cage



Fig. 2: group feeding with 10 bees (tagged with RFID chip) per cage



Fig. 3: group feeding with 2 bees per cage (OECD 213)



Fig. 4: group feeding with 10 bees per cage (OECD 213)

## Results

**RFID Homing flight** success rate, at 1 ng thiamethoxam per bee, was significantly lower in the group of ten bees compared to the two bees approach, as well as the control (fig. 5). Obviously, a large variability was found in the ten bees feeding group. For the other doses similar trends were obtained. **Acute toxicity data** with dimethoate showed that group feeding scheme with ten bees per cage resulted in higher mortality values when compared to the two bees feeding scheme (at same dosing levels). As a consequence the LD<sub>50</sub> value is higher for the latter (fig. 6).

RFID: Homing success per treatment and feeding scheme

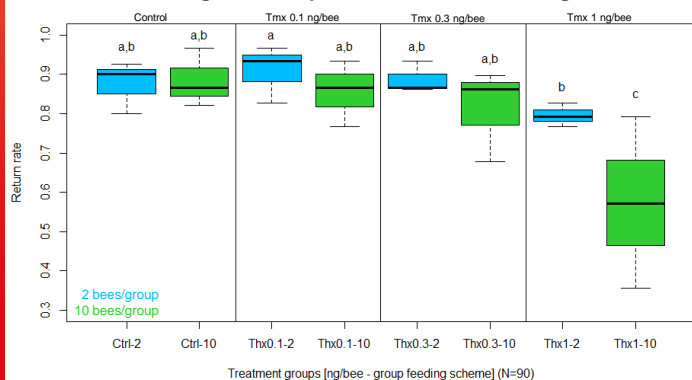


Fig. 5: Boxplot: Homing flight success per treatment and feeding scheme. Literals differentiate statistically significant (p<0.05) groups, validated by Chi-Square-Tests.

OECD 213: 24h mortality per group feeding scheme

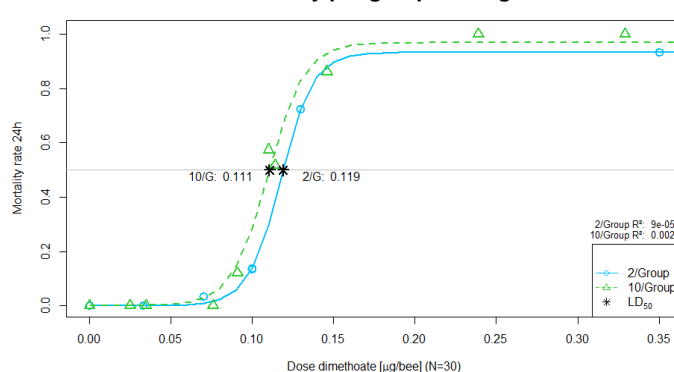


Fig. 6: LD<sub>50</sub> dose-response model for dimethoate with two, resp. 10 group feeding schemes. 2 group feeding showed a more accurate and closer LD<sub>50</sub> value compared to the reported LD<sub>50</sub> value of 0.1257µg/bee by Baskar et al.<sup>2</sup>

## Conclusion

High variability of homing success or mortality rate observed with the ten bee feeding scheme is most likely caused by inhomogeneous dose distribution among bees, or either by over- or underdosing of single bees within replicates. In contrast, food intake with the two bees feeding scheme is generally faster and more homogenous as the chance to feed directly on the offered sugar solution is increased. Hence, a more accurate and uniform dosing distribution can be expected resulting in less variable data between runs, replicates and treatments. We highlight that feeding (treatment of interest) in smaller groups of honeybees should be discussed and considered to **minimize the trophallaxis dependency** regarding food distribution in group dosed honeybees. Moreover, to compare endpoints of toxicological studies with single dosed wild bees for regulatory purposes.

**REFERENCE** <sup>1</sup>Brodtschneider R, Libor A, Kupelwieser V, Crailsheim K, PloS ONE 12(3) (2017), <sup>2</sup>Baskar K, Sudha V, Tamilselvan C ScholeReps 1(1) (2016)

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