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1.22 Weight differences of honey bees after administration of sublethal doses of dimethoate

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Abstract

Background: The aim of this work was to assess honey bee body weight as a possible further parameter to detect effects in a 10 day chronic feeding study according to OECD 245[1] following exposure to sublethal concentrations of a plant protection product (*i.e.* dimethoate). This investigation is based on the assumption that weight differences might be caused by chronic feeding of dimethoate. Two set of tests in two different laboratories (Lab 1 and Lab 2) were conducted in order to investigate possible weight changes of complete adult honey bees and/or parts of their body (honey stomach and intestine) following treatment of dimethoate. Bees were weighed before and after chronic feeding of sub-lethal concentrations of dimethoate.

Results: Differences in the number of bees which lost weight following treatment of sublethal concentrations of dimethoate was found in Lab 1, but could not confirmed in Lab 2.

The difference in weight between the control group and the dimethoate treatment could only be detected as a statistical significant difference in one lab at the highest concentration (0.4 mg/kg).

Assessment of weight changes of parts of the bee body (honey stomach and intestine) shows a very high variation (CV) which makes interpretation of the data of the total body weight questionable.

Conclusion: The results of the two laboratories were contradictory and no conclusive assessment can be done following the two sets of experiments. Assessment of bee body weight within a 10-day chronic feeding study is considered questionable for the detection of sublethal effects. Further work with other active ingredients is needed to clarify if body weight change of honey bees can be used as a parameter for sublethal effects.

Keywords: chronic toxicity, sublethal effects, weight differences, honey bees

Introduction

Testing of chronic effects of Plant Protection Products (PPP) on adult honey bees by continuous feeding of contaminated sugar solution over a period of 10 days is an integral part of the current risk assessment for honey bees.

According to the OECD Guideline 245 mortality and food consumption have to be assessed in order to detect possible side-effects of PPP to honey bees. Additionally, sublethal parameters like behavioural abnormalities should be quantitatively recorded.

The tests were performed in two independent contract laboratories providing bees of two different breeding lines of *Apis mellifera carnica*. In each laboratory an experiment was conducted in order to investigate possible weight changes of complete adult honey bees following treatment of dimethoate. In the experiments bees were colour coded and thereafter weighed before and after chronic feeding of sub-lethal concentrations of dimethoate. Bees were either weighed in groups of 5 or individually. Additionally one lab dissected the bees after test end and weighed parts of the honey bee body (honey stomach and intestine). This was done in order to show the relation between the complete honey bee body weight and body parts. It was assumed that due a differing filling level of these entrails the body weight could be influenced by a certain degree.

Experimental Methods

The study followed OECD TG 245 and was performed with young adult worker bees (*Apis mellifera*) (1 to 2 days old) which were kept in the laboratory under controlled test conditions (dark, 33°C, 60 ± 10% rel. humidity). The bees were fed *ad libitum* with pure 50 % (w/v) aqueous sucrose solution either untreated or containing the insecticide dimethoate at concentration levels of 0.1, 0.2 and 0.4 mg dimethoate/kg feeding solution over a period of 10 days. Per treatment group 5 replicates (cages) each containing 10 bees were used. About one hour before test start all bees were cooled at 6±2°C in order to immobilise them before weighing. Half of the bees (5 per cage) were individually colour-marked in order to compare their weight at start and end of the test period (10 days). Thereafter, all bees were weighed before start of exposure. After 10 days all surviving bees were shock frozen by using dry ice and weighed again in order to calculate their possible weight difference.

During the test period daily assessments on mortality and food consumption were conducted. The following parameters were assessed and statistically evaluated:

a) number of bees with weight losses: number of treated bees which had lost weight at test end (< 0 mg) compared to the control group (Fisher Exact Test, α=0.05, one sided-greater). See Table. 1

b) weight differences: comparison of the extent of weight differences in the dimethoate treated groups compared to the control group (Step-down Jonkheere-Terpstra Test Procedure, α=0.05, one sided-smaller). See Figure 1 and 2.

c) weight of honey stomach/intestine: determination of the weight of the honey stomach and intestine of the bees after dissection and put it into relation to the total body weight at test end. See Table 2.

For a) and b) only coloured and surviving bees were used; c) was conducted in one lab only.

Results

3.1 Number of bees with weight losses

One lab (Lab 1) showed a statistical significant difference in the number of bees which had a negative weight balance at test end at 0.2 and 0.4 mg/kg dimethoate (Fisher Exact Test, α=0.05, one sided-greater).

The data of the other lab (Lab 2) did not show any statistical significant difference in the loss of weight of the bees at test end in any of the dimethoate treatments (i.e. 0.1, 0.2 and 0.4 mg dimethoate/kg) (Fisher Exact Test, α=0.05, one sided-greater). See Table 1.

Table 2 Weight differences of adult bees at test end

	Lab 1				Lab 2			
		bees with weight diff. [3]				bees with weight diff. [3]		
Treatment [1] (dimethoate)	# of surviving bees [2]	< 0 mg	> 0 mg	[%] [4]	# of surviving bees [2]	< 0 mg	> 0 mg	[%] [4]
control	21	**8**	13	**38**	23	**12**	11	**52**
0.1 mg/kg	25	**15 (n.s.)**	10	**60**	24	**16 (n.s.)**	8	**67**
0.2 mg/kg	24	**20 (*)**	4	**83**	24	**15 (n.s.)**	9	**63**
0.4 mg/kg	13	**11 (*)**	2	**85**	23	**11 (n.s.)**	12	**48**

25 bees were initially marked and used for the assessment

[1] mg/kg = mg dimethoate/kg feeding solution

[2] number of bees which survived at test end

[3] weight difference at test end; < 0 mg = weight loss; > 0 mg = weight increase

[4] percentage of bee which showed a loss of weight at test end

* = statistical significant different compared to the control; n.s. = not statistical significant different to the control

3.2 Weight differences

The weight differences of the honey bees at test start and at test end were considerable. A comparison of the weight differences between the dimethoate treated bees to the control bees showed a statistically significant difference at 0.4 mg dimethoate/kg feeding solution in Lab 1. All other weight differences of the dimethoate treatments in both labs were not statistically significant different (Step-down Jonkheere-Terpstra Test Procedure, α=0.05, one sided-smaller). See Figure 1 and 2.

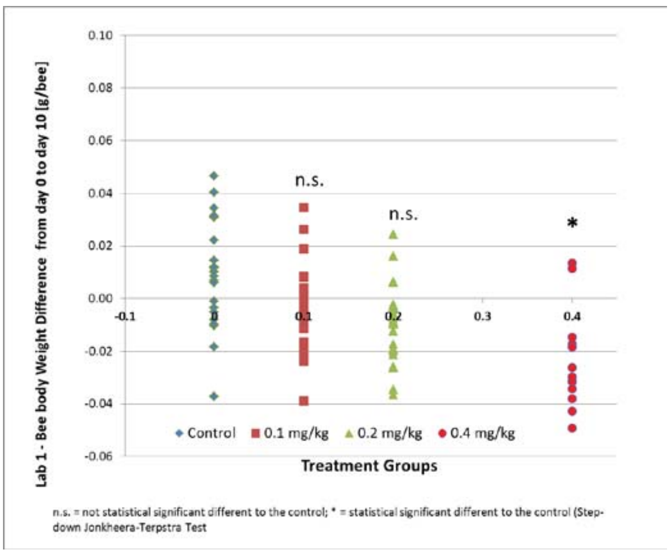

Figure 1 Lab 1: Bee body weight differences between day 0 and day 10 under the impact of 3 different concentrations of dimethoate

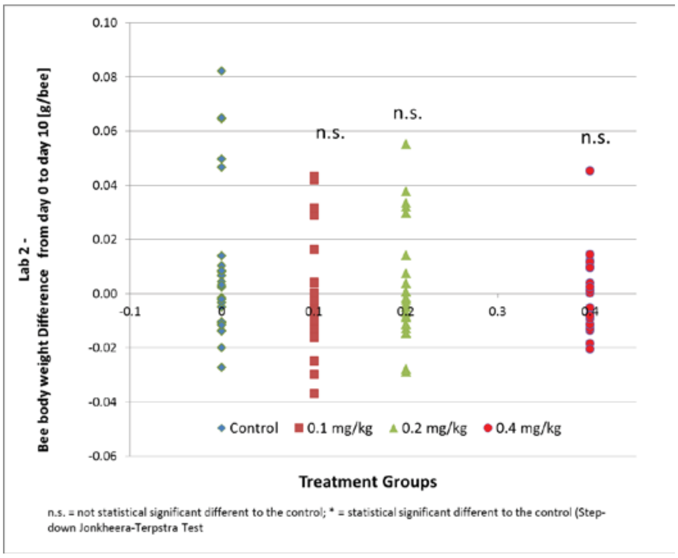

Figure 2 Lab 2: Bee body weight differences between day 0 and day 10 under the impact of 3 different concentrations of dimethoate

3.3 Preparation of honey stomach and intestine

The preparation showed a great variation regarding the size, content and weight of the prepared entrails (i.e. honey stomach, intestine). The Coefficient of Variation (CV) of the weight of honey stomach plus intestine is very large (47 to 48 %) which means largely scattered data and therefore a statistical evaluation is considered not reasonable. See Table 2.

Table 3 Weights and proportions of adult bees and entrails (honey stomach plus intestine)

Treatment	mean total weight of bee at day 10 [g]	Honey stomach + intestine					
		mean [g]	min [g]	max [g]	CV	% of total bee weight min	% of total of bee weight max
C	0.0973	0.0201	0.0051	0.0537	48%	4.8	43.0
T1	0.0967	0.0198	0.0078	0.0508	48%	8.4	44.3
T2	0.0952	0.0188	0.0044	0.0437	47%	6.9	37.1
T3	0.0878	0.0174	0.0033	0.0439	48%	5.1	34.4

Furthermore, for all treatments a correlation was found between the body weight difference (start to end) and the weight of the prepared honey stomach + intestine (R2 = 0.4715). This means that a bee which had a low body weight at the end of the test, likely had a low content (weight) of the honey stomach + intestine. See Figure 3

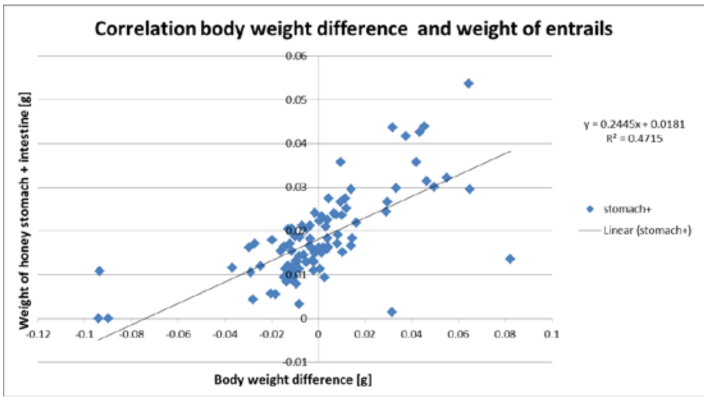

Figure 3 Lab 2: Difference of body weight in correlation to the weight of honey stomach plus intestine.

4. Discussion and Conclusions

Based on these results the following can be concluded:

- No conclusive statement can be done following the two sets of experiments as the results are contradictory.

- Differences in the number of bees which lost weight following treatment of sublethal concentrations of dimethoate were found in Lab 1, but could not be confirmed in Lab 2.

- Statistical significant difference in weight between the control group and the dimethoate treatment could only be detected in one lab at the highest concentration (0.4 mg/kg).

- The variation (CV) of the total weight of the prepared entrails (honey stomach + intestine) was very high which means a great and varying factor influencing the total weight of a bee body. There is a high probability that possible dimethoate-related effects on the weight of other bee parts (e.g. fat body) could be overlapped by the content/weight of the honey stomach and intestine

Therefore, assessment of bee body weight within a 10-day chronic feeding study is considered questionable for the assessment of sublethal effects. Further work is needed and with other active ingredients to clarify if body weight change of honey bees can be used as a parameter for sublethal effects.

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462
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Hazards of pesticides to bees

13th International Symposium of the ICP-PR Bee Protection Group

18. - 20. October 2017, València (Spain)

- Proceedings -

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1st Symposium, Wageningen, the Netherlands, 1980
2nd Symposium, Hohenheim, Germany, 1982
3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
7th Symposium, Avignon, France, 1999
8th Symposium, Bologna, Italy, 2002
9th Symposium, York, UK, 2005
10th Symposium, Bucharest, Romania, 2008
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12th Symposium, Ghent, Belgium, 2014
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Job & Margreet van Praagh with award,
Anne Alix (secretary of the board)

Foto

Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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