4.10 Chlorantraniliprole: Lack of effects on bumblebee reproduction (*Bombus terrestris*) under semi-field conditions in *Phacelia tanacetifolia*

Axel Dinter[1], Kristin E. Brugger[2]

[1] DuPont de Nemours (Deutschland) GmbH, DuPont Str. 175, D-63263 Neu-Isenburg, Germany. Corresponding author: Tel. +49(0)6175797211, Email: axel.dinter@dupont.com.
[2] DuPont Crop Protection, Stine-Haskell Research Center, 1090 Elkton Road, Newark, DE 19714, USA

Abstract

Background: In a semi-field trial the effect of chlorantraniliprole spray application on *Phacelia tanacetifolia* on the bumblebee, *Bombus terrestris* L. (Hymenoptera, Apidae), was studied.

Results: Chlorantraniliprole applied twice at 60 g a.s./ha as a spray application on flowering *Phacelia* with a 9-day spray interval during daily bumblebee flight did not have any pertinent effects regarding all parameters assessed, i.e. mortality, flight activity, hive weight, condition of colonies, development of bumblebee brood, production of young queen offspring and vigor relative to the water treated control. Similar numbers of young queens and drones were determined in the chlorantraniliprole and control treatments. No residues above the level of quantification (LOQ) of 0.001 mg/kg were found in any of the control samples in pollen or nectar. Residues of chlorantraniliprole above the LOQ level were found for all matrices after application in the chlorantraniliprole treatment. Residues in pollen samples were generally higher compared to the nectar samples, while chlorantraniliprole residue levels declined rapidly in both matrices after each spray application.

Conclusion: In a semi-field trial no effects of chlorantraniliprole applied twice at 60 g a.s./ha on the bumblebee, *Bombus terrestris*, including reproduction was found.

Key words: chlorantraniliprole, insecticide, side-effects, bumblebee, *Bombus terrestris*

1. Introduction

Chlorantraniliprole is an anthranilic diamide insecticide[1,2] and is registered in many countries worldwide. Chlorantraniliprole has proven to have negligible effects on numerous beneficial non-target arthropod species or to have a rather low and transient impact on some beneficial species[3,4,5]. Also, chlorantraniliprole and its formulated products[8] demonstrated low intrinsic toxicity for honeybees and bumblebees *Bombus terrestris* L. (Hymenoptera, Apidae) and in worst-case semi-field tunnel and greenhouse trials no significant effects on pollinating bees were found, even when bees were directly over-sprayed during foraging activity[6]. For *Bombus impatiens* Cresson (Hymenoptera, Apidae) a laboratory study concluded that chlorantraniliprole is safe for greenhouse use in the presence of bumblebees[7].

This paper summarizes the results of a semi-field tunnel trial with chlorantraniliprole and the bumblebee, *Bombus terrestris* L. (Hymenoptera, Apidae), where flowering *Phacelia tanacetifolia* was sprayed twice at 60 g a.s./ha.

2. Experimental Methods

A semi-field tunnel test with *Bombus terrestris* L. (Hymenoptera, Apidae) was conducted based on general Setac/escort recommendations and EPPO No. 170 (4)[8,9]. The trial was conducted in Southern Germany with the formulated product Coragen® and an application rate of 60 g a.s./ha plus a water treated control and a toxic reference. Each of the three treatments consisted of four separate tunnels with one bumblebee colony (delivered by Koppert BV., The Netherlands) for biological assessment. The individual tunnels covered an area of 60 m²/tunnel (Figure 1).

[8] Chlorantraniliprole 200 g/L formulation is Coragen® and Chlorantraniliprole 35WG formulation is Altacor®.

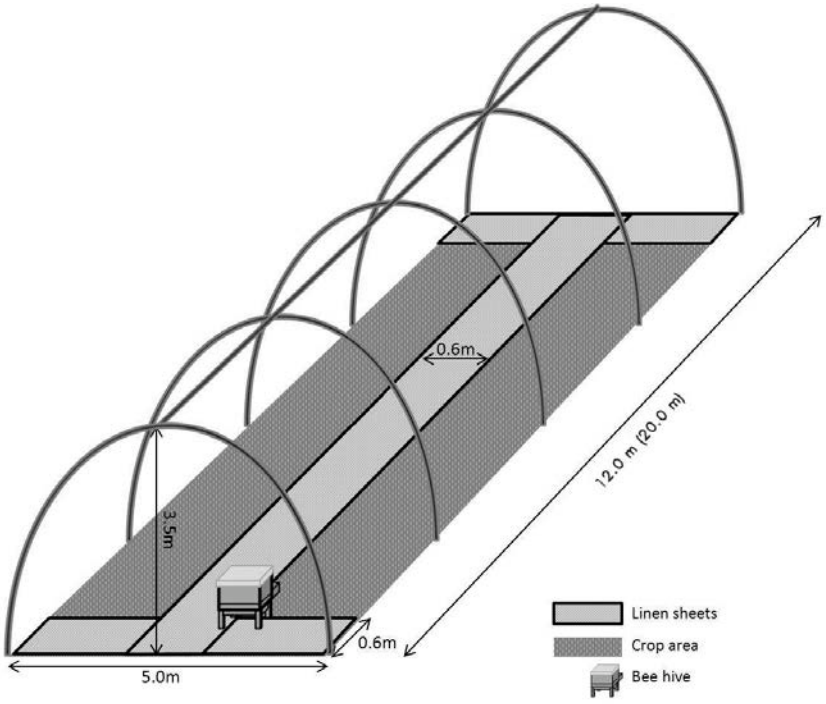

Figure 1 Setup of bumblebee tunnel (Tunnel length of 12 m for the biological assessment tunnels and of 20 m for the residue tunnels)

Additionally, four 100-m²-tunnels (two for the control and two for the chlorantraniliprole treatment) were set-up similarly but with two bumblebee hives plus one honeybee (*Apis mellifera* L. (Hymenoptera, Apidae)) hive (small queen right colonies with approx. 4000 to 6000 worker bees and all brood stages) to serve for pollen and nectar residue sampling. Analysis of residues of chlorantraniliprole was carried out for honeybee nectar sampled directly from combs and prepared from forager honeybees (stomach content), for honeybee pollen sampled directly from combs and prepared from forager honeybees, and for bumblebee nectar sampled from nectar cells in the hives. Residue samples were taken from control and chlorantraniliprole replicates at 7 dates (DAA1-1, DAA1+1, DAA1+3, DAA1+8, DAA1+10, DAA1+11 and DAA1+17. DAA1 = Day after the 1st application) and analysed for residues of chlorantraniliprole with a level of quantification (LOQ) of 0.001 mg/kg.

After the initial brood assessment (09 August 2013) the bumblebee colonies were set-up in the tunnels and left for 3 days before exposure to the first spray application to acclimate to the new environment. The spray applications were performed with a hand-held boom sprayer at 400 L spray volume/ha during full flowering of the *Phacelia* crop and during foraging activity of the bees (1st spray at 12 August 2013 (BBCH 63) and 2nd spray at 21 August 2013 (BBCH65)). The control (tap water) and chlorantraniliprole treatment were spayed twice, while the toxic reference (dimethoate) was only sprayed once at the first spraying date at 2000 g dimethoate/ha. The bumblebee colonies were exposed to the treated flowering *Phacelia* crop for 29 days in the tunnel tents. After the exposure phase in the tunnels the bumblebee hives for the biological assessments were kept closed in a climatic chamber at 25 °C (± 3 °C) from 11 to 12 September 2013 and then bumblebee hives were anaesthetised with dry ice (CO_2) and deep-frozen in a deep-freezer for the final brood assessment at 12 September 2013. Bumblebees were supplied with auxiliary food (sugar solution supplied with the hives, and pollen pellets) before set-up of the hives in the

tunnels and after the exposure phase when they were kept closed in the climatic chamber. During the exposure phase the sugar solution supply was closed except that additional feeding with sugar solution was performed from 17 to 20 August 2013 and at 30 August 2013 in order to keep larval mortality (observed in control hives) as low as possible.

The influence of chlorantraniliprole and the toxic reference was evaluated by comparing the results to the data in the control treatment regarding the following observations: Number of living worker bumblebees and larvae, mortality of bumblebees (workers, queens and larvae), flight activity within the crop, development of the bumblebee brood, condition of the bumblebee colonies and residue levels of the different analysed matrices.

3. Results

3.1 Bumblebee flight intensity

The bumblebee colonies were placed in the tunnels 3 days before the first application in order to acclimate the bumblebees to their new environment. In all treatment groups the bumblebees immediately started foraging the crop (Figure 2). The flight intensity increased to approximately 5 bumblebees/4 m^2 at the application day (control value). In the control and the chlorantraniliprole treatment a more or less continuous increase of the foraging activity was observed during the course of the study up to DAA1+17 when a maximum of flight activity was reached (> 20 bumblebees/4 m^2). Significant differences ($p \leq 0.05$, t-test) in the flight activity of the chlorantraniliprole group were observed at DAA1+8 (increase) and at DAA1+9 after the 2nd spray application (decrease). The significant increase at DAA1+8 was probably due to the cloudy conditions and low temperature in the early morning (< 10 °C until 6:30 AM) where the control assessments were performed approximately one hour before the chlorantraniliprole assessments. The significant decrease in flight activity at DAA1+9 just after the 2nd spray application was probably due to a combination of increased foraging activity the day before application (18.2 bumblebees/4 m^2) and the application of chlorantraniliprole before the assessment. However, from DAA1+10 (= +1 day after the 2nd spray application) on, there were no differences between control and chlorantraniliprole in flight activity. Decreasing flight activity in control tunnels mainly was due to the weather conditions as i.e. at DAA1+15 with a clouding of 100 %. The flight activity of the toxic reference was significantly reduced ($p \leq 0.05$, t-test, Mann Whitney exact test) for all samplings after spray application of the toxic reference on 12 August 2013, resulting in very low flight activities several days after application and reaching maximum values of approximately of 5 bumblebees/4 m^2 at the end of the exposure phase.

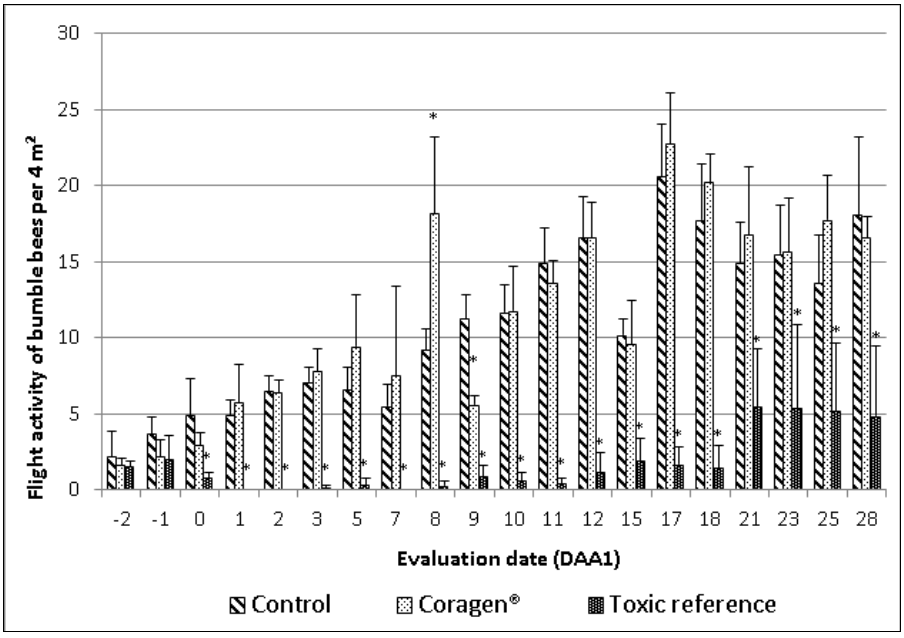

Figure 2 Mean bumblebee flight intensity (number of forager bees/4 m^2/minute ± STD) in the control (C), chlorantraniliprole at 2-times 60 g a.s./ha (Coragen), and toxic reference treatment (1-time 2000 g dimethoate/ha) (Toxic reference) during bee flight in flowering *Phacelia tanacetifolia*.. (1st application in C, chlorantraniliprole and toxic reference at 12 August 2014 (BBCH 63, DAA1±0)), 2nd application in C and chlorantraniliprole at 21 August (BBCH 65, DAA1+9) (DAA1 = Days after 1st application during bee flight) of bumblebees in the test tunnel tents. * = statistical significant difference to control, $p \leq 0.05$, t-test, Mann Whitney exact test).

3.2 Bumblebee mortality

Total mortality including dead adult bumblebees and larvae observed in the tunnels, in front of the bumblebee hives and inside the hives (mean values per day) for the control and the chlorantraniliprole treatment values were generally low with exception of the assessments after the 2nd spray application (DAA1+10 until DAA1+18) where a slightly higher mortality was found (Table 1). However, these differences were not significant ($p \leq 0.05$, t-test) if compared to the control observations.

Table 1 Mean number of dead workers and larvae per day per bumblebee hive (in the tunnels in front of and inside the bumblebee hives) following 2 spray applications of chlorantraniliprole at 60 g a.s/ha during bee flight in flowering *Phacelia tanacetifolia*.

Mean number of dead workers and larvae per day and per bumble bee hive (in the tunnel, in front of and inside the bumble bee hives)							
Date	**DAA1**	**Treatment groups**					
		Control		**Chlorantraniliprole**		**Toxic Reference**	
		workers	**larvae**	**workers**	**larvae**	**workers**	**larvae**
Applications of test item at 12 Aug 2013 (0 DAA1) and 21 Aug 2013 (9 DAA1)							
09 Aug 2013	-3	1.25	1.00	1.50	0	0	0
10 Aug 2013	-2	0.25	0.50	0	0	0	0
11 Aug 2013	-1	1.75	0.25	1.00	0	1.00	0
12 Aug 2013	**0**	0.50	0	0.25	0	4.75* [a)]	0.50
13 Aug 2013	+1	0	2.00	5.50	0.25	92.50* [b)]	3.25
14 Aug 2013	+2	0.75	5.50	0.25	3.00	20.75* [a)]	0.75
15 Aug 2013	+3	0.75	2.50	0.25	0	5.25* [b)]	0
17 Aug 2013	+5	0.63	1.50	0.38	0.88	6.25* [b)]	0.13* [a)]
19 Aug 2013	+7	0.88	0.75	0.75	0.75	4.88* [a)]	4.13
20 Aug 2013	+8	0	1.00	0.75	1.25	0.50	5.00
21 Aug 2013	**+9**	2.00	1.75	0.25	1.75	5.25	1.25
22 Aug 2013	+10	2.00	2.00	21.50	5.75	2.50	0* [b)]
23 Aug 2013	+11	0.75	5.75	4.25	13.75	1.50	0
24 Aug 2013	+12	2.00	6.50	2.00	12.25	4.75	13.50
27 Aug 2013	+15	1.50	2.42	0.58	5.58	1.50	0* [a)]
29 Aug 2013	+17	3.25	7.00	2.13	12.88	1.50	0.25
30 Aug 2013	+18	2.25 [c)]	5.50	2.25	14.00	0.25	4.25
02 Sep 2013	+21	3.42	2.50	0.33* [a)]	5.67	1.33	2.83
04 Sep 2013	+23	1.75	1.38	1.13	2.38	0.75	0* [a)]
06 Sep 2013	+25	2.63	1.25	0.63	1.75	2.00	0* [b)]
09 Sep 2013	+28	3.42	0.33	1.33	1.17	1.83	1.42
12 Sep 2013	+31	6.00	1.08	1.67	1.08	1.75	0.92
Mean per day and hive after application (DAA1 0 to DAA1 +9)		**0.69**	**1.88**	**1.05**	**0.99**	**17.52**	**1.88**
		2.56		**2.03**		**19.39**	
Mean per day and hive after application (DAA1 0 to DAA1 +31)		**1.80**	**2.67**	**2.43**	**4.43**	**8.41**	**2.01**
		4.47		**6.86**		**10.42**	

DAA1 = days after application 1 (**bold** indicates dates of applications)
* statistically significant different to control ($p \leq 0.05$)
[a)] t-test
[b)] Mann Whitney exact test
including 1 dead young queen
Calculations based on unrounded values

Total mortality was higher in the toxic reference group with a maximum at DAA1+1. Mortality was significantly higher at DAA1±0, DAA1+1, DAA1+2 and DAA1+7 compared to the control ($p \leq 0.05$, t-test, Mann Whitney exact test). A total mean mortality of adult bumblebees of 189 was observed for the toxic reference compared to 76 in the control hives and 62 in the chlorantraniliprole hives. Queen mortality (original queens) was observed in all four replicate hives of the toxic reference after several days (DAA1+2, DAA1+3, DAA1+5 and DAA1+15). No mortality of queens (original queens) was observed in the control and the chlorantraniliprole treatment.

3.3 Bumblebee hive weight

The weight development of the control and chlorantraniliprole hives was similar (Figure 3). Strong increases in weight of the hives (measured including hive box) occurred when the sugar solution supply was opened and allowed consumption by the bumblebees. No significant differences ($p \leq 0.05$, t-test, Mann Whitney exact test) were detected between the control and the chlorantraniliprole treatment. From DAA1+1 to the last assessment date on DAA1+31 the mean weight in the colonies of the control and chlorantraniliprole treatment increased clearly. In view of the total observation period from DAA1-3 until DAA1+31 the colonies increased their mean weight by 558 g in the control and 700 g in the chlorantraniliprole treatment.

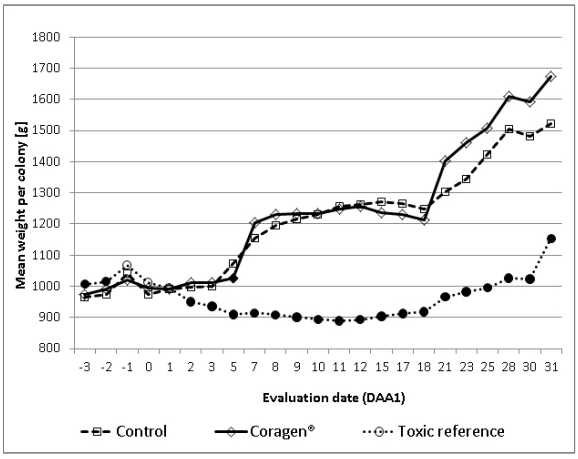

Figure 3 Mean weight of the bumblebee hives (g) (including hive box) in the control (C), chlorantraniliprole at 2-times 60 g a.s./ha (Coragen), and toxic reference treatment (1-time 2000 g dimethoate/ha) (Toxic reference) during bee flight in flowering *Phacelia tanacetifolia*.. (1st application in C, chlorantraniliprole and toxic reference at 12 August 2014 (BBCH 63, DAA1±0)), 2nd application in C and chlorantraniliprole at 21 August (BBCH 65, DAA1+9) (DAA1 = Days after 1st application during bee flight).

In contrast, the weight development of the toxic reference showed a decrease in weight starting from the 1st spray application on with significant differences compared to the control ($p \leq 0.05$, t-test) from DAA1+2 onwards till the end of the exposure period. In view of the total observation period from DAA1-3 until DAA1+31 the toxic reference colonies increased their mean weight by 146 g only.

3.4 Bumblebee colony and brood size

The initial colony assessment (09 August 2013) revealed that the bumblebee colonies were all queen-right and in good condition with a mean number of 159 workers/hive. Additionally, the hives of the different treatment groups showed similar strength with regard to brood stages and food storage (Table 2).

Table 2 Summary of results of initial and final bumblebee colony assessments following 2 spray applications of chlorantraniliprole at 60 g a.s./ha during bee flight in flowering *Phacelia tanacetifolia*.

Initial colony assessment: 09 Aug 2013 (pre-application)						
Treatment group	**Control**		**Chlor-antraniliprole**		**Toxic Reference**	
	Mean	*STD*	**Mean**	*STD*	**Mean**	*STD*
Living queen	**1**	-	**1**	-	**1**	-
Number of alive worker bees	**151.8**	*17.2*	**161.8**	*16.4*	**163.8**	*18.4*
Number of brood cells with eggs	**18.3**	*4.0*	**21.8**	*4.6*	**17.5**	*5.3*
Number of brood cells with larvae (workers)	**152.3**	*8.8*	**145.3**	*38.4*	**151.0**	*44.0*
Number of alive pupae (workers)	**150.8**	*33.0*	**140.8**	*52.3*	**153.8**	*62.2*
Number of filled nectar cells	**48.8**	*13.8*	**52.5**	*11.7*	**62.0**	*11.3*
Number of filled pollen cells	**0**	-	**0**	-	**0**	-
Weight of hive (without hive box) [g]	**317.7**	*8.6*	**327.7**	*35.0*	**360.3*** [a)]	*24.6*
Total number of alive brood stages (eggs, larvae, pupae)	**321.3**	*34.6*	**307.8**	*48.2*	**322.3**	*54.9*
Total number of alive stages (alive brood and adult bees)	**473.0**	*20.6*	**469.5**	*39.8*	**486.0**	*66.9*
Final colony assessment: 12 Sep 2013 (post-application)						
Treatment group	**Control**		**Chlor-antraniliprole**		**Toxic Reference**	
	Mean	*STD*	**Mean**	*STD*	**Mean**	*STD*
Number of alive young queens	**113.0**	*32.2*	**84.3**	*18.8*	**0.0*** [a)]	*0.0*
Weight of alive young queens [g]	**107.7**	*31.4*	**81.9**	*20.7*	**0.0*** [a)]	*0.0*
Number of alive workers	**239.5**	*121.9*	**298.3**	*99.0*	**126.8**	*35.1*
Number of alive drones	**60.5**	*12.8*	**75.3**	*21.7*	**0.0*** [a)]	*0.0*
Number of brood cells with eggs	**16.3**	*7.3*	**30.0**	*24.9*	**8.0**	*4.5*
Number of brood cells with larvae (workers/males)	**67.0**	*46.9*	**77.3**	*62.6*	**61.5**	*53.0*
Number of brood cells with larvae (queens)	**2.5**	*2.1*	**4.0**	*4.1*	**0.0**	*0.0*
Number of pupae (workers/drones)	**138.3**	*30.0*	**211.5*** [a)]	*34.9*	**40.5*** [a)]	*42.6*
Number of pupae (queens)	**28.8**	*26.9*	**17.5**	*31.0*	**0.0**	*0.0*
Number of filled nectar cells	**255.8**	*89.1*	**330.5**	*102.0*	**107.0**	*104.4*
Number of filled pollen cells	**5.8**	*3.5*	**0.5**	*0.6*	**8.0**	*9.1*
Weight of hive (without cage) [g]	**773.0**	*162.3*	**837.8**	*74.7*	**383.5*** [a)]	*143.2*
Total number of alive brood stages (eggs, larvae, pupae)	**252.8**	*41.6*	**340.3**	*65.1*	**110.0*** [a)]	*68.5*
Total number of alive adult bees (alive young queens, workers, drones)	**413.0**	*106.1*	**457.8**	*90.4*	**126.8*** [b)]	*35.1*
Total number of alive stages (alive brood and adult bees)	**665.8**	*118.3*	**798.0**	*118.0*	**236.8*** [a)]	*88.8*
Weight / young alive queen [g]	**0.95**	*0.04*	**0.97**	*0.06*	**-**	-

Mean = mean values of all 4 replicates (hives) per treatment group. STD = standard deviation
* Statistically significant difference compared to control ($p \leq 0.05$): [a)] t-test. [b)] Mann Whitney exact test

At the final assessment (12 September 2013), all colonies of the control and chlorantraniliprole treatment groups still had their original living queen. In the toxic reference all original queens were dead. The mean numbers of young queens, workers and drones produced in the control and the chlorantraniliprole group did not show significant differences ($p \leq 0.05$, t-test, Mann Whitney exact). However, the number of young queens and drones differed significantly ($p \leq 0.05$, t-test) between control and toxic reference, where no drones and young queens were found. The number of young queens, workers and drones was 113.0, 239.5, and 60.5 in the control and 84.3, 298.3 and 75.3 in the chlorantraniliprole group, respectively. Considering the total number of adults and brood the chlorantraniliprole treatment group produced slightly higher number of offspring with 457.8 adults, 340.3 brood stages and a total of alive stages of 798.0 compared to 413.0 adults, 252.8 brood stages and 665.8 total alive stages in the control. Significant reductions

($p \leq 0.05$, t-test, Mann Whitney exact) were found for the toxic reference compared to the control. Only 126.8 adults, 110.0 brood stages resulting in a total of 236.8 total alive stages were counted in the toxic reference.

Also with regard to the individual brood stages, the final brood assessment did not show significant differences between the control and the chlorantraniliprole treatment group with exception of the significantly ($p \leq 0.05$, t-test) higher number of pupae in the chlorantraniliprole treatment group. The production of pupae was significantly ($p \leq 0.05$, t-test) reduced in the toxic reference. Also the weight per adult young queen was approximately the same for the control and the chlorantraniliprole treatment group. The mean weight of the hives was slightly higher in the chlorantraniliprole treatment group and significantly lower in the toxic reference compared to the control.

3.4 Chlorantraniliprole residue concentrations in pollen and nectar

No chlorantraniliprole residues above the LOQ level of 0.001 mg/kg were found in any of the pollen or nectar control samples taken at all 7 dates (DAA1-1, DAA1+1, DAA1+3, DAA1+8, DAA1+10, DAA1+11 and DAA1+17). Also, no chlorantraniliprole residues above the LOQ level of 0.001 mg/kg were found in any of the pollen or nectar chlorantraniliprole samples taken at or before the 1st chlorantraniliprole spray application (DAA1-1).

Residues of chlorantraniliprole above the LOQ level were found for all matrices after the 1st and 2nd chlorantraniliprole spray application (Table 3). Chlorantraniliprole residues in pollen samples were generally about two orders of magnitude higher compared to the nectar samples. Maximum chlorantraniliprole residue values in pollen were measured 1 day after the 1st or 2nd chlorantraniliprole spray application at 1.546 mg/kg (from honeybee forager bees) and at 2.160 mg/kg (from honeybee combs), respectively. Chlorantraniliprole residue values in pollen decline rapidly after the 1st and 2nd spray application. Maximum chlorantraniliprole residue values in nectar were also measured directly (1 day) after the 1st or 2nd chlorantraniliprole spray application at 0.023 mg/kg (from honeybee forager bees) and at 0.037 mg/kg (from bumblebee hive cells), respectively. Residue levels detected in honeybee and bumblebee nectar were similar.

Table 3 Maximum residue concentrations of chlorantraniliprole (mg/kg) detected in pollen and nectar collected by honeybees or bumblebees (pollen loads or nectar stomach content from forager bees, or collected from inside the hives) following 2 spray applications of chlorantraniliprole at 60 g a.s./ha during bee flight in flowering *Phacelia tanacetifolia*.

Timing of sampling	**Chlorantraniliprole residues (mg/kg)**				
	Honeybees				Bumblebees
	Pollen		**Nectar**		**Nectar**
DAA1 (DAA2)	Forager bees	Hive combs	Forager bees	Hive combs	Hive cells
-1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
+1	1.546	1.575	0.023	< LOQ	0.001
+3	0.335	1.822	0.005	0.001	0.008
+8 (-1)	0.082	0.075	0.001	0.002	0.003
+10 (+1)	0.832	2.160	0.018	0.001	0.037
+11 (+2)	0.349	0.112	0.007	0.003	0.010
+17 (+8)	0.027	0.583	0.002	0.003	0.012

(LOQ = Level of quantification = 0.001 mg/kg. DAA = Days after application (1 or 2))

4. Discussion

Experiences with bumblebee testing to determine the hazard and toxicity of pesticides to bumblebees, including semi-field approaches, were reviewed by Van der Steen (2001)[10] summarizing technical challenges, e.g. in sufficient food resources in small tents. In the current bumblebee semi-field trial with large 60-m^2-tunnels it was possible to expose *B. terrestris* colonies with starting sizes of over 400 individuals over a period of 29 days to treated flowering *Phacelia*.

Additional transient short-term feeding with sugar solution was only performed from 17 to 20 August 2013 (DAA1+5 to DDA1+8) and at 30 August 2013 (DAA1+18, equivalent to +9 days after the 2nd spray application of chlorantraniliprole) in order to avoid larval stress, because slightly increased larval mortality was observed in control colonies. The impact of the additional feeding of the bumblebee colonies with untreated sugar solution with regards to the effects of chlorantraniliprole on the tested bumblebee colonies is considered low, because the chlorantraniliprole residue levels detected in nectar were relatively low versus those found in pollen, highlighting that the main route of chlorantraniliprole exposure for bees is via pollen and not via nectar. Also a rapid decline of the chlorantraniliprole concentrations in nectar (as well as for pollen) from one to two or three days after chlorantraniliprole spraying was observed. Therefore, the bumblebee colonies in the chlorantraniliprole treatment were exposed to a worst-case scenario, because the bees could only forage on a highly bee-attractive crop (*Phacelia*) treated twice at 60 g a.s./ha.

The maximum chlorantraniliprole residue levels detected in this trial as well as the rapid decline of residue concentrations are very much in line with residue results found in an earlier semi-field *Phacelia* honeybee trial with a maximum pollen and nectar concentration of 2.863 and 0.0472 mg chlorantraniliprole/kg, respectively[6]. The pollen and nectar chlorantraniliprole residue data of both bee studies highlight that bees foraging in chlorantraniliprole treated crops will only temporarily be exposed to high levels of chlorantraniliprole.

The biological findings of this bumblebee study show that the control colonies developed well under the experimental test conditions with a significant increase in colony strength and resulting in production of significant numbers of drones and queens. At the same time it could be shown by spraying a toxic reference that the test system was able to show complete impairment of reproduction, which was due to high initial worker mortality and lack of queen survival.

In contrast to the toxic reference, chlorantraniliprole applied twice via spray application on flowering *Phacelia* with a 9 day interval during bumblebee flight activity did not have any pertinent effects regarding all parameters assessed, i.e. mortality, flight activity, hive weight, condition of colonies, development of bumblebee brood, production of young queen and drone offspring and vigor relative to the water treated control.

In a worst-case chronic oral exposure experiment with small artificial *B. terrestris* colonies – without a queen – under laboratory conditions, bumblebees were constantly exposed to Coragen via pollen dosed between 0.4 to 40 mg a.s./kg over 7 weeks resulting in suppression of reproduction in worker bumblebees[11]. The measured magnitude and rapid decline of chlorantraniliprole pollen concentrations measured in the current semi-field bumblebee trial show that the laboratory experiment was highly over-dosed and represented an unrealistic exposure scenario for chlorantraniliprole, which is also confirmed by the chlorantraniliprole pollen residue data of a previous honeybee semi-field study[6].

Lack of effects on foraging activity, adult mortality, colony weight and queen production were found for bumblebees, *Bombus impatiens,* foraging on flowering white clover in lawns that were treated with 230 g chlorantraniliprole/ha followed by irrigation , while for another tested insecticide (clothianidin) effects were found[12].

5. Conclusions

Low toxicity for honeybees and bumblebees was demonstrated for chlorantraniliprole and its formulated products in worst-case semi-field and greenhouse trials[6]. The current semi-field bumblebee study with chlorantraniliprole applied twice via spray application on flowering *Phacelia* at 60 g a.s./ha during bumblebee flight confirms the previous findings; no pertinent effects were observed in all parameters assessed, i.e. mortality, flight activity, hive weight, condition of colonies, development of bumblebee brood, production of young queen and drone offspring and vigor relative to the water treated control. As chlorantraniliprole has also proven to have negligible effects on numerous beneficial non-target arthropod species or to have a rather

low and transient impact on some beneficial species, it provides an excellent tool for integrated pest management (IPM) programmes.

6. Acknowledgments

Sincere thanks to all co-operators performing the bumblebee semi-field study.

7. References

1. Cordova D, Benner EA, Sacher MD Rauh JJ, Sopa JS, Lahm GP, Selby TP, Stevenson TM, Flexner L, Gutteridge S, Rhoades DF, Wu L, Smith RM, Tao Y, Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pestic Biochem Phys* **84**: 196-214 (2006).
2. Lahm GP, Stevenson TM, Selby TP, Freudenberger JH, Cordova D, Flexner L, Bellin CA, Dubas CM, Smith BK, Hughes KA, Hollingshaus JG, Clark CE, Benner EA. Rynaxypyr® : A new insecticide anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Bioorg Med Chem Lett* **17**: 6274-6279 (2007).
3. Dinter A, Brugger K, Bassi A, Frost N-M, Woodward M (2008): Chlorantraniliprole (DPX-E2Y45, DuPont™ Rynaxypyr®, Coragen® and Altacor® insecticide) - a novel anthranilic diamide insecticide - demonstrating low toxicity and low risk for beneficial insects and predatory mites. WG Pesticides and Beneficial Organisms, Proceedings of the Meeting at Berlin, Germany, 10th - 12th October 2007, Editors: Heidrun Vogt, Jean-Pierre Jansen, Elisa Vinuela & Pilar Medina. *IOBC/wprs Bull* **35**: 128-135 (2008).
4. Brugger KE, Cole PG, Newman IC, Parker N, Scholz B, Suvagia P, Walker G, Hammond TG (2010) Selectivity of chlorantraniliprole to parasitoid wasps. *Pest Manag Sci* **66**: 1075-1081.
5. Larson JL, Redmond CT, Walker D (2012) Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass. *Pest Manag Sci* **68**: 740-748.
6. Dinter A, Brugger KE, Frost N-M, Woodward MD (2009): Chlorantraniliprole (Rynaxypyr): A novel DuPont™ insecticide with low toxicity and low risk for honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) providing excellent tools for uses in integrated pest management. Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group. *Julius-Kühn-Archiv* **423**: 84-96.
7. Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G (2010) Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Aphidae). *Pest Manag Sci* **66**: 142-146.
8. EPPO, Guideline for the efficacy evaluation of plant protection products – Side effects on honeybees. OEPP/EPPO, PP 1/170 (4) update 2010. *Bulletin OEPP/EPPO Bulletin* **40**: 313-319.
9. Barrett KL, Grandy N, Harrison EG, Hassan S, Oomen P (eds.), Guidance Document on Regulatory Testing Procedures for Pesticides with Non-Target Arthropods. From the ESCORT Workshop (European Standard Characteristics of Beneficial Regulatory Testing); Wageningen, Holland, 28 - 30 March, 1994. 51pp (1994)
10. Van der Steen JM (2001) Review of the methods to determine hazards and toxicity of pesticides to bumblebees. *Apidologie* **32**: 399-406
11. Smagghe G, Deknopper J, Meeus I, Mommaerts V (2013) Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. *Pest Manag Sci* **69**: 787-791.
12. Larson JL, Redmond CT, Walker DA (2013) Assessing insecticide hazard to bumble bees foraging on flowering weeks in treated lawns. *Pest Manag Sci* **68**: 740-748. *PLOS ONE* **8**: e66375.