1.5 Cyantraniliprole: Pollinator profile of the novel insecticides under laboratory, semi-field and field conditions

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Abstract

Background: The pollinator profile of cyantraniliprole, a systemic anthranilic diamide insecticide, with foliar or soil applications between 12.5 to 150 g a.s./ha, was investigated.

Results: Cyantraniliprole - tested up to maximum water solubility level – caused no increased acute oral or contact honeybee mortality. The lowest LD50 values for formulated cyantraniliprole were 0.39 (oral) and 0.63 (contact) µg cyantraniliprole/ honeybee, respectively. The oral toxicity of 4 plant metabolites was maximally similar to cyantraniliprole or no oral toxicity was determined up to maximal water solubility level. Cyantraniliprole spray deposits at 150 g a.s./ha and aged for ≥ 3 hours pose low risk for honeybees. Cyantraniliprole use may results in residues in pollen and nectar, but oral honeybee risk assessments indicate low risk for honeybees via oral exposure. In semi-field and field honeybee tests low risk for honeybees was confirmed. Tomato greenhouse study results demonstrate that there is an excellent fit between the use of bumblebees (*Bombus terrestris*) for pollination and cyantraniliprole – applied either via spray or drip irrigation.

Conclusion: Based on a comprehensive data package it was found that the intended uses of DuPont cyantraniliprole formulations pose low risk for pollinators.

Key words: Cyantraniliprole, insecticide, side-effects, honeybee, bumblebee

1. Introduction

Cyantraniliprole (DPX-HGW86, DuPont™ Cyazypyr®) is the second the anthranilic diamide insecticide (IRAC Group 28) discovered by E.I du Pont de Nemours and Company, Inc., next to chlorantraniliprole, which is known for its low intrinsic toxicity for honeybees and bumblebees and negligible effects on numerous beneficial non-target arthropod species[1,2,3,4,5]. Cyantraniliprole is the first anthranilic diamide insecticide to control a cross-spectrum of chewing and sucking pests, and being developed by DuPont and Syngenta. Cyantraniliprole is a systemic insecticide and DuPont products containing cyantraniliprole are optimized for foliar or soil applications and are effective on a wide range of crops (i.e. for vegetable and top fruit crops). Application rates may vary for different pests and crops between 12.5 to 150 g a.s./ha with up to 2 applications per crop. Cyantraniliprole spray formulations, cyantraniliprole 100 g/L OD and cyantraniliprole 100 g/L SE[2], may be mixed with up to 2.5 L Codacide Oil (oil seed rape oil, developed by Microcide Ltd.) per ha. The cyantraniliprole soil application formulation, cyantraniliprole 200 g/L SC[3], is intended to be used via drip irrigation applied twice at up to 75 g a.s./ha. Also, cyantraniliprole is effective as a seed treatment in e.g., rape. For seed treatment use, cyantraniliprole 625 g/L FS[4], is effective at 50 µg a.s./rape seed.

2. Experimental Methods

The effects of cyantraniliprole and its four formulations on pollinators were investigated in GLP studies using adopted test guidelines for honeybees (i.e., OECD or EPPO test methods) or with modifications to address specific questions or to study effect on bumblebees.

[2] Cyantraniliprole 100 g/L OD formulation is Benevia® and Cyantraniliprole 100 g/L SE formulation is Exirel®.
[3] Cyantraniliprole 200 g/L SC formulation is Verimark®.
[4] Cyantraniliprole 625 g/L FS formulation is Lumiposa®.

2.1 Acute honeybee and bumblebee testing

The acute toxicity to the honeybee (*Apis mellifera* L.) (Hymenoptera, Apidae) was investigated in oral and contact tests following OECD Guideline No. 213 and No. 214[6,7]. Cyantraniliprole technical material was tested up to the maximum water solubility level. The maximum achievable dose rate was 0.11 µg cyantraniliprole/honeybee in the oral test. In the contact test applying 5-µL-droplets a maximum rate of 0.09 µg cyantraniliprole/honeybee could be applied. Oral and contact tests with the formulated products were performed without the use of any additional organic solvents. The acute toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera, Apidae) was studied in oral and contact tests following Van der Steen (2001)[8] and OECD 213/214 (1998)[6,7] with modifications and adaptions according the recommendations of the ICPPR non-*Apis* ring test group in the year 2014. Additionally acute oral honeybee tests with four cyantraniliprole metabolites (IN-HGW87, IN-J9Z38, IN-K5A78 and IN-DBC80) were performed, at least up to the maximum water solubility level of the metabolites, partly including 1% acetone to get the metabolite into stable solutions[6].

2.2 Foliage residue honeybee toxicity

The duration of the toxicity of cyantraniliprole foliage residues was evaluated in a study with cyantraniliprole 100 g/L OD following USEPA OPPTS 8503030 test guideline[9]. Honeybees were exposed under laboratory conditions for 24 hours to alfalfa foliage after cyantraniliprole spray application at 150 g a.s./ha and after different ageing periods.

2.3 Semi-field tunnel honeybee testing

Several semi-field tunnel tests were conducted following the EPPO 170 (3) & (4) test design with flowering *Phacelia tanacetifolia* Benth., rape (*Brassica napus* L.) or melon (*Cucumis melo* L.), as a model crop[10,11].

2.3.1 Semi-field tunnel honeybee testing to assess effects from pre-flowering spray applications

In a semi-field test the potential systemic impact of cyantraniliprole on honeybees was studied by spraying non-flowering winter oil seed rape twice and later exposure of honeybees in tunnels during the rape flowering period. This study was conducted in Southern Germany in April to May 2009 and included four treatment groups. Pre-flowering sprays in the control (2-times tap water at 300 L/ha), cyantraniliprole 100 g/L OD and cyantraniliprole 100 g/L SE treatment (each 2-times at 150 g a.s./ha plus 2.5 L Codacide Oil per ha) were made at 15 April (BBCH 51/52, DAE-14) and 21 April (BBCH 55, DAE-8) before setup of honeybee hives inside tunnels on 28 April (DAE-1) in the evening. Spraying in the toxic reference (400 g dimethoate/ha) was done at 1 May (BBCH 63-65, DAE2) (DAE = Days after exposure of honeybees in the test tunnels). Mortality, foraging activity, behaviour, and brood and colony strength were assessed during the 1-week tunnel exposure period, and/or at a remote site up to 4 weeks later.

2.3.2 Semi-field tunnel honeybee testing to assess effects from spray application during flowering (including a pre-flowering spray)

In a *P. tanacetifolia* tunnel study conducted in Northern Germany in June/July 2008, cyantraniliprole was studied following a pre-flowering spray followed by a spray during flowering 14 days later with cyantraniliprole 100 g/L OD. Cyantraniliprole was treated twice – once before flowering and once 14 days later during bee-flight – at a rate of 10 g a.s./ha (T1) or 100 g a.s./ha (T2). The control was sprayed once with tap water (400 L/ha) as well as the toxic reference (400 g dimethoate/ha) at the same day as cyantraniliprole treatments T1 and T2 were sprayed the 2nd time during bee flight and full flowering at 18 June 2008 (BBCH 65). Mortality, foraging activity, behaviour and brood and colony strength were assessed during the tunnel exposure period (before and after the 2nd cyantraniliprole spray), and/or at a remote site up to 4 weeks later.

2.3.3 Semi-field tunnel honeybee testing to assess effects from spray application during flowering (including a pre-flowering spray) on bee brood

The potential effect of cyantraniliprole on the honeybee brood development was investigated in semi-field study following EPPO 170 (3) and the OECD Guidance Document No 75 recommendations s[12]. The study encompassed 3 treatment groups (control, cyantraniliprole and toxic reference), each with 3 replicate tunnels. Cyantraniliprole (cyantraniliprole 100 g/L OD at 150 g a.s./ha plus Codacide Oil at 2.5 L/ha) was sprayed twice with an application interval of 15 days on *P. tanacetifiolia* plots in growth stages BBCH 58 and BBCH 65, respectively. The 1st spray application onto the non-flowering *Phacelia* crop (20 June 2009) was only performed in the control and cyantraniliprole treatment. The 2nd spray application (5 July 2009) was performed in all 3 treatment groups in the evening after daily bee flight (spray volume of 400 L/ha). The toxic reference was sprayed at 300 g fenoxycarb/ha.

2.3.4 Semi-field tunnel honeybee testing to assess effects from soil application (drip irrigation) during flowering

The effect of cyantraniliprole applied directly to the soil as drip application was investigated in a tunnel trial conducted in the province of Valencia in Spain from July to September 2010. Two cyantraniliprole drip applications with cyantraniliprole 200 g/L SC at a rate of 100 g a.s./ha with an application interval of 7 days were tested and with the last application (05 Aug 2010) during full flowering of the melon plants when enough flowers are present to allow foraging of the bees. The second application was done in the evening after bee flight the day before the application in the control and in the toxic reference. The applications were carried out with a drip volume of 2500 L water/ha, plus an irrigation volume of 2500 L water/ha before application and of 5000 L water/ha after the application. During the drip applications in cyantraniliprole treatment, the control and toxic reference groups received an irrigation of 10000 L water/ha. The toxic reference was sprayed at 400 g dimethoate/ha during bee flight and full flowering of the melons (on the day after the second drip application in the test cyantraniliprole treatment). The application was carried out with a spray volume of 1000 L water/ha. The control application with tap water was made the same day as the spray in the toxic reference with a spray volume of 1000 L/ha. The effects were examined on small honeybee colonies in tunnel tents (5.0 m x 40.0 m and a height of 3.5 m) placed over two rows of melon plants. The honeybee colonies were placed in the tunnels at the flowering of the melons in the night between the 01 Aug 2010 and 02 Aug 2010, 5 days before the application in C and R. The semi-field test comprised 3 replicate tunnels in each of the treatment groups.

2.4 Field honeybee testing

2.4.1 Field honeybee testing in rape

The effects of cyantraniliprole were tested on the honeybee under field conditions following EPPO 170 (3) plus recommendations by Lewis *et al.* (2009)[13]. This study was conducted in Southern Germany starting in April 2010 and was continued until end of overwintering in spring 2011 (rape field size about 1 ha). Cyantraniliprole treatment group T1 had two applications. The first application was performed before set-up of the honeybee colonies at the experimental fields and before flowering of *B. napus* L. The second application was performed during flowering of rape and after set-up of the honeybee colonies at the experimental fields, in the evening after daily honeybee flight. Each application was carried out with cyantraniliprole 100 g/L OD at a rate of 90 g a.s./ha. An untreated rape field served as control field. The first spray application in T1 was performed of non-flowering rape on the 26 April 2010 (BBCH 59). The honey bee colonies were set up at these pre-treated experimental fields on the 03 May 2010 during rape flowering (BBCH 63 on the control field and BBCH 63-65 on the field T1, recorded on 04 May 2010). The second application was performed on flowering rape on the 16 May 2010 (BBCH 65-67) in the evening,

after daily honey bee-flight. On 14 June 2010 (DAA+28), all bee colonies were removed from the field sites and transported to a monitoring site. The colony condition was assessed every 7±1 days until the end of the swarming period (ca. 12 July 2010). After the swarming season and until the end of the bee season (05 October 2010) the colony condition was assessed every 21±2 days. An additional assessment was made at the end of overwintering period on 05 April 2011. Applications were carried out with a spray volume of 300 L/ha. The effects cyantraniliprole were examined on 6 commercial honeybee colonies placed at each test field.

2.4.2 Field honeybee testing in melon

The melon field study was conducted in Southern Spain from July 2011 to March 2012. The effects of cyantraniliprole were examined on 6 commercial honeybee colonies placed at each test field. The study comprised 1 replicate melon field for each of the treatments following EPPO 170 (4)[11]. The study included three treatment groups. Cyantraniliprole with two applications of cyantraniliprole 100 g/L OD at a rate of 90 g a.s./ha plus 2.5 L Codacide Oil per ha sprayed in the evening after bee-flight (T1). The first application was performed at start of melon flowering and after set-up of the honeybee colonies at the experimental fields, and the second application was performed 7 days after the first application during flowering of melon. Cyantraniliprole sprays made in treatment T2 were made with cyantraniliprole 100 g/L OD during daily honeybee flight at similar application rates and dates, while the control field was untreated. The honeybee colonies were set up at the experimental fields on the 31 Jul 2011 to 01 August 2011 during night at early flowering of melon (BBCH 61-62). The first cyantraniliprole application was performed onto fields of flowering melons on the 04 August 2011 in the evening after bee flight in T1 and on 05 August 2011 during daily bee flight in T2 (BBCH 61-62). The second applications were performed on 11 August 2011 in the evening after bee flight in T1 (BBCH 66-67) and on the 12 August 2011 during daily bee flight in T2 (BBCH 65-67). All applications were carried out with a spray volume of 1000 L/ha. On 27 August 2011 (DAA2+15; DAA2 = Days after 2nd application of T2) all bee colonies were removed from the field sites and transported to a monitoring site. The colony condition was assessed every 7±1 days until DAA2+28. Until the end of the bee season (21 October 2011) the colony condition was assessed every 14±2 days. On 01 March 2012 the last brood evaluation was made to check overwintering success of the test colonies.

2.5 Greenhouse bumblebee testing

In a the semi-field tomato greenhouse trial in Southern Spain the effects cyantraniliprole applied via drip irrigation or applied as spray solution on colonies of the bumblebee *B. terrestris* were studied based on general SETAC/ESCORT recommendations (Barrett *et al.* 1994)[14] and EPPO No. 170 (3). The study was comprised of 4 cyantraniliprole treatments and a control group. In two treatments cyantraniliprole 200 g/L SC was applied via drip irrigation 3-times at 100 g a.s./ha (T1: drip irrigation 21, 14 and 7 days before release of the bumblebees in the greenhouse compartment, and T2: drip irrigation 14, 7 and 1 day before release of the bumblebees in the greenhouse compartments). The drip application volume was 5000 L/ha (followed by 3-times 5000 L tap water per ha, that was done in all treatments and in the control). In the other two treatments cyantraniliprole 100 g/L OD was sprayed 3-times at 10.0 g a.s./hL plus 0.25 % (v/v) Codacide Oil/ha and at a target application volume of 800 to 1100 L/ha (equivalent to application rates of 80 to 110 g a.s./ha; T3: spray application 15, 8 and 2 days before release of the bumblebees in the greenhouse compartments (the last application 2 days before release of the bumblebees was performed in the evening = about 37-38 hours before release), and T4: spray application 14, 7 and 1 day before release of the bumblebees in the greenhouse compartments (the last application 1 day before release of the bumblebees will be performed in the evening = about 15-16 hours before release)). The control was sprayed with tap water performed 1 day before release of the bumblebees in the greenhouse compartments together with the last spray application in T4 and the last drip application in T2; all applications were performed with closed bumblebee hives and

no bumblebees in the plots. Each treatment group was divided in 4 plots of about 400 m² each separated by a net with one bumble bee colony, each consisting of a young queen plus 25 worker bumblebees plus brood stages. The influence of cyantraniliprole was evaluated by comparing the results in the four treatments to the control regarding the following observations: Number of living and dead worker bees and larvae, foraging activity as measured by flower visits (bite marks), consumption of sugar solution, development of the bumblebee brood and condition of the colonies. To assess the foraging/pollination activity of the bumblebees the tomato blossoms were classified in 4 categories and each category received points (category 1: no bite mark = 1 point; category 2: 1-3 bite marks/blossom = 2 points; category 3: > 3 bite marks/blossom = 3 points; category 4: blossom with brown pistil = 4 points).

2.6 Field honeybee testing with seed-treated rape

The effects of winter oil seed rape grown from seeds treated with cyantraniliprole 625 g/L FS were tested on the honeybee (*Apis mellifera* L.) under field conditions following EPPO No. 170 (3) plus recommendations by Lewis *et al.* (2009).

Two field studies were conducted (one in France and one in Germany) starting in September 2010 and were continued until October 2011. Both trials comprised 3 treatments: cyantraniliprole with rape seed loading of 50 µg a.s./seed (Treatment T1), another cyantraniliprole seed treatment (T2) and control (C, without insecticide seed treatment, just fungicides). The effects of cyantraniliprole seed treatment was examined on 6 commercial honeybee colonies placed at each of the experimental flowering rape fields the following spring. The field tests comprised 1 replicate field in each of the treatments (1 to 2 ha field size). The following parameter were assessed: Number of dead honeybees on the linen sheets and in the dead honeybee traps in front of the hives, foraging activity on the rape crop, condition of the colonies and development of the brood and behaviour of the honeybees in the crop area and around the hives. Samples of guttation liquid were taken from rape plants after emergence of the seedlings and once during flowering of the plants. Rape flowers were collected once from each field shortly after set-up of the honey bee colonies. Samples of sealed honey, pollen and wax were taken from each hive once during the exposure period. Also forager bees were collected in each treatment twice during the honeybee exposure period in spring. From these forager bees the pollen loads and nectar stomach contents were separated later in the laboratory. Samples of guttation liquid, flowers, honey, pollen and wax from hives, pollen loads from forager bees and nectar from stomachs of forager bees were analyzed for residues of cyantraniliprole and its metabolites with a level of quantification (LOQ) of 5.0 µg/kg.

Also, residues in pollen and nectar were analyzed from summer oil seed rape grown from seeds treated with cyantraniliprole 625 g/L FS at 100 µg a.s./seed at 4 sites in Canada in 2009.

2.7 Additional tests to quantify residue in pollen and nectar

Cyantraniliprole and metabolite residue concentrations were determined i.e., in nectar and pollen following application with all cyantraniliprole formulation applied pre- and/or during flowering in many different crops partly above the intended application rates to quantify the level of oral exposure for bees. Studies were conducted under laboratory conditions (radiolabeled translocation tests) or under field conditions in different i.e., EU countries applying different sampling techniques (e.g., hand sampling or sampling by bees (pollen load, stomach content)).

3. Results

3.1 Acute honeybee and bumblebee toxicity

No increased mortality was observed, when honeybees or bumblebees were exposed orally or by contact to the active substance cyantraniliprole at the maximum solubility in water. The oral and contact honeybee LD_{50} values using water as solvent were >0.11 and >0.09 µg cyantraniliprole/bee, respectively. The oral and contact bumblebee LD_{50} values using water as

solvent were >0.28 and >0.09 µg cyantraniliprole/bee, respectively (Table 1). The higher oral endpoint for the bumblebees is a result of the 2-fold oral dose being offered in the oral bumblebee test versus the oral honeybee test. The three formulations tested demonstrated similar toxicities for honeybees. The lowest oral and contract honeybee LD_{50} values were determined for the cyantraniliprole 100 g/L OD formulation with 0.39 and 0.65 µg a.s./honeybee, respectively. The cyantraniliprole 100 g/L SE formulation was slightly less honeybee toxic with LD_{50} values of 0.92 and 2.78 µg a.s./honeybee, respectively. The two formulated products applied as spray formulations meet the EU oral and contact hazard quotient (HQ) criteria of 50 up to application rates of 19.5 and 32.5 g a.s./ha (cyantraniliprole 100 g/L OD) or 32.5 and 139 g a.s./ha (cyantraniliprole 100 g/L SE), respectively.

Table 1 Acute oral and contact toxicity of cyantraniliprole and formulated products on honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*).

Test material	**Oral LD_{50}** (µg a.s./bee)	**Contact LD_{50}** (µg a.s./bee)	**Oral LD_{50}** (µg a.s./bee)	**Contact LD_{50}** (µg a.s./bee)
	Honeybee (*Apis mellifera*)		**Bumblebee (*Bombus terrestris*)**	
Cyantraniliprole technical (in water)*	>0.11	>0.09	>0.28	>0.09
Cyantraniliprole 100 g/L OD	0.39	0.65	46.00	92.52
Cyantraniliprole 100 g/L SE	0.92	2.78	>0.47	>100
Cyantraniliprole 200 g/L SC	0.40	0.66	>0.53	>100

* = tested up to maximum water solubility limit

In comparison to the honeybee, *A. mellifera*, the bumblebee species *B. terrestris* was clearly less sensitive to cyantraniliprole (Table 1). Definitive oral and contact LD_{50} endpoints were determined for the cyantraniliprole 100 g/L OD formulation with 46.00 and 92.52 µg a.s./bumblebee, respectively, which are about two orders of magnitude higher than the corresponding honeybee endpoints. For the other 2 formulations (cyantraniliprole 100 g/L SE and cyantraniliprole 200 g/L SC) no increased bumblebee mortality was determined up to the highest dose rates tested.

For the cyantraniliprole metabolite, IN-HGW87, which may be found in plant matrices, an oral LD_{50} of 0.298 µg/honeybee was determined, similar to the lowest definitive endpoint determined for parent substance, cyantraniliprole (tested as cyantraniliprole 100 g/L OD) (Table 2). The three other metabolites resulted in no honeybee mortality increase up to the tested maximum water solubility level of the individual metabolites.

Table 2 Acute oral toxicity of cyantraniliprole and plant metabolites on honeybees (*Apis mellifera*).

Test material	**Oral LD_{50}** (µg cyantraniliprole or metabolite per honeybee)
Cyantraniliprole technical (in water)*	>0.11
Cyantraniliprole 100 g/L OD	0.39
IN-HGW87**	0.298
IN-J9Z38***	>0.008
IN-K5A78	>45.61
IN-DBC80*	>49.29

* = tested up to maximum water solubility limit
** = tested in water plus 1% acetone
*** = tested at maximum solubility in water plus 1% acetone

3.2 Foliage residue honeybee toxicity

Honeybees showed no treatment related mortality or behaviour abnormalities when exposed to alfalfa foliage which was treated at 150 g cyantraniliprole/ha and aged for 3, 8, 24, 48, or 72 hours.

3.3 Results of semi-field tunnel honeybee tests

3.3.1 Results of semi-field tunnel honeybee testing to assess effects from pre-flowering spray application

Flight activity in the control was between and 1.1 to 12.3 forager bees/m^2 during DAE+1 to DAE+8 in the flowering rape tunnels (Figure 1a). There were no significant differences of the daily flight activity in the two treatments which were sprayed twice with cyantraniliprole onto the pre-flowering rape compared to the control during this period (DAE+1 to DAE+8; Bonferroni-U-test for data on DAE+7 and two-sided Dunnett's t-test for all other days, $p > 0.05$; no analysis was performed for DAE0 because there was no flight activity in any treatment detectable in the crop). There were no statistically significant differences of the daily mortality in the cyantraniliprole treatments compared to the control during the whole exposure period, except for the value of 3.0 dead honey bees in the cyantraniliprole 100 g/L SE treatment (T1) on the day after set-up of the colonies (DAE0) (one-sided 'upper' Dunnett's t-test, $p \leq 0.05$), but this slight difference on DAE0 is not being viewed as treatment related and not colony relevant (Figure 1b). In contrast, spraying of the toxic reference (R) during rape flowering and during bee flight at DAE+2 resulted in significantly reduced foraging intensity (one-sided pooled t-test for data on DAE+3, DAE+7 and DAE+8; Satterthwaite (Welch) test for data on DAE+4, DAE+5 and DAE+6, $p \leq 0.05$) and increased mortality (DAE+3 to DAE+8) were statistically significant (one-sided Satterthwaite (Welch) test for data on DAE+3 and one-sided pooled t-test for all other days, $p \leq 0.05$; logarithmic values were used for data on DAE+4 and DAE+5) on all days except for the assessment on DAE+7. In both cyantraniliprole treatment groups T1 and T2 and also in the control group, normal honeybee behaviour was recorded throughout the observation period (DAE0 to DAE+8). Overall, the pre-flowering sprays with cyantraniliprole had no negative effect on the flight activity, mortality, behaviour, or brood/colony development up to DAE+28 at a remote site, where the bee hives were kept after the exposure phase.

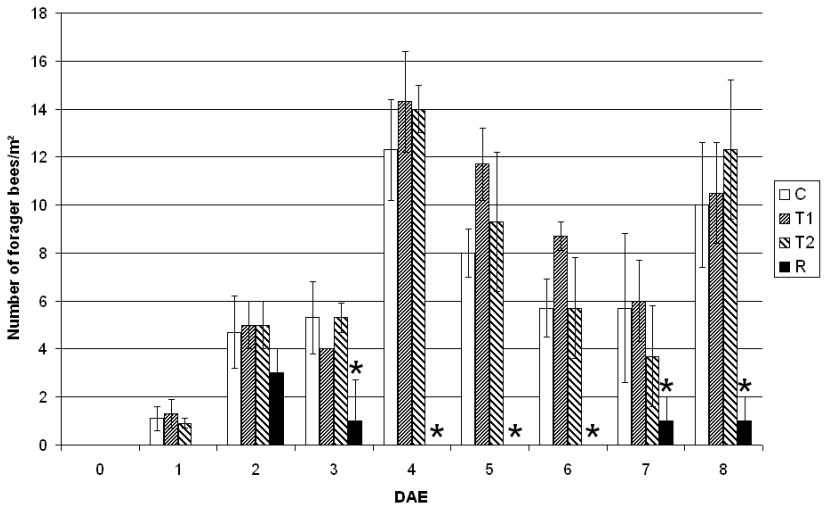

Figure 1a Flight intensity

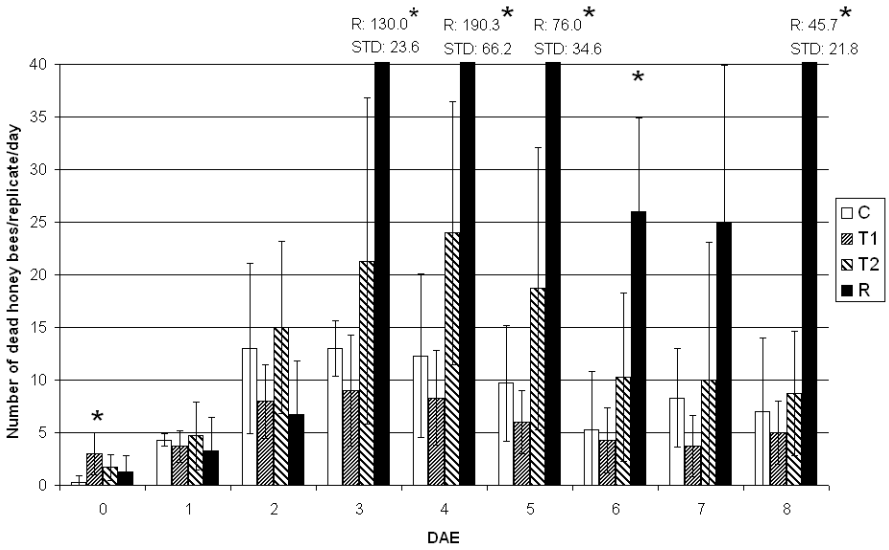

Figure 1b Mortality

Figure 1: Mean honeybee flight intensity (number of forager bees/m² ± STD) (a.) and mean honeybee mortality (number of dead honeybees/replicated tunnel/day ± STD) (b.) in the control (2-times water at 300 L/ha) (C), cyantraniliprole 100 g/L SE (2-times at 150 g a.s./ha plus 2.5 L Codacide Oil per ha) (T1), cyantraniliprole 100 g/L OD (2-times at 150 g a.s./ha plus 2.5 L Codacide Oil per ha) (T2) and toxic reference treatment (1-time 400 g dimethoate/ha) (R) after pre-flowering spray application in winter oilseed rape in Germany, 2009. (Pre-flowering sprays in C, T1 and T2 at 15 April (BBCH 51/52, DAE-14) and 21 April (BBCH 55, DAE-8) before setup of hive inside tunnels on 28 April (DAE-1) in the evening. Spray in R at 1 May (BBCH 63-65, DAE2) (DAE = Days after cxposure of honeybees in the test tunnel tents. * = statistical significant difference to control)

3.3.2 Semi-field tunnel honeybee testing to assess effects from spray application during flowering (including a pre-flowering spray)

No indications were found that the first application of cyantraniliprole (before start of flowering) at rates of 10 g a.s./ha (T1) or 100 g a.s./ha (T2) had any negative effect on the flight activity or mortality of the honeybee colonies that were set up at the treated plots during flowering (9 days after the first application) and observed from the 10th to the 14th day after the first application (i.e., until the day of the second application) (Figure 2). Cyantraniliprole, applied during full flowering and honeybee flight at rates of 10 g or 100 g a.s/ha, had an effect on honeybee flight activity. If the application rate was 10 g a.s./ha (T1), there was a significant reduction of honey bee flight activity on the day of the application. If the application rate was at 100 g a.s./ha (T2), flight activity in the crop was significantly reduced on the day of the application and on the next day (two-sided Dunnett's t-test, $p \leq 0.05$) (Figure 2a). At application rates of 100 g a.s./ha, honeybee mortality increased on the day of the second application (during full flowering and honeybee flight) and on the next day (one-sided 'upper' Dunnett's t-test, $p \leq 0.05$) (Figure 2b). At application rates of 10 g a.s./ha, there was no increase in honeybee mortality. In contrast to both the cyantraniliprole treatment and the control, the toxic reference had a clear effect on bee flight activity and morality. At a rate of 10 g a.s./ha (T1), intoxication symptoms were detectable ca. 1-2 hours after the second application (during full flowering and honeybee flight). At a rate of 100 g a.s./ha (T2), intoxication symptoms were detectable ca. 2 hours after the application during full flowering and honeybee flight until the morning of the next day. It was found that cyantraniliprole, applied twice (once before flowering and set-up of the honeybee colonies, and 14 days later during full flowering and

bee-flight), with each application at rates of 10 g or 100 g a.s./ha showed no obvious test item related impact on the honeybee brood development.

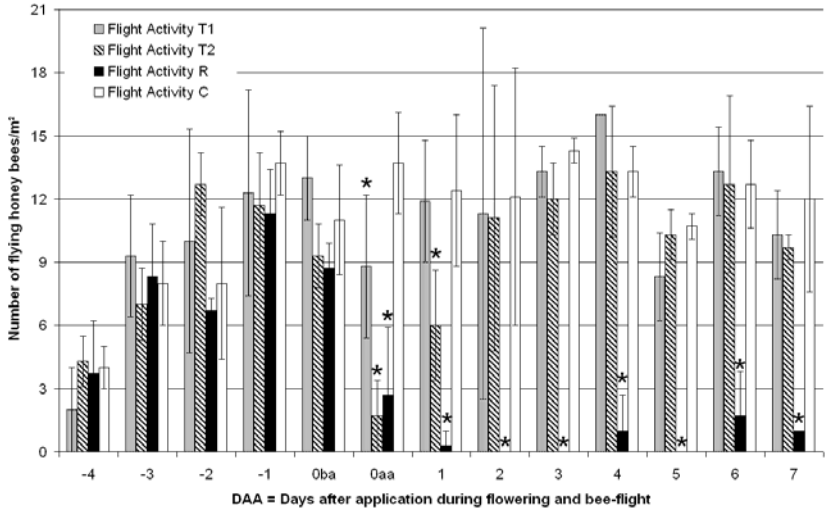

Figure 2a Flight intensity

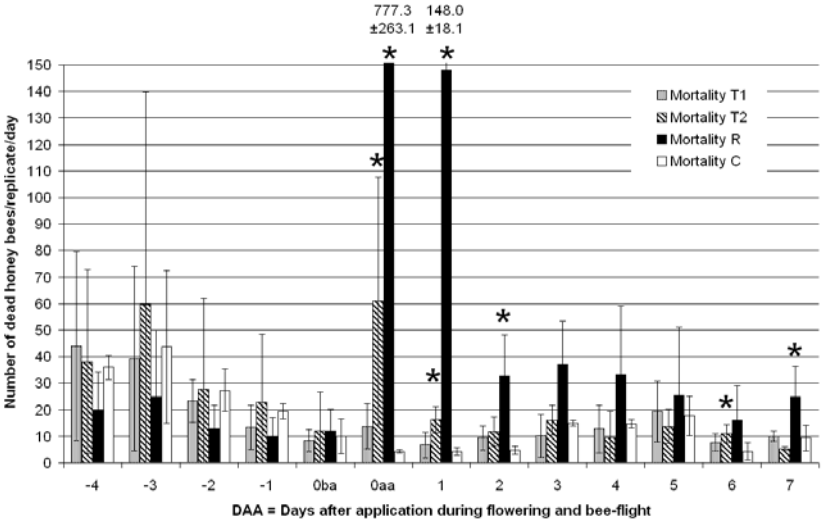

Figure 2b Mortality

Figure 2: Mean honeybee flight intensity (number of forager bees/m^2 ± STD) (a.) and mean honeybee mortality (number of dead honeybees/replicated tunnel/day ± STD) (b.) in the control (1-time water at 400 L/ha) (C), cyantraniliprole 100 g/L OD (2-times at 10 g a.s./ha) (T1), cyantraniliprole 100 g/L OD (2-times at 100 g a.s./ha) (T2) and toxic reference treatment (1-time 400 g dimethoate/ha) (R) after pre-flowering spray application and spray application during flowering and during bee flight in *Phacelia* in Germany, 2008. (1 pre-flowering spray in T1 and T2 at 4 June (BBCH 55/57, DAA-14) before setup of hive inside tunnels on 13 April (DAA-5) in the evening. Spray in T1 and T2 (2nd spray each), C and R at 18 June (BBCH 65, DAA0) (DAA = Days after application during bee flight) of honeybees in the test tunnel tents. * = statistical significant difference to control).

3.3.3 Semi-field tunnel honeybee testing to assess effects from spray application during flowering (including a pre-flowering spray) on bee brood

Following the 2nd application there was a distinct but short-term effect on honeybee mortality and foraging activity due to cyantraniliprole; no effects on colony development or colony strength were observed. With respect to the honeybee brood development, cyantraniliprole caused no effects on the brood nest size (brood stages in cm^2/colony), survival of marked eggs (brood termination rate), brood development from eggs into adult bees (brood index) and brood compensation ability (brood compensation index) (Figure 3). The calculated mean brood termination rate 23 days after brood fixing data was 28.1% and 15.2% in the control and cyantraniliprole treatment, respectively and therefore on a level typically for healthy honeybee colonies under semi-field conditions. Thus no cyantraniliprole effect on the brood development was detected. The high termination rate of 72.2% in the toxic reference indicated the suitability of the test system to detect potential effects of the test item on the brood development. Overall, based on the results of this study, cyantraniliprole applied twice at rate of 150 g a.s./ha, does not adversely affect honeybee colonies.

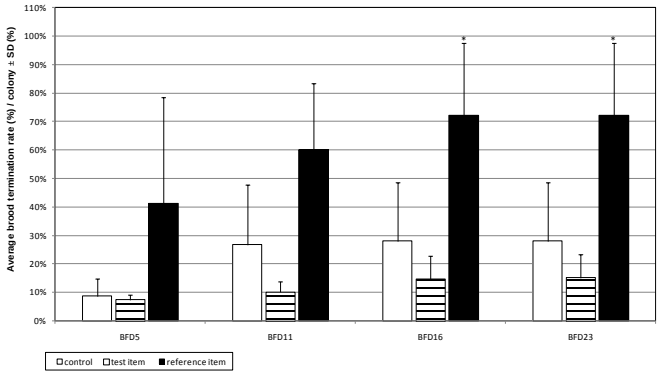

Figure 3 Mean termination rate of honeybee brood (% ± STD) in the control (1-time water at 400 L/ha) (control), cyantraniliprole 100 g/L OD (2-times at 150 g a.s./ha) (test item) and toxic reference treatment (1-time 250 g fenoxycarb/ha) (reference item) after pre-flowering spray application and spray application during flowering and after honeybee flight in *Phacelia* in Germany, 2008.
(1 pre-flowering spray in the test item treatment at 20 June (BBCH 58) before setup of hive inside tunnels on 2 July April (DAA-3) in the early morning. Spray in test item (2nd spray), control and reference item at 5 July (BBCH 65, DAA0) (BFD = Brood fixing date. DAA = Days after 2nd application after daily bee flight) of honeybees in the test tunnel tents. * = statistical significant difference to control)).

3.3.4 Semi-field tunnel honeybee testing to assess effects from soil application (drip irrigation) during flowering

The mean flight activity during the pre-application period (DAA-4 to DAA-1) was 1.7 honeybees/10 flowers in the control, 1.2 honeybees/10 flowers in the cyantraniliprole treatment, and 2.3 honeybees/10 flowers in the toxic reference. The mean flight activity over the whole post-application period was 1.2 honeybees/10 flowers in the control, 1.2 honeybees/10 flowers in the cyantraniliprole treatment and 0.9 honeybees/10 flowers in the toxic reference (Figure 4a). During the pre-application period before the second application means of 8.8 dead honeybees/day in the control, 16.8 dead honeybees/day in the cyantraniliprole treatment and 14.3 dead honeybees/day in the toxic reference were observed. The mean mortality during the whole post-application period was 3.6 dead honeybees/day in the control, 2.7 dead honeybees/day in the cyantraniliprole treatment and significantly higher numbers of 58.9 dead honeybees/day in the toxic reference (t-

test pooled, 1-sided, $p \leq 0.05$) (Figure 4b). The honeybees in the control and cyantraniliprole treatment group showed normal behaviour throughout the observation period. No behavioural abnormalities could be detected at any assessment date during exposure of the honeybees to the cyantraniliprole treated crop. In the toxic reference group bees were aggressive and showed intoxication symptoms on the day of the application and the day afterwards. No effect on the brood development was observed due to cyantraniliprole. The colony strength and the presence of the different brood stages and food resources was in the normal range throughout the study in the cyantraniliprole treatment and in the control.

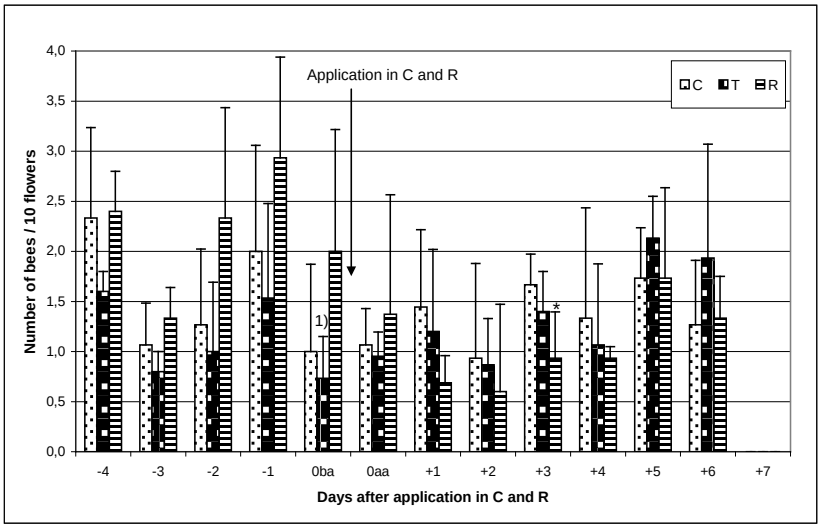

Figure 4a Flight intensity

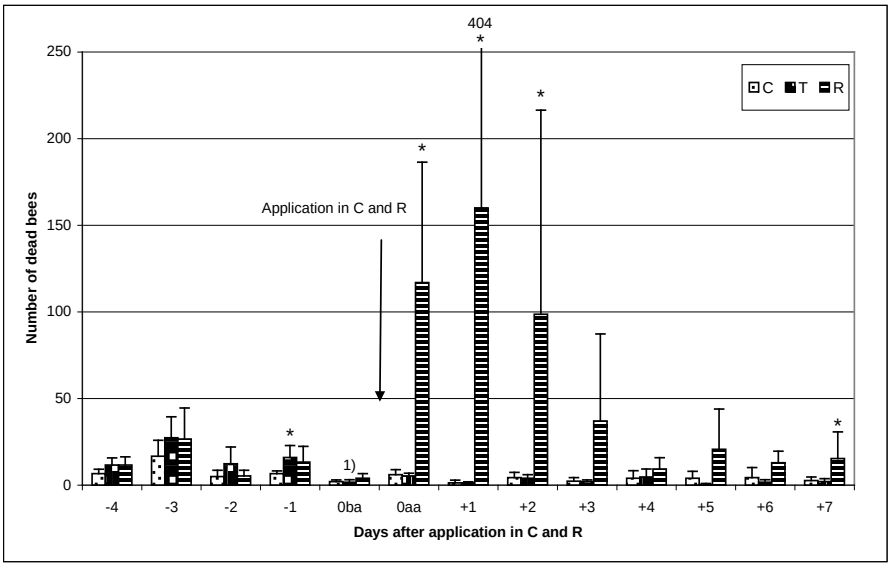

Figure 4b Mortality

Figure 4: Mean honeybee flight intensity (number of forager bees/10 flowers ± STD) (a.) and mean honeybee mortality (number of dead honeybees/replicated tunnel/day ± STD) (b.) in the control (C), cyantraniliprole 200 g/L SC (2-times at 100 g a.s./ha) (T), and toxic reference treatment (1-time 400 g dimethoate/ha) (R) during flowering and during honeybee flight in melons in Spain, 2010. (1st drip irrigation in T at 29 July (BBCH 61-63, DAA-8) before setup of hive inside tunnels during the night of 1-2 August (DAA-5 to DAA-4). 2nd drip irrigation in T at 5 August (BBCH 65, DAA-1) in the evening after daily bee flight, and spray in C and R at 6 August (BBCH 65, DAA0) (DAA = Days after application during bee flight) of honeybees in the test tunnel tents. * = statistical significant difference to control).

3.4 Field honeybee testing

3.4.1 Field honeybee testing in rape

It was found both cyantraniliprole applications (once before flowering, and once during flowering after honeybee flight) at 90 g a.s./ha had no effects on honeybee mortality and flight activity (Figure 5). Only slight effects on the behaviour of the honeybees were detected on DAA0aa. Both applications with cyantraniliprole had no short-term or long-term effect on honeybee colony condition and brood development throughout the whole season until start of overwintering in October. Survival rate of the honeybee colonies in the cyantraniliprole treatment during overwintering was comparable to the control.

Residues of cyantraniliprole were only found in honey samples of the first sampling date (DAA+16 and DAA+29) and were in a range from 0.0059 to 0.0069 mg cyantraniliprole/kg in 2 of 6 samples. In pollen, no quantifiable residues of cyantraniliprole were found at DAA+11, DAA+49 and DAA+323. Cyantraniliprole residues were found in two of six wax samples at each sampling date and were in a range from 0.0086 to 0.0334 mg cyantraniliprole/kg (DAA+11 and DAA+49), while no residues were found at DAA+323. There were no residues of any of the cyantraniliprole metabolites detected above LOQ in honey, pollen and wax of the cyantraniliprole treatment.

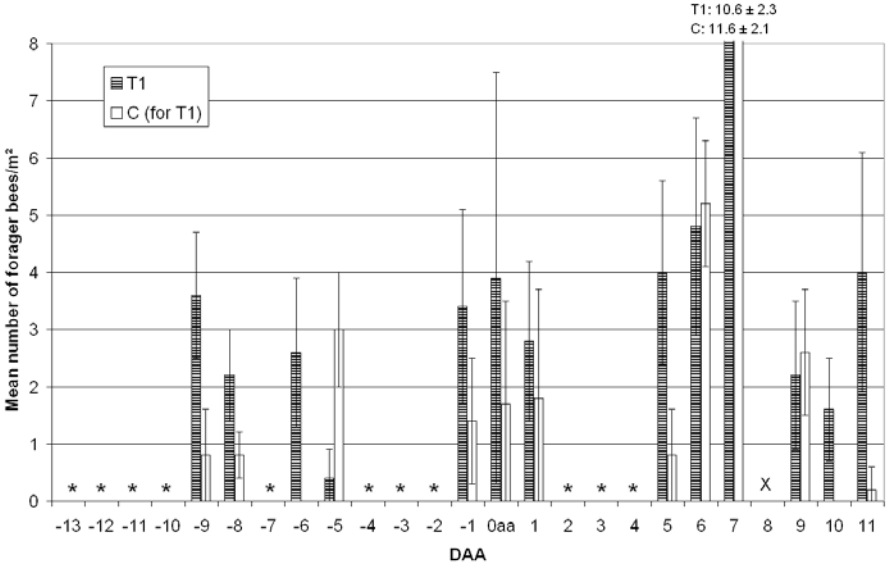

Figure 5a Flight intensity

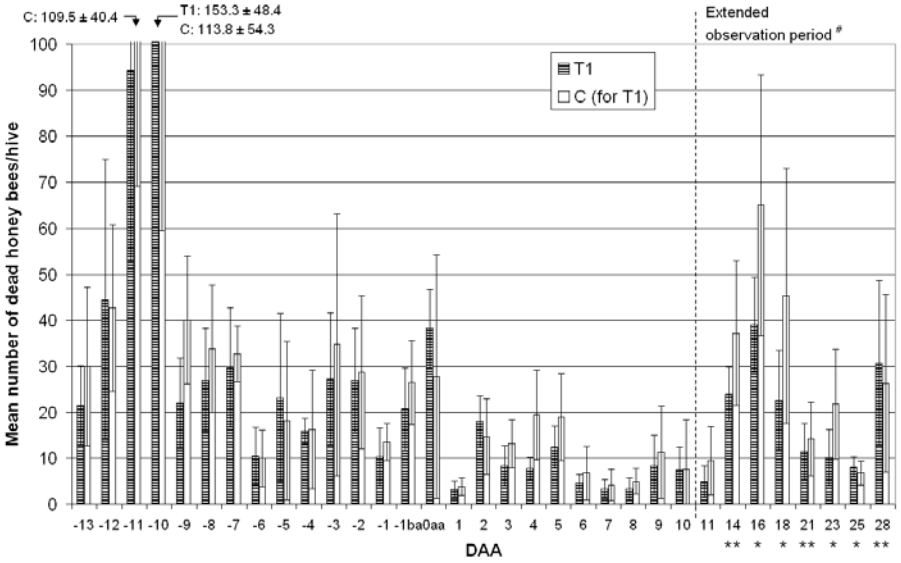

Figure 5b Mortality

Figure 5: Mean honeybee flight intensity (number of forager bees/m² ± STD) (a.) and mean honeybee mortality (number of dead honeybees/hive ± STD) (b.) in the untreated control (C) and cyantraniliprole 100 g/L OD (2-times at 90 g a.s./ha: 1st spray pre-flowering (26 April 2010 BBCH 59, before hive setup on 3 May 2010), 2nd spray after daily honeybee flight, 16 May 2010 BBCH65-67) (T1) in a winter oilseed rape field trial in Germany.

3.4.2 Field honeybee testing in melons

Two spray applications of cyantraniliprole (plus Codacide Oil) sprayed at rates of 90 g a.s./ha after daily honeybee flight (T1) and during honeybee flight (T2) had no effect on honey bee mortality, flight activity and behaviour. Sprayed during honeybee flight, mortality was slightly elevated on the day of first application; this observation was not considered to be biologically relevant (Figure 6). In both treatments T1 and T2, cyantraniliprole had no short-term or long-term effect on honeybee colony condition and brood development throughout the whole season until end of overwintering in March 2012. Pollen source determination detected that between the two applications, the experimental colonies foraged mainly for pollen and nectar in wild flowers, but on DAA2+2 a significant amount of melon pollen could be found in the forager bee samples of T2 (12 % in pollen loads and 13 % in nectar extracted from honey stomachs).

No quantifiable residues of cyantraniliprole or any metabolite were found in any of the nectar samples of the three sampling dates (DAA2+4, DAA2+7 and DAA+48). Quantifiable residues of cyantraniliprole in pollen were found in samples of the first and second sampling date (DAA2+4 and DAA2+7) in T1 and T2 (up to a maximum of 0.0196 mg cyantraniliprole/kg). Quantifiable residues of the metabolite IN-MLA84 were only found in pollen samples of the first sampling date (DAA2+4) in T1 (up to a maximum of 0.0086 mg/kg); no residues were found in samples taken at the third sampling date on DAA2+48. No quantifiable residues of cyantraniliprole or any metabolite were found in any of the wax samples of the three sampling dates.

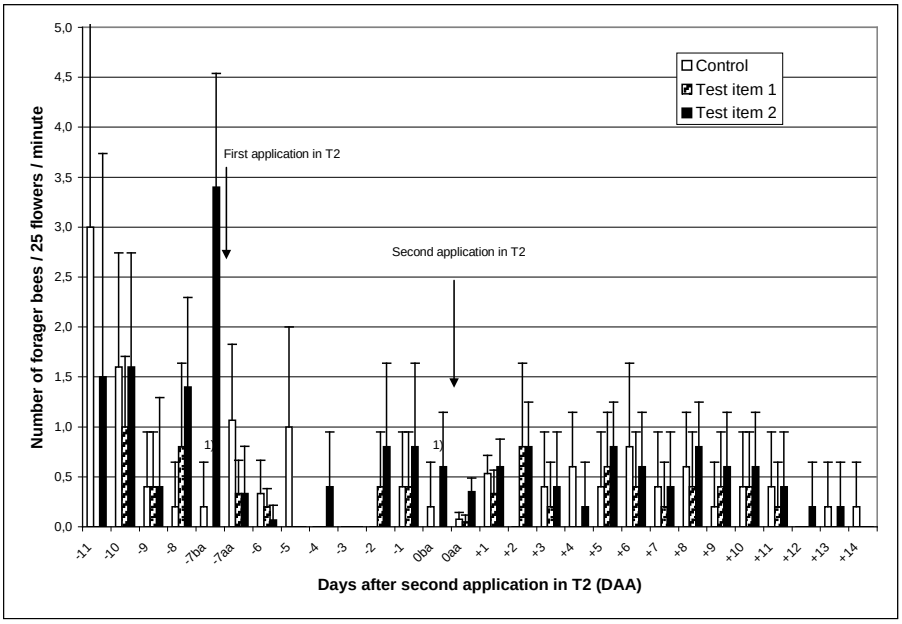

Figure 6a Flight intensity

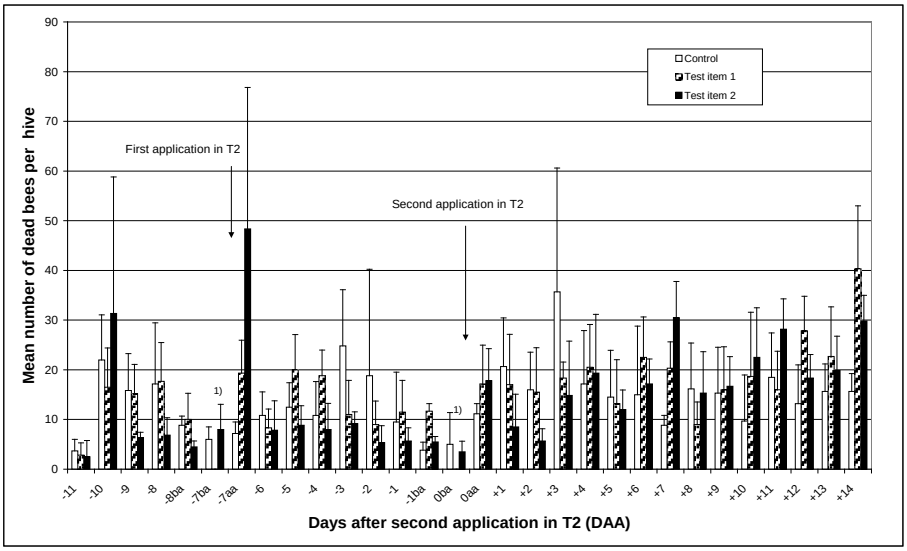

Figure 6b Mortality

Figure 6: Mean honeybee flight intensity (number of forager bees/m² ± STD) (a.) and mean honeybee mortality (number of dead honeybees/hive ± STD) (b.) in the untreated control (C), and 2-times cyantraniliprole 100 g/L OD at 90 g a.s./ha plus 2.5 L Codacide Oil/ha with 1st spray 4 August 2011, BBCH 61-62 and 2st spray 11August 2011, BBCH 66-66, both sprayed after honeybee flight in the evening (Test item 1), and 2-times cyantraniliprole 100 g/L OD at 90 g a.s./ha plus 2.5 L Codacide Oil/ha with 1st spray 5 August 2011, BBCH 61-62, and 2st spray 12August 2011, BBCH 66-66, both sprayed during honeybee flight (Test item 2) in a melon field trial in Spain.

3.5 Greenhouse bumblebee testing

Cyantraniliprole applied via drip irrigation or applied via spray application with the last application made 1 day before release and exposure of the bumblebees (worst-case scenario tested) had no negative effect on bumblebee foraging intensity and mortality (Figure 7). Overall, cyantraniliprole did not have any effects regarding all parameters assessed, i.e., mortality, foraging activity, consumption of sugar solution, condition of colonies and development of bumblebee brood relative to the water treated control.

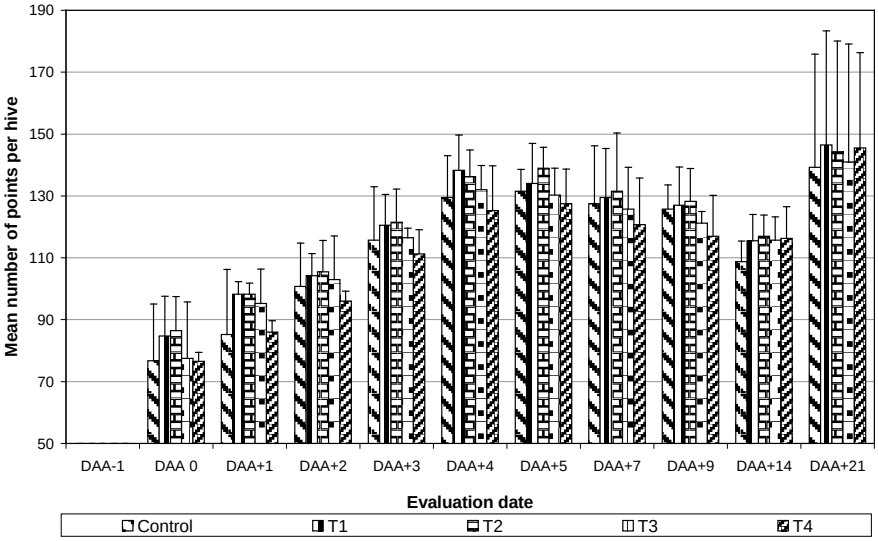

Figure 7a Flight intensity

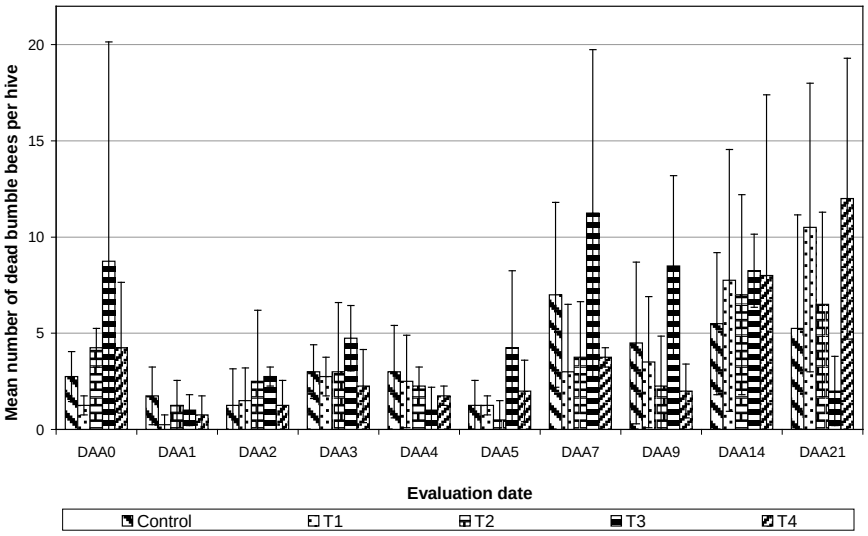

Figure 7b Mortality

Figure 7: Mean bumblebee flight intensity (number of points ± STD) (a.) and mean bumblebee mortality (number of dead bumblebees/hive ± STD) (b.) in the control and in two treatments with cyantraniliprole 200 g/L SC applied via drip irrigation 3-times at 100.0 g a.s./ha (T1: drip irrigation 21, 14 and 7 days before release of the bumble bees in the greenhouse compartment, and T2: drip irrigation 14, 7 and 1 day before release of the bumble bees in the greenhouse compartments), and in two treatments with cyantraniliprole 100 g/L OD sprayed 3-times at 10.0 g a.s./hL plus 0.25 % (v/v) Codacide Oil/ha) and at a target application volume of 800 – 1100 L/ha (equivalent to application rates of 80 to 110 g a.s./ha (T3: spray application 15, 8 and 2 days before release of the bumble bees in the greenhouse compartments (the last application 2 days before release of the bumble bees was performed in the evening = about 37-38 hours before release), and T4: spray application 14, 7 and 1 day before release of the bumble bees in the greenhouse compartments (the last application 1 day before release of the bumble bees will be performed in the evening = about 15-16 hours before release)) in a tomato greenhouse semi- field trial in Spain.

3.6 Field honeybee testing with seed-treated rape

Honeybee colonies were exposed to *Brassica napus* L. plants grown from seeds dressed with cyantraniliprole 625 g/L FS with a load of 50 µg a.s./seed (T1) or from seeds dressed with another cyantraniliprole seed treatment product (T2). In both field trials – in France and Germany – no negative effect on honeybee mortality and flight activity (Figure 8), behaviour, colony condition and brood development were determined in both cyantraniliprole treatments T1 and T2. At the last assessment at the end of the honey bee season in October, all hives were in good condition for overwintering at both locations.

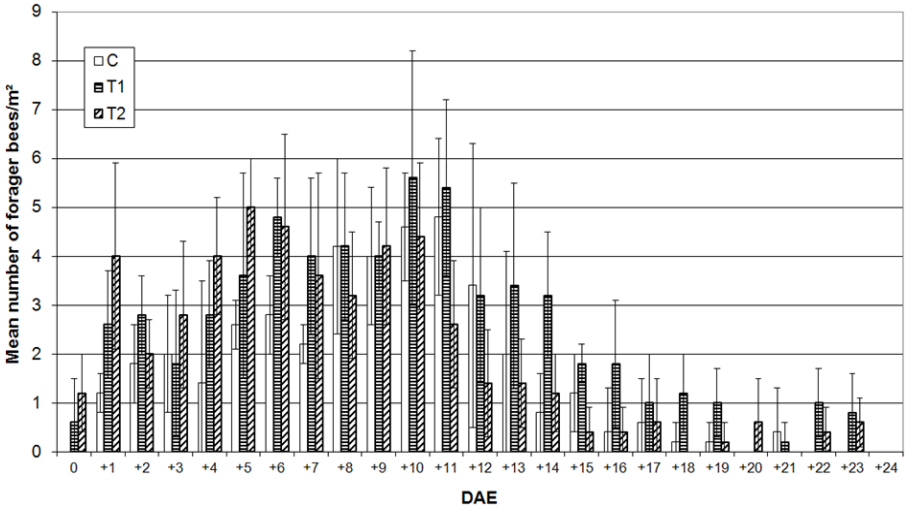

Figure 8a Flight intensity

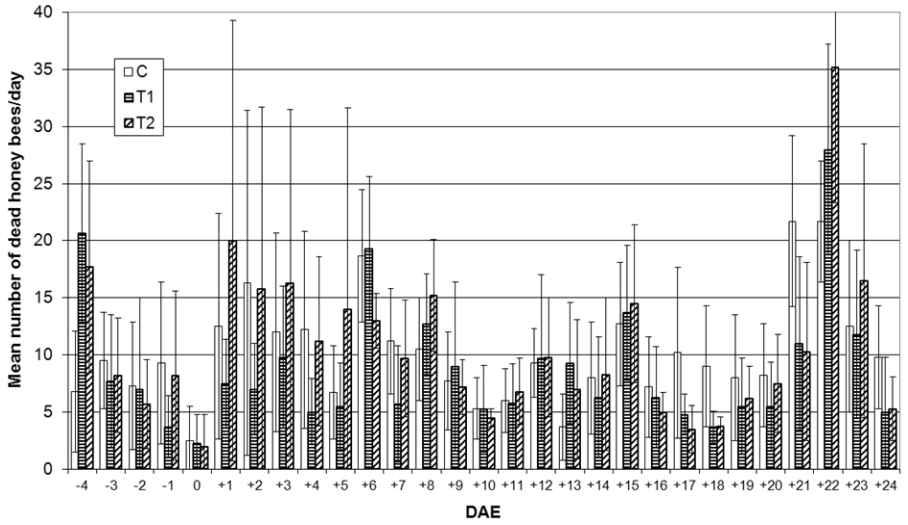

Figure 8b Mortality

Figure 8: Mean honeybee flight intensity (number of forager bees/m^2 ± STD) (a.) and mean honeybee mortality (number of dead honeybees/hive ± STD) (b.) in the control (C), cyantraniliprole 625 g/L FS with 50 µg a.s./seed (T1) and another cyantraniliprole seed treatment (T2) (France) (DAE = Days after exposure starting at BBCH63. DAE-4 to DAE-1 = Pre-exposure mortality before hive set-up at test site).

No residues above the LOQ level of 0.005 mg cyantraniliprole/kg were found in any of the control samples. Cyantraniliprole residues in guttation liquid were the highest directly after emergence of rape seedlings in autumn (maximum value: 0.265 mg cyantraniliprole/kg), but the residue level declined rapidly to level just above or below LOQ within 3 to 4 weeks (Table 3). In the following spring, no cyantraniliprole residue were determined in any bee matrices i.e., guttation liquid, whole rape flowers, nectar and pollen from forager bees, or from pollen, wax or honey samples taken inside the hives. No residues of any of the cyantraniliprole metabolites were detected in any sample at any time.

Table 3 Cyantraniliprole residues (mg/kg) determined in different bee matrices in 2 winter oil seed rape honeybee field trials in France and Germany.

Cyantraniliprole residues in different bee matrices (mg/kg)				
Timing	WOSR trial in France		WOSR trial in Germany	
(France / Germany)	Control	Cyantraniliprole 50µg/rape seed	Control	Cyantraniliprole 50 µg/rape seed
Guttation liquid				
DAD+9 / DAD+15	<LOQ	0.2650	<LOQ	0.0976
DAD+20	<LOQ	0.0068	<LOQ	0.0151
DAD+24 / DAD+30	<LOQ	0.0069	<LOQ	<LOQ
DAE+1	<LOQ	<LOQ	<LOQ	<LOQ
Whole winter oil seed rape flowers				
DAE+1	<LOQ	<LOQ	<LOQ	<LOQ
Nectar from stomach of forager bees				
DAE+2	<LOQ	<LOQ	<LOQ	<LOQ
DAE+6	<LOQ	<LOQ	<LOQ	<LOQ
Pollen from stomach of forager bees				
DAE+2	<LOQ	<LOQ	<LOQ	<LOQ
DAE+6	<LOQ	<LOQ	<LOQ	<LOQ
Pollen from hives				
DAE+14 / DAE+7	<LOQ	<LOQ	<LOQ	<LOQ
Wax from hives				
DAE+7	<LOQ	<LOQ	<LOQ	<LOQ
Honey from hives				
DAE+21 / DAE+8	<LOQ	<LOQ	<LOQ	<LOQ

(DAD = Days after drilling of rape. DAE = Days after exposure of bee hive in flowering rape)
(LOQ = 0.005 mg cyantraniliprole/kg, except for pollen in forager bees (LOQ = 0.050 mg/kg))

Also no residue of cyantraniliprole or its metabolites were determined in pollen and nectar sampled 45 to 58 day after planting from flowering summer oil seed rape in Canada that was seed-treated at 100 µg cyantraniliprole/seed.

3.7 Residue in bee matrices, i.e. in pollen and nectar

The maximum cyantraniliprole and metabolite residue concentrations determined in nectar and pollen following applications with all different cyantraniliprole formulations applied pre- and/or during flowering are summarized in Table 4. The highest cyantraniliprole concentrations found in pollen and nectar were 3.450 and 0.1458 mg cyantraniliprole/kg, respectively; both resulting from spray applications. Typically, cyantraniliprole residue concentrations in nectar were much lower than in corresponding pollen samples. Cyantraniliprole drip applications to soil resulted in 2 orders of magnitude lower pollen and nectar residue concentrations compared to spray applications. Residues of cyantraniliprole metabolites were rarely found and typically more likely in pollen, but rarely in nectar samples.

Table 4 Maximum residue concentrations of cyantraniliprole and its plant metabolites (mg/kg) detected in pollen and nectar following different modes of application (spray, soil mixing or drip irrigation) of different cyantraniliprole test substances (Technical material, cyantraniliprole 100 g/L OD, cyantraniliprole 100 g/L SE, or cyantraniliprole 200 g/L SC) at different maximum application rates under laboratory or field conditions. (LOQ = 0.005 mg/kg).

Substance analysed	Application mode	Cyantraniliprole test substance	Max rate (g cyantraniliprole/ ha)	Pollen (mg/kg)	Nectar* (mg/kg)
Cyantraniliprole	Spray	Technical	3 x 150 (lab)	3.9200	na
Cyantraniliprole	Spray	100 g/L OD, 100 g/L SE	2 x 100 (field) 2 x 150 (field)	1.9330 3.4500	0.0550 0.1458
Cyantraniliprole	Soil mixing	Technical	1 x 450 (lab)	0.1500	na
Cyantraniliprole	Drip	200 g/L SC	3 x 100 (field)	0.0121	0.0262
IN-J9Z38	Soil mix	Technical	1 x 450 (lab)	0.2020	<0.005
IN-MLA84	Spray	Technical	3 x 150 (lab)	0.0480	<0.005
IN-HGW87	Spray	200 g/L OD	1 x 120 (field)	0.0283	<0.005
IN-K5A77	Spray	Technical	3 x 150 (lab)	0.0210	na
IN-DBC80	Spray	Technical	3 x 150 (lab)	0.0160	na
IN-MYX98	Spray	200 g/L OD	1 x 120 (field)	0.0155	<0.005
IN-K5A78	Soil mixing	Technical	1 x 450 (lab)	0.0050	na
IN-N7B69	Spray/Drip	100 g/L OD, 100 g/L SE, 200 g/L SC	2 x 150 (field) 3 x 100 (field)	<0.005	<0.005
IN-JCZ38	All above	All above	All above	<0.005	<0.005

* = Nectar residue data only generated in field studies
na = not assessed

4. Discussion

Cyantraniliprole has demonstrated to have intrinsic oral and contact honeybee toxicity, but of moderate magnitude with little variation among the different formulated products.

The hazard quotient (HQ) values for the cyantraniliprole spray formulations, cyantraniliprole 100 g/L OD and cyantraniliprole 100 g/L SE meet the EU-relevant trigger value of 50 for contact exposure up to 32.5 and 139.0 g a.s./ha and for oral exposure up to 19.5 and 46 g a.s./ha, respectively. Based on this worst-case Tier 1 risk assessment cyantranilprole uses at low intended use rate are predicted to pose a low risk for honeybees (including the rape seed treatment use with an intended use rate equivalent to 25 g a.s./ha, for 50 µg a.s./seed and 500000 rape seeds/ha). This conclusion is also supported by the honeybee semi-field and field trial results with spray applications made during flowering and during daily honeybee flight activity at 10 or 12.5 g a.s./ha demonstrating lack of effects on honeybee colonies.

Risk resulting from residual exposure

The risk for honeybees resulting from residual exposure via contact with treated foliage was found to be of short duration, which was proven in the treatment with spray deposits aged for 3 hours or longer and resulting in no increased honeybee mortality. Therefore spraying of cyantraniliprole after daily bee flight is unlikely to pose a risk of residual effects for honeybees the following day up to the highest intended use rate of 150 g a.s./ha.

Risk resulting from oral exposure of honeybee to pollen and nectar, and guttation liquid

Residue of cyantraniliprole may be found in bee matrices (i.e. in pollen and nectar) with a clear trend of decreasing concentration after application, but rarely metabolites, and if so only in significantly lower levels.

Oral risk assessments – considering published honeybee consumption assumptions by Rortais et al. (2005)[15] – do not indicate a risk for honeybees resulting from oral exposure to residues of cyantraniliprole and its metabolites resulting from any cyantraniliprole use, because even the maximum measured residue amounts in any pollen and nectar samples and the resulting calculated oral uptake dose rates are not high enough to indicate a risk for honeybees on the basis of the laboratory toxicity endpoints for cyantraniliprole, as well as for the metabolites. Also, the maximum cyantraniliprole residue concentrations detected in guttation liquid from emerging cyantraniliprole seed-treated rape seedlings and assuming that the complete water needs of adult honeybees (~ 10 µL water/day according Free & Spencer-Booth Y (1958)[16]) would be consumed solely via guttation liquid do not indicate a risk for honeybees.

Risk resulting from drip irrigation uses

Low risk for honeybees for the intended drip irrigation uses was found on the basis of a worst-case semi-field tunnel trial dosed above the intended use rates, and on the basis of low levels of residues in pollen and nectar and low oral risk prediction on basis of honeybee consumption assumptions by Rortais et al. (2005)[15].

Similarly, lack of risk was found for bumblebees tested under tomato greenhouse drip irrigation conditions if the bumblebees were released the day after the last drip event. This finding is also supported by the results that adult *B. terrestris* worker bees are about 2 orders of magnitude less sensitive to cyantraniliprole in acute oral and contact laboratory tests than honeybees.

Risk resulting from rape seed treatment use

Only cyantraniliprole (but no metabolite) residues were detected in guttation liquid from emerging rape seeds, and the residue concentrations were identified to be too low for an actual risk for forager bees (see discussion above). In whole flowers, pollen and nectar of flowering rape no residues of cyantraniliprole were found grown out of summer and winter oil seed rape cyantraniliprole-treated seeds, and therefore low risk for honeybees was proven (No exposure = no risk). The lack of effects on honeybees was confirmed in two corresponding biological field trial parts in Germany and France where honeybee colonies were exposed to flowering rape grown out of cyantranilprole-treated rape seeds and lack of effects on mortality, foraging activity, brood and colony development and over-wintering success. The lack of residue in bee matrices resulting from flowering rape is explained by the rapid degradation of cyantraniliprole; typical DT50 values in soil range between 13 to 87 days (DuPont unpublished data). The risks from potential seed treatment off-field dust drift during drilling of rape is considered low, because the contact Tier 1 EU HQ quotient of 50 is met up to 32.5 g a.s/ha (see discussion above) and because semi-field and field studies have shown low risk for honeybees resulting from spray application during flowering and during honeybee flight at 10 and 12.5 g a.s./ha, which are rates far above the expected dust drift off-field rate resulting from drilling of seed treated rape at 50 µg a.s./seed, which is equivalent to 25 g a.s./ha assuming an intended seeding rate of 500000 rape seeds/ha.

Risk resulting from pre-flowering sprays

For pre-flowering cyantraniliprole sprays at the highest intended use rate of 2-times 150 g a.s./ha proved lack of effects on honeybees in the highly bee-attractive rape model crop. This finding is supported by results of laboratory translocation data with radio-labelled cyantraniliprole with rape plants demonstrating that cyantraniliprole residues in rape pollen are found following soil application. Also for pre-flowering spraying in rape in the field it was demonstrated that cyantraniliprole is being translocated into pollen and nectar of flowering rape. Furthermore, the low risk assumption for honeybees resulting from any pre-flowering cyantraniliprole spraying is supported by the general oral risk assessment for honeybees based on the maximum pollen and nectar residue concentrations, which included pre-flowering sprays. Lack of effects was confirmed

in honeybee semi-field and field trials, which included a pre-flowering spray and an assessment period before intended sprayings and observations during flowering.

Risk resulting from flowering sprays

Sprays made at 10 or 12.5 g cyantraniliprole/ha with one of two sprays made during flowering and during daily honeybee flight proved low risk for honeybees.

Field trials in highly-bee attractive rape with sprays made at 90 cyantraniliprole/ha with one of two sprays made during flowering and after daily honeybee foraging activity proved low risk for honeybees. A field trial in moderately bee-attractive melons with 2 sprays made at 90 a.s./ha made during flowering and during daily honeybee foraging activity proved low risk for honeybees, while a field trials in rape with sprays made at 90 a.s./ha with one of two sprays made during flowering and during daily honeybee foraging activity detected increased acute forager bee mortality, but no longer-term effects. Therefore, it is recommended to perform cyantraniliprole sprays in actively blooming crops when bees are not actively foraging, i.e., after daily bee flight.

Also for greenhouse uses and bumblebees, it is recommended to close the hive during application and to re-open those again the next day to avoid direct exposure of bumblebees in line with good agricultural practices.

5. Conclusions

Cyantraniliprole has been demonstrated to have intrinsic honeybee toxicity, but of moderate magnitude. Individual cyantraniliprole metabolites are maximally similar in toxicity to the parent compound. Residues of cyantraniliprole have been found in bee matrices (i.e., pollen and nectar). Residues of metabolites have been found rarely, and if so, only in significantly lower levels than the parent compound. Risk assessments do not indicate a risk for bees resulting from oral exposure to residues of cyantraniliprole and its metabolites. Exposure of bees to residues is of short duration, as determined in numerous field studies. Worst-case tunnel tests, field tests and risk assessments do not indicate a significant, biologically relevant impact on honeybee colonies (adults and brood), if cyantraniliprole sprays are being made after daily bee flight or applied via drip irrigation in several crops, or used as a seed treatment in rape. Cyantraniliprole can also be used in combination with bumblebees in greenhouses. Overall, the effects of cyantraniliprole on bees are well understood and it is unlikely that the intended uses of DuPont cyantraniliprole formulations will pose a risk to bees.

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