

B.9.4.2 Semi-field studies

Abbreviation used:

DAA = days after application

ba = before application

aa = after application

Studies with the formulation

Study M-054090_0003228099

Reference/notifier	: 5.1.2.e (2002)	GLP statement	: yes
Type of study	: Honeybee semi-field tunnel test	Guideline	: OEPP/EPPO guideline No. 170 (3), 2001
Year of execution	: 2002	Acceptability	: acceptable
Test substance	: Thiacloprid OD 240 Batch no. 07690/0086(0082)		

Substance	Species	Method	Duration [d]	Application rate [L/ha]	Value [g as/ha]	Parameter	Adverse effect
Thiacloprid OD 240	<i>Apis mellifera</i>	Tunnel test in flowering <i>Brassica napus</i> , 1x foliar spray during flowering and active foraging of bees	Exposure 7 d Observation duration 7 d.	0.3	73.19	Mortality Flight intensity Brood development Behaviour	N Y N N

Methods

Location

Semi-field experiment was conducted on a test field which was located in the south of Germany near Pforzheim.

Sizes field and crop, test condition (tunnel or field)

The overall size of the test field was approximately 700 m². The crop was oil seed spring-rape *Brassica napus*. The crop was treated twice with Karate (active ingredient: lambda-cyhalothrin), on April 16, 2002 and May 2, 2002.

Treatments and test concentrations and application

Three replicates (= three gauze tunnels) were set up for each experimental group, consisting of an untreated control, a toxic reference (Perfekthion (dimethoate) which was applied at a concentration of 650 g product/ha (251.97 g as/ha) in 300 L water/ha and one concentration of the test item (0.3 L/ha equivalent to 73.19 g as/ha), respectively. Nine tunnels were used in total. The area covered by each

tent was about 40 m² per tent. The size of each tent (covered plot) was 8 m long, 5 m wide and approx. 3.5 m high in the centre. The tunnels were randomly assigned to the different treatments. The following conditions were met for application:

- the oil-seed spring-rape crop was in full bloom
- more than 10 bees/m² were visiting flowers
- wind speed was below 2 m/sec.

Application was performed at June 17, 2002.

Species tested

Honey bees of the race *Apis mellifera*.

Population size

For the test, small healthy colonies with three combs containing approx. 4000 to 6000 bees were used. Furthermore the following criteria for the nuclei were guaranteed:

- at least two brood combs containing eggs, larvae and capped cells
- at least one honey and pollen comb
- bees were free of *Nosema* and other bee diseases.

Timing placement hives under test conditions

Hives were placed inside the tunnels 3 days before the application of the test substance.

Parameters observed and timing thereof

The following parameters were observed:

- Mortality: 2ba, 1ba, 0ba, 0aa (1 and 5 h after application and in the evening), daily 1-7DAA.
- Flight intensity: 2ba, 1ba, 0ba, 0aa (1 and 5 h after application and in the evening), daily 1-7DAA. At each assessment time the number of bees that were both foraging on flowering oil-seed spring-rape and flying over the crop were counted on a square of 1 m².
- Condition of the colonies, development of bee brood: 4ba (=1 day before moving the colonies in the tunnels) and 7DAA. The following parameters were assessed:
 - Strength of the colony (number of combs covered with bees)
 - Presence of a healthy queen (presence of eggs, presence of queen cells)
 - Estimate of the pollen storage area and area with nectar
 - Estimate of the area containing eggs, larvae and capped cells
- Behaviour of the bees was observed prior to and after application.

Statistical evaluation

The data of mortality and flight activity were analysed for significant differences in comparison to the control group. The normality of data was tested following Shapiro Wilk test. If data were normal distributed, differences of the test substance treatment to the control were analysed by using the Dunnett test (Multiple-t test, probability 95 % ($p < 0.05$)). If data were not normal distributed differences of means were analysed by the pairwise U-test according to Mann-Whitney, probability 95 % ($p < 0.05$).

Results

Actually applied concentrations

Amounts actually applied were 72.31, 77.46 and 72.31 g thiacloprid/ha and 247.38, 254.55 and 261.40 g dimethoate/ha. These concentrations are all within the allowed range of 10%.

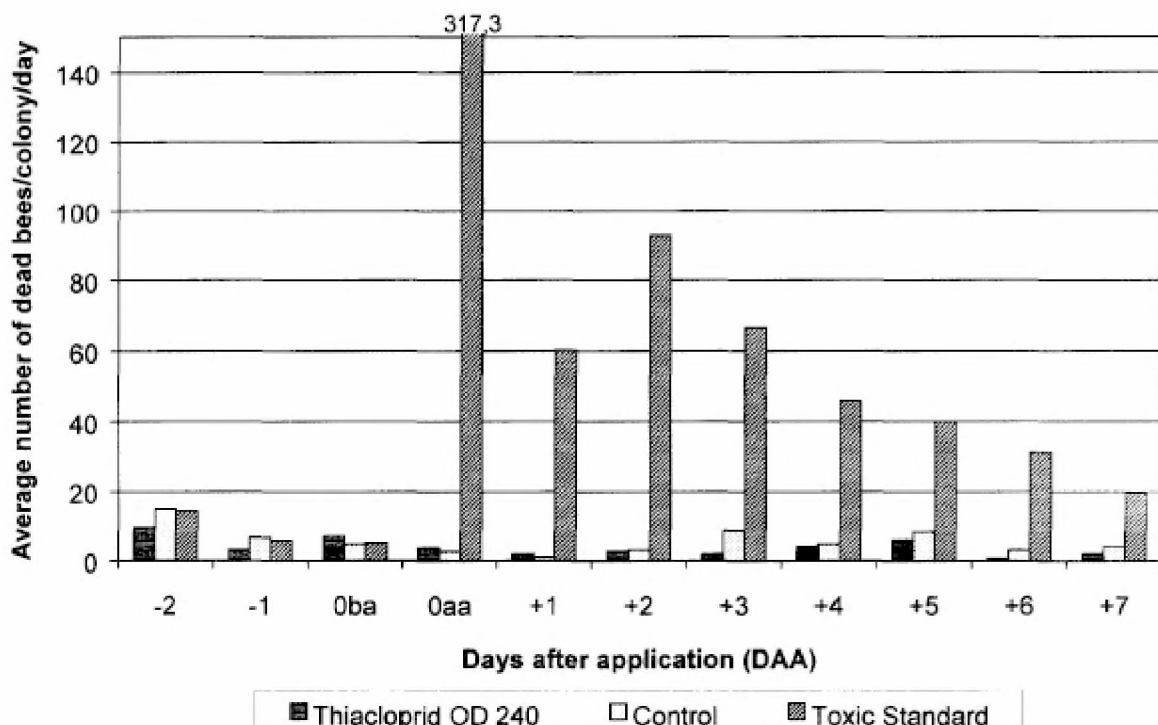
Climatic conditions

Measured minimum temperatures ranged between 14.8 and 18.6°C and maximum temperatures between 24.6 and 33.9°C, the relative humidity (RH) ranged between 24.5 and 100%. Precipitation was limited to 2.2 mm at 6DAA.

Mortality

Results on foraging activity and mortality are reported in the following table (copied from the report). When comparing the average pre-application mortality and the average post-application mortality using $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) no increase of mortality occurred in the test substance treatment group Thiachloprid OD 240. An obvious increase was observed in the toxic standard group. The values for $C_{M(average)}$ were 0.4 for the test substance treatment, 0.5 for the control group and 10.2 for the toxic standard treatment. Mortality in the treatment did not differ significantly from control. Mortality in the toxic standard group differed significantly from the control at 0aa and 1-7DAA.

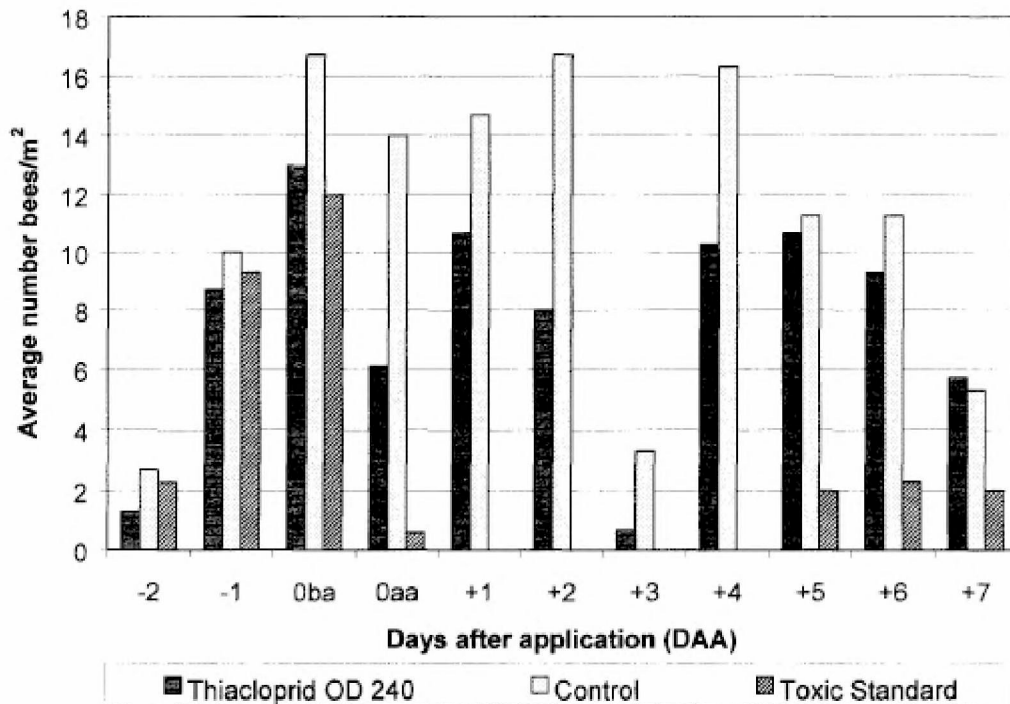
Figure x. Average number of dead bees/replicate/day collected in the dead bee traps and on the edge of the treated oil-seed spring-rape area in the test substance treatment with Thiachloprid OD240, the control and the toxic standard group (Oba =evaluation on the day of treatment shortly before application; Oaa = evaluation on the day of treatment after application)



Flight intensity

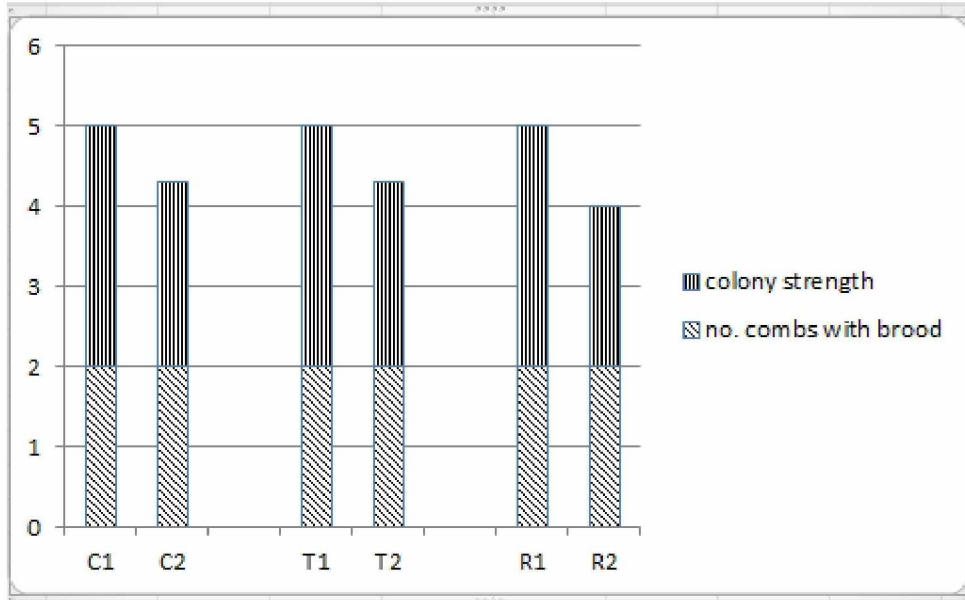
Flight intensity in the treatment differed significantly from control at 0aa and 1-4DAA. In the toxic standard group flight intensity differed significantly from the control at 0aa and 1-7DAA.

Figure x. Average flight intensity in the test substance treatment group with Thiocloprid OD 240, the control and the toxic standard group prior to and after application



The mean colony strength (no. of combs covered by adult bees) and the mean number of combs containing brood is given in the figure below. Largest decrease of the strength of the colonies was observed in the toxic standard group. No differences were observed between the control and the treatment.

Figure x. Colony strength and number of combs containing brood (figure prepared by reviewer using data in the study)



Brood development

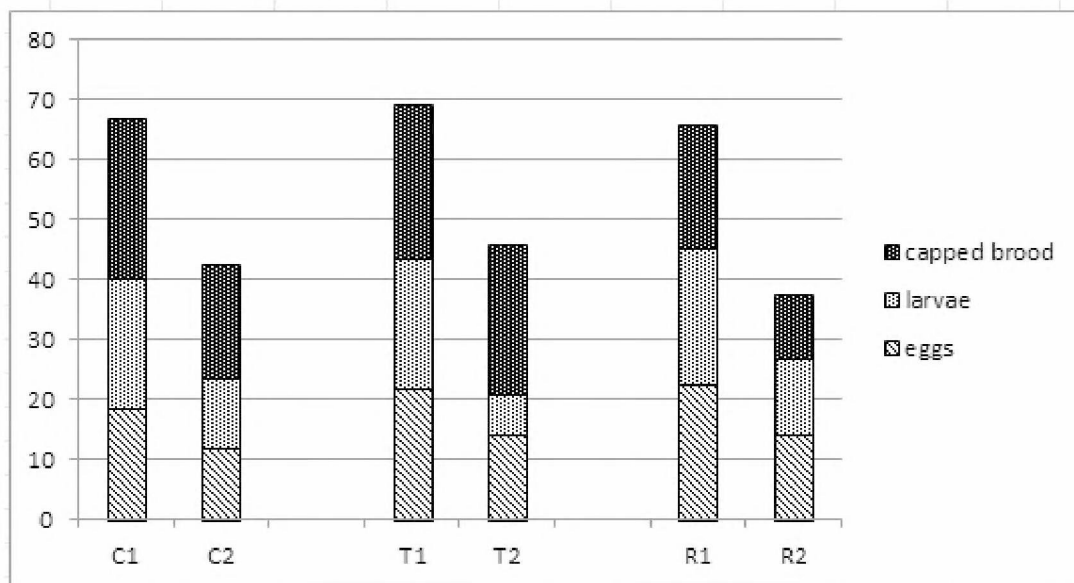
Egg-laying activity was assessed and recorded at test initiation (June 17, 2002) and at 7DAA .

The data were expressed as

- Average comb area containing eggs in %
- Average comb area containing larvae in %
- Average comb area containing capped brood in %

Decreases in brood development were observed for all three treatments and were explained by the author by the restricted spaces the bees were kept in for 10 days and the limited food supply within the small area of crop in each tent.

Figure x. Mean brood development (figure prepared by reviewer using data in the study)



C1, T1 and R1 = Control, Treatment, Toxic reference at June 17

C2, T2 and R2 = Control, Treatment, Toxic reference at June 24

Behaviour

Directly after application of Thiacloprid OD 240 and during the postapplication assessments no behavioural differences were observed in the Thiacloprid OD 240 treatment group compared to the control group and the bees continued with foraging on the treated oil-seed springrape flowers.

Conclusions of the author

The results of the study revealed that Thiacloprid OD 240 can be applied via foliar application at a rate corresponding to 73.19 g as/ha into a full-flowering and highly bee attractive crop, as represented here by oil seed spring rape, during honey bees actively foraging on the crop, without adverse effects on mortality, behaviour, brood-, and population development.

However, a significant decrease of flight intensity was observed on 0aa and the following 4 days after application as compared to the control group and a repellent effect caused by the test substance in the first four days after application can be assumed.

Remarks by reviewer

A positive control was included in the test and showed that the test system was behaving as expected. Author concluded that behaviour was not affected. This could not be evaluated by RMS as observations on behaviour were not reported. Development of pollen and nectar stores were not recorded. Colony strength was determined as the number of combs covered by adult bees. This parameter has little distinctive capacity as the intensity of the coverage cannot be included. An estimate of the total number of bees was not made. Concerning the number of combs with brood the author made more differentiation by also discriminating between full and halve combs. More differentiation could have been possible according to reviewer.

According to the recent EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Brassica napus* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of thiacloprid on brood related parameters, mortality and flight intensity.

The results of the study show that only flight intensity seems to be affected. There were no test item related effects in any other endpoint (except for behaviour as these data were not reported). This study can be used in the risk assessment for Thiacloprid OD 240 .