2.14 Single versus double field rate: Do different rates of fenoxycarb in chronic Oomen bee brood feeding tests cause different effects sizes?

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Abstract

Background: EFSA (2013)1 recommends to modify the Oomen bee brood feeding test (Oomen et al., 19922) from an acute to a chronic feeding test, but proposals regarding the concentration of the reference item fenoxycarb in such trials are missing. For the chronic Oomen bee brood feeding ring-test (see Lückmann & Schmitzer 2014[3]) the double field rate was used. Due to the lack of information about the effect size of the single field rate two separate bee brood feeding tests (following the method given by the ring-test protocol) were conducted: one in July 2013 (study 1) and one in April 2014 (study 2). The single and the double field rate of fenoxycarb were applied each at both times. As endpoints effects on brood termination rate (BTR) of marked eggs, young and old larvae, pupal mortality and colony development (i.e. number of brood cells and colony strength) were recorded and evaluated.

Results: The chronic administration of the double field rate caused reproducible results whereas those of the single field rate were more variable. Distinct (*i.e.* ≥ 50%) and statistically significant increased BTRs of eggs were observed for the single rate in study 2 only, and for the double rate in both studies. Pupal mortality was statistically significantly increased at both rates in both studies and also bee brood and colony strength development was affected at both rates in both studies. Distinct dose-related differences between the two test rates were present for the BTRs of eggs in study 1 and for pupal mortality and colony development in study 2.

Conclusion: The chronic feeding of the single rate of fenoxycarb did not cause reproducible, dose-related effects. Therefore it is recommend using the double field rate of fenoxycarb as the toxic reference item dose in chronic Oomen bee brood feeding studies as long as no further data are available on the effect size of the single rate.

Key words: honeybees, chronic Oomen bee brood feeding test, fenoxycarb, single rate, double rate

Introduction

The preliminary 'Guidance Document on the risk assessment of plant protection products on bees' (EFSA 2013[1]) proposes to change the Oomen bee brood feeding test (Oomen *et al.,* 1992[3]) from a single-day-testing to a chronic-feeding test. Based on this, the feeding period of honeybee colonies with a product-spiked sugar solution should be extended from one to nine days to guarantee chronic exposure of bee brood. However, EFSA gives no recommendations on the concentration of the reference substance (fenoxycarb), which Oomen suggested to use due to its known insect growth regulator properties. As no practical experiences were available regarding the chronic feeding for this study type, the 'Oomen-brood method ring-test group' of the German 'AG Bienenschutz' prepared a ring-test protocol for a chronic feeding test under field conditions (for details see AG Bienenschutz, unpublished 2013[4]). The results are presented by Lückmann & Schmitzer (2014)[3]. Because no information was available about the effectiveness, e.g. size of the Brood Termination Rate (hereafter BTR) or pupal mortality of the reference item fenoxycarb, the protocol suggested to use the ninth part of the double field rate of 300 g a.s./400 L water/ha which equals to 42 mg a.s. administered in 0.5 L sugar solution per feeding day and colony. As no data were excisting about the effects of the single rate on the parameters given above, the study intended to investigate effect sizes of the single and double field rate.

Experimental methods

Two separate bee brood feeding studies following the method given by the ring-test protocol of the 'AG Bienenschutz' and summarized by Lückmann & Schmitzer (2014[3]) were conducted: one in July 2013 (study 1) and one in April 2014 (study 2). Over a period of nine days the colonies were daily fed with 0.5 L of a freshly prepared 50:50 (w:v) sugar solution which was administered to the colonies by feeders placed on the top of each hive. Food uptake was assessed daily. Each study consisted of three treatment groups: a control and two concentrations of the insect growth regulator fenoxycarb as the test item. The daily concentrations of fenoxycarb were 1/9 of the single (150 g a.s./400 L water/ha) and double field rate (300 g a.s./400 L water/ha) which corresponded to 21 and 42 mg a.s./0.5 L/colony/day, respectively. Study 1 comprised of four and study 2 of three replicates for each treatment group. On the Brood area Fixing Day (hereafter BFD) 200 cells either filled with eggs, young or old larvae were marked. Feeding started on the day of brood fixing in 2013 (*i.e.* food administration evenings) and one day after in 2014 (*i.e.* food administration mornings). As the main endpoints the BTRs of the marked cells with the respective brood stages, pupal mortalities and colony developments (*i.e.* number of brood cells and number of bees (colony strength)) were determined.

Based on the time the respective brood stages need to complete the development BTRs of the marked cells filled with old larvae were assessed on BFD 0, 5, 9 and 15 in study 1, and on BFD 0, 4, 10 and 16 in study 2. For the cells filled with eggs and young larvae the BTRs were also assessed on BFD 22. For purposes of clarity only the BTRs at the last assessments will be presented. For colony development, *i.e.* number of brood cells and number of bees (colony strength) was estimated on the same days as the BTR assessment and as well on BFD 28, but not on BFD 15 in study 1. Pupal mortality was recorded daily for a period of 28 days via dead bee traps.

For both studies calculation of descriptive statistics was performed. For statistical analysis of BTRs and pupal mortalities were tested on normality using Shapiro-Wilks, followed by a one-way ANOVA and in case of statistical differences by the post-hoc Tukey test for multiple comparisons, $\alpha = 0.05$.

Results and discussion

The results of the two studies are summarised in Table 1 and Figure 1 to Figure 4. In the control the mean BTRs for all brood stages in both studies were on a low level (Table 1). They were comparable to the data of the ring-test (Lückmann & Schmitzer 2014[3]) which amounted to 14.7%, 12.6% and 7.6 % for eggs, young and old larvae, respectively.

The BTRs for eggs were statistically significantly higher in the double fenoxycarb rate in study 1 (July 2013, $p = 0.022$) and study 2 (April 2014, $p = 0.024$) compared to the control, whereas this was observed for the single rate in study 2, only ($p = 0.009$). In contrast the BTRs for young and old larvae were not statistically significantly different between the control and both fenoxycarb rates in both studies; exception: young larvae at the double rate in study 1 ($p = 0.028$).

Further on, the data of the fenoxycarb groups displayed a distinct dose-response relationship of the mean BTRs for the eggs in study 1 but not in study 2. For the other brood stages BTRs were on comparable levels.

Table 1 Summary of brood termination rates and daily mortalities

	Study 1 (July 2013)			Study 2 (April 2014)		
Mean BTR ± SD [%]	**C**	**FOX, 1x**	**FOX, 2x**	**C**	**FOX, 1x**	**FOX, 2x**
- eggs*	17.0 ± 19.0a	28.3 ± 5.7ab	62.9 ± 27.6b	8.9 ± 3.8a	60.8 ± 17.8b	50.6 ± 15.8b
- young*	3.5 ± 1.1a	8.9 ± 4.1ab	10.3 ± 3.0b	0.4 ± 0.3a	0.6 ± 0.2a	2.2 ± 2.7a
- old larvae**	2.3 ± 1.6a	2.4 ± 2.9a	3.9 ± 2.3a	1.9 ± 2.0a	2.1 ± 1.7a	1.2 ± 0.8a
Mean daily pupal mortality ± SD [n/colony/day]	0.1 ± 0.2a	82.9 ± 18.4b	67.9 ± 29.3b	1.1 ± 0.2a	161.0 ± 12.1b	190.2 ± 12.5c

Both fenoxycarb rates caused a high and statistically significant increased daily pupal mortality compared to the control in both studies (single rate: $p < 0.001$ in both studies; double rate: $p = 0.003$ in study 1 and $p < 0.001$ in study 2). A statistically significant difference between both rates was observed in study 2 ($p = 0.028$).

In addition, both rates caused a distinct and similar reduction of the total mean number of brood cells (Figure 1 and Figure 2) and mean colony strength (Figure 3 and Figure 4).

C= Control, FOX = Fenoxycarb, 1x = single rate, 2x = double rate, * at BFD 22, ** at BFD 15/16
Statistical analysis via one-way ANOVA and post-hoc Tukey test for multiple comparisons, α = 0.05; a,b,c: same letters indicate that groups are not statistically significantly different

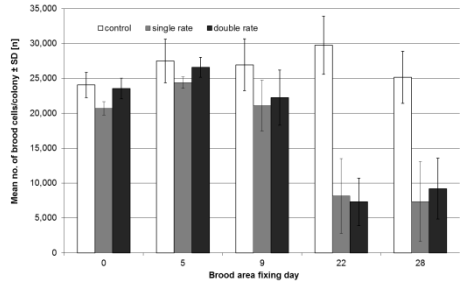

Figure 1 Bee brood development in study 1 (July 2013)

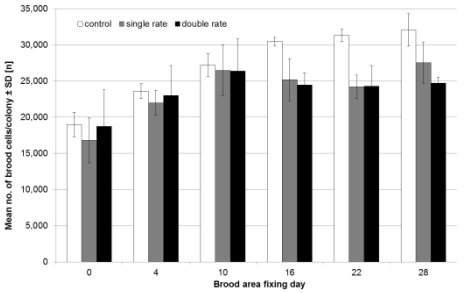

Figure 2 Bee brood development in study 2 (April 2014)

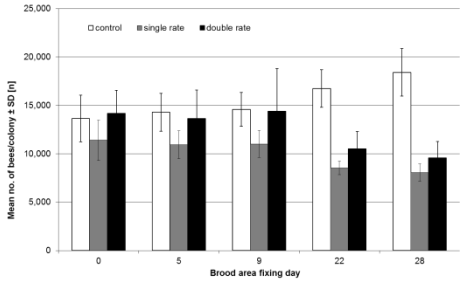

Figure 3 Bee colony strength development in study 1 (July 2013)

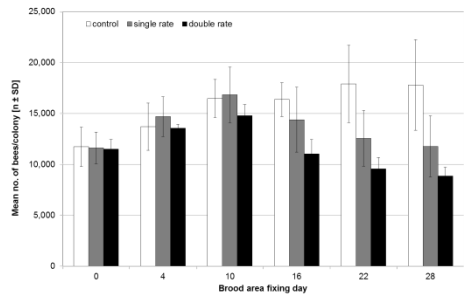

Figure 4 Bee colony strength development in study 2 (April 2014)

Conclusions

The chronic administration of the double field rate of fenoxycarb caused reproducible results whereas those of the single field rate were more variable. In fact clear effects on pupal mortality and colony development (*i.e.* number of brood cells and colony strength) were recorded at both rates in both studies, whereas distinct (i.e. ≥ 50%) and statistically significant effects on BTRs of eggs were observed for the single rate in study 2 only, and for the double rate in both studies. Obvious dose-related differences in effect sizes were found for the BTRs of the eggs in study 1 and for pupal mortality and colony development in study 2. Thus the chronic feeding of the single rate of fenoxycarb did not cause clear reproducible dose-related effects. Therefore it is recommended to use the double field rate of fenoxycarb as the toxic reference substance dose in chronic Oomen bee brood feeding studies as long as no further data are available on the effect size of the single rate.

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