

Summaries of the 'residue' studies provided by Bayer.

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5.1.2.e

01-08-2013

A field study on oilseed rape *Brassica napus* was carried out in Poland in 2010 (winter rape) and 2012 (spring rape). The aims of the study were 1) to measure concentrations of thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid in nectar, pollen, honey and bee bread, following realistic dosages to control oilseed rape pests; and 2) to assess the effect on the involved bee colonies. The treatments are in the table below.

The oilseed rape crops treatments

Group - field/ treatment	A winter rape	B winter rape	C spring rape	D spring rape	E spring rape
Seed dressing	CRUISER OSR 322FS (I,F)* thiamethoxam 280 g metaxyl-M 32.15 g fludioxonil 8 g/l dose: 11.25 ml/kg seeds	CHINOOK PLUS 500FS (I) imidacloprid 420 g beta-cyfluthrin 100 g/l dose: 5 ml/kg seeds	CRUISER OSR 322FS (I,F) thiamethoxam 280g metaxyl-M 32.15, fludioxonil 8g/l dose: 11.25 ml/kg seeds	MODESTO 480 FS (I) clothianidin 400 g beta-cyfluthrin 80 g/l dose: 12.5 ml/kg seeds FUNABEN T 480FS (F) thiuram 332 g carbendazim 148 g/l dose: 5.5 ml/ kg seeds	CHINOOK 200 FS (I) imidacloprid 100 g beta-cyfluthrin 100 g/l dose: 20 ml/kg seeds FUNABEN T 480 FS (F) thiuram 332 g carbendazim 148 g/l dose: 5.5 ml/kg seeds
Spraying during the growing period	MOSPILAN 20 SP (I) acetamiprid 20% dose: 0.1 kg/ha spray: 24.04.2010 CYPERKILL SUPER 25 EC (I) cypermethrin 250 g dose: 0.15 l/ha spray: 13.04.2010	MOSPILAN 20 SP acetamiprid 20% dose: 0.1 kg/ha spray: 18.04.2010 PROTEUS 110 OD (I) thiacloprid 100 g deltamethrin 10 g/l dose: 0.5 l/ha spray: 18.04.2010 CYPERKILL SUPER 25 EC cypermethrin 250 g dose: 0.15 l/ha spray: 10.04.2010	PROTEUS 110 OD thiacloprid 100 g deltamethrin 10 g/l dose: 0.5 l/ha spray: 06.06.2012 GALERA 334 SL(H)* clopyralid 267 g pikloram 67 g/l dose: 0.35 l/ha spray: 21.05.2012 TARGA 10EC (H)* quizalofop-p- ethyl 100 g/l dose: 1.0 l/ha spray: 21.05.2012	FURY 100 EW (I) zeta-cypermethrin 100 g/l dose: 0.1 l/ha spray: 06.06.2012 GALERA 334 SL clopyralid 267 g pikloram 67 g/l dose: 0.35 l/ha spray: 20.05.2012	PROTEUS 110 OD thiacloprid 100 g deltamethrin 10 g/l dose: 0.5 l/ha spray: 05.06.2012 FURY 100 EW azeta-cypermethrin 100 g/l dose: 0.1 l/ha spray: 08.05.2012 GALERA 334 SL clopyralid 267 g pikloram 67 g/l dose: 0.35 l/ha spray: 08.05.2012

*I-insecticide, F- fungicide, H-herbicide

Fields A, B, C, D, and E were 41, 35, 29, 21, and 17 ha, respectively. Blooming period winter rape from April 30 to May 21, 2010; spring oilseed rape from June 14 to July 2, 2012. Ten healthy bee colonies (*A. mellifera carnica* and *caucasica*) were placed in the vicinity of each field during bloom, plus 5 additional colonies for pollen load data only. Two control groups (one per year) were located in an area where no rape grew. The exact locations were not given in the paper. Colony sizes: about 14 combs in 2010; 12 combs in 2012. Colony size (# combs covered by bees and the size of the brood area) was determined before treatment, and every 3 weeks after treatment until the end of the season. Bee mortality was counted in the hive bottom boards and in bee traps in front of the hives. Honey yield was determined.

In September 2010 (not done end of 2012) colonies from treatment and control groups were prepared for overwintering. Each colony received approx. 18-20 liters of sugar solution. Also, formulations containing amitraz were applied to fight Varroa mites. Colony status was checked in April 2011.

Nectar was collected 6-7 times directly from the flowering fields. Nectar flow from combs was taken 7 and 14 days after colonies were placed in the fields. Honey was harvested about one week after the blooming period. Pollen was collected every 3-4 days. Bee bread was sampled once after the blooming period. All matrices were analysed for pollen (species) and residues.

One sample of approx. 100 bee workers was taken from the brood chamber, after the blooming period.

Residue analysis based on a modified QuEChERS method with LC-MS/MS. Recovery 70-110%, SD <20%. Thiacloprid LOD/LOQ 0.1/0.5 ng/g in nectar and honey; 0.4/2 in pollen and bee bread; and 0.1/0.5 in bees. Acetamiprid LOD/LOQ 0.1/0.5 ng/g in nectar and honey; 0.2/1 in pollen and bee bread; and 0.3/1 in bees.

Results

Regardless of the residues measured, all colonies developed comparably and there were not significant differences in honey production. The 2010 hives overwintered without problems.

Population size of honeybee colonies (2010)

Date of measurement	Number of combs covered by bees (meant±sd)			p-value ^a	Brood area (dm ²) (meant±sd)			p-value ^a
	Group A	Group B	The control group		Group A	Group B	The control group	
28.04.10	14.4±1.6	14.1±1.0	14.3±1.1	0.859	69.4±15.0	65.7±11.5	63.8±18.0	0.703
31.05.10	19.7±0.7	19.8±0.6	19.1±1.2	0.172	70.1±14.9	77.2±8.6	65.8±12.2	0.162
21.06.10	20.0±0.0	19.8±0.6	19.4±1.0	0.142	71.8±8.6	77.1±10.1	66.5±10.1	0.105
12.07.10	20.0±0.0	19.8±0.6	19.4±1.0	0.142	60.9±9.1	60.2±20.3	57.2±13.3	0.865
27.07.10	9.0±0.0	9.0±0.0	9.0±0.0		50.4±8.1	52.1±11.3	44.6±10.1	0.229
24.08.10	9.0±0.0	9.0±0.0	9.0±0.0		36.1±6.0	33.8±5.3	32.3±11.1	0.556
21.09.10	9.0±0.0	9.0±0.0	9.0±0.0		10.3±7.0	8.9±4.2	9.3±5.0	0.840
18.10.10	7.6±0.5	7.3±0.7	7.7±0.9	0.477	2.2±0.7	1.9±0.4	2.1±1.5	0.813

^aANOVA test.

Table 10.

Population size of honeybee colonies (2012)

Date of measurement	Number of combs covered by bees (meant±sd)				p-value ^a	Brood area (dm ²) (meant±sd)				p-value ^a
	Group C	Group D	Group E	The control group		Group C	Group D	Group E	The control group	
14.06.12	11.8±0.6	12.0±0.0	12.0±0.0	11.6±0.8	0.424	65.9ab±25.0	59.0ab±24.6	70.8b±10.9	39.1a±19.7	0.03
16.07.12	12.0±0.0	12.0±0.0	12.0±0.0	12.0±0.0		55.3a±24.2	54.8±7.2	60.2±9.4	46.8±14.3	0.420
01.08.12	12.0±0.0	12.0±0.0	12.0±0.0	11.7±0.8	0.171	53.9±17.1	55.6±12.5	52.5±14.5	49.4±12.1	0.863
20.08.12	7.9±0.6	8.4±0.5	10.0±0.0	8.5±0.5	<0.000	46.1±10.0	51.7±14.2	48.5±8.1	54.1±3.3	0.433
11.09.12	5.8±0.4	6.1±0.3	5.9±0.3	5.8±0.4	0.290	2.2±2.2	5.1±4.6	3.6±3.8	7.3±3.6	0.058
27.09.12	5.8±0.4	6.1±0.3	5.9±0.3	5.8±0.4	0.290	3.2±2.3	2.7±2.1	2.7±2.5	2.2±1.6	0.838

^aANOVA test. The different letters (a, b) indicate significant differences between the means

Apart from the test substances (see table below), more than 15 other pesticides were found frequently and sometimes in high concentrations in the matrices.

Neonicotinoids incidence and level of their concentration (ng/g) in analyzed samples collected from winter and spring oilseed rape (2010, 2012)

active substance	Samples of nectar (Σ samples of nectar from flowers and combs, honey) n=212				Samples of pollen (Σ samples of pollen loads and bee bread) n=205			
	% positive	mean	max.	median	% positive	mean	max.	median
imidacloprid	21	0.6	2.0	0.6	0	0	0	0
clothianidin	17	2.3	10.1	1.6	11	1.8	3.7	1.2
thiamethoxam	65	4.2	12.9	3.1	37	3.8	9.9	2.9
acetamiprid	51	2.4	13.3	1.1	45	4.1	26.1	2.5
thiacloprid	64	6.5	208.8	2.5	62	89.1	1002.2	4.1

The table below summarizes the data on pollen origin. These data are important, since they correlate the measured concentrations with their origin.

Table 3.
Contents of *Brassica napus* pollen in different types of samples collected from honeybee colonies placed in the rape crops (2010, 2012)

Field	Period of blooming; period of supplying forage	Average content of <i>Brassica napus</i> pollen grains (%±sd)			
		Pollen loads	Bee bread	Nectar flow	Honey
A - winter rape	30.04-21.05.10	92.3±4.6	25.3±19.3	52.7±19.0	31.7±21.6
B - winter rape		6.0±2.3	33.4±26.9	49.1±22.7	51.3±13.9
C - spring rape	14.06-02.07.12	98.0±1.9	74.6±5.6	67.2±19.6	79.4±12.8
D - spring rape		85.1±12.2	60.5±11.6	61.2±14.2	84.2±8.4
E - spring rape		78.3±16.3	48.7±18.7	75.7±10.3	67.2±14.4

Table 4.
Botanical origin of nectar and pollen flow harvested by honeybee colonies from the control groups - results of pollen analysis (2010, 2012)

Control group/year	Type of sample	Predominant pollen (>10%)
Control group in 2010	Nectar flow, honey	<i>Prunus</i> type, <i>Malus</i> type, <i>Brassica napus</i> , <i>Rubus</i> type, <i>Aesculus</i> , <i>Salix</i> , Brassicaceae, Asteraceae,
	Pollen loads, bee bread	<i>Prunus</i> type, <i>Malus</i> type, <i>Rubus</i> type, <i>Aesculus</i> , <i>Salix</i> , Brassicaceae, Asteraceae, <i>Fragaria</i>
Control group in 2012	Nectar flow, honey	<i>Tilia</i> , Brassicaceae, <i>Fagopyrum</i> , <i>Trifolium repens</i> , <i>Phacelia</i> , <i>Prunus</i> type, <i>Rubus</i> type, <i>Anthriscus</i> type, <i>Brassica napus</i>
	Pollen loads, bee bread	<i>Trifolium repens</i> , <i>Brassica napus</i> , <i>Phacelia</i> , <i>Fagopyrum</i> , Brassicaceae, <i>Tilia</i> , <i>Rubus</i> type, <i>Anthriscus</i> type, <i>Achillea</i> type, <i>Prunus</i> type,

The results per matrix per field (n, mean±sd) are given in the tables below, first for 2010, then for 2012. Thiacloprid was applied in fields B, C, and E. Acetamiprid was applied in fields A and B.

Incidence of neonicotinoids and level of their concentration (ng/g) in analyzed samples collected from winter rape (2010)

Field/analyzed samples	n	Imidacloprid		Clothianidin		Thiamethoxam		Acetamiprid		Thiacloprid		
		% pos.	Mean ±sd	% pos.	Mean ±sd	% pos.	Mean ±sd	% pos.	Mean ±sd	% pos.	Mean ±sd	
A	Nectar ff*	6	0	nd	0	nd	0	nd	100	7.6±2.9	0	nd
	Nectar fc**	10	100	0.5±0.1	0	nd	100	2.4±0.2	100	6.8±0.5	0	nd
	Honey	10	100	0.5±0.1	0	nd	100	1.8±0.3	100	6.8±0.7	100	0.3±0.3
	Pollen loads	20	0	nd	0	nd	0	nd	100	10.5±2.4	0	nd
	Bee bread	10	0	nd	0	nd	0	nd	100	4.7±1.9	100	0.8±1.3
	Bees	10	0	nd	0	nd	0	nd	90	0.3±0.2	0	nd
B	Nectar ff*	7	0	nd	0	nd	0	nd	0	nd	100	65.6±75.7
	Nectar fc**	10	100	0.6±0.1	0	nd	0	nd	100	0.1±0.02	100	5.5±1.3
	Honey	10	100	0.8±0.3	0	nd	0	nd	100	0.1±0.03	100	5.5±0.8
	Pollen loads	20	0	nd	0	nd	0	nd	0	nd	100	3.1±0.7
	Bee bread	10	0	nd	0	nd	0	nd	100	0.6±1.1	100	2.7±1.4
	Bees	10	0	nd	0	nd	0	nd	0	nd	100	0.4±0.3
K1	Nectar fc**	10	0	nd	0	nd	0	nd	100	0.2±0.1	100	3.4±1.6
	Honey	10	0	nd	0	nd	0	nd	100	0.1±0.1	100	2.3±1.6
	Pollen loads	20	0	nd	0	nd	0	nd	0	nd	100	646.0±274.0
	Bee bread	10	0	nd	0	nd	0	nd	100	0.1±0.1	100	25.6±14.2
	Bees	10	0	nd	0	nd	0	nd	100	0.3±0.2	100	0.1±0.03

*nectar collected directly from rape flowers;

** nectar flow from combs; K1- the control group; nd - not detected

- red color indicates substances used as seed dressing or as a spray.

In the 2010 winter rape, thiacloprid was only applied in field B. The average content of *B. napus* pollen was only 6%, and the mean concentration in the pollen load was 3.1 ng/g. Mean concentrations in field nectar were 66 ng/g, an order of magnitude higher than in comb-nectar and honey. Concentrations in pollen equal that of the bread. Whether by cross-contamination from field B, or from other sources, some thiacloprid (mean values under the LOQ and close to the LOD) was found in honey and bee bread in field A.

Remarkably, the concentration of thiacloprid in the control pollen load was about 10x that of the nectar in field B (646 ng/g vs. 66 ng/g). Also bee bread, nectar and honey from the control field contained residues above the LOQ. This was thought to be caused by use of thiacloprid in nearby fruit orchards (pollen from *Prunus* spp were dominant), but also oilseed rape pollen were found. The 2010 results for acetamiprid are remarkable, since it seems that in field B no acetamiprid was present in the nectar in the plants, while application was at the same time as for thiacloprid. Application of acetamiprid in field A was 6 days later, with clear presence of the a.s. in all matrices.

In the 2012 spring rape, thiacloprid was applied (at the same day) in field C and E. Remarkably, the mean residue in pollen load in field C reached a value of 82 (\pm 124) ng/g, while no a.s. was found in pollen of field E. The difference between residues in bee bread was however smaller: 22 vs 7.5 ng/g. The field nectar of field C and E contained some (mean 0.9 ng/g) or no thiacloprid, while levels in comb nectar and honey were comparable. Compared to the results of C and E, the control and field D contained relatively high loads of thiacloprid. Although acetamiprid was not applied in 2012, the a.s. was found in all fields at levels comparable to those of the 2010 control field, or to the thiacloprid values in 2012.

Incidence of neonicotinoids and level of their concentration (ng/g) in analyzed samples collected from spring rape (2012)

Field/analyzed samples	n	Imidacloprid		Clothianidin		Thiamethoxam		Acetamiprid		Thiacloprid		
		% pos.	mean \pm sd	% pos.	mean \pm sd	% pos.	mean \pm sd	% pos.	mean \pm sd	% pos.	mean \pm sd	
C	Nectar ff*	7	0	nd	0	nd	100	5.4 \pm 3.7	14	0.2 \pm 0.6	43	0.9 \pm 1.6
	Nectar fc**	20	0	nd	0	nd	100	10.3 \pm 2.3	0	nd	95	2.8 \pm 1.4
	Honey	10	0	nd	0	nd	100	7.7 \pm 2.8	70	0.7 \pm 0.5	100	2.1 \pm 0.4
	Pollen loads	25	0	nd	0	nd	100	6.6 \pm 2.0	0	nd	100	81.6 \pm 123.6
	Bee bread	10	0	nd	0	nd	100	3.6 \pm 1.5	80	7.6 \pm 9.1	100	21.8 \pm 25.8
	Bees	10	0	nd	0	nd	0	nd	0	nd	0	nd
D	Nectar ff*	6	0	nd	50	2.6 \pm 4.0	100	2.7 \pm 1.1	0	nd	67	3.8 \pm 4.5
	Nectar fc**	20	0	nd	100	1.3 \pm 0.3	100	3.3 \pm 0.3	0	nd	25	0.3 \pm 0.6
	Honey	10	0	nd	100	3.4 \pm 1.0	100	3.9 \pm 0.5	30	0.3 \pm 0.5	0	nd
	Pollen loads	20	0	nd	50	0.6 \pm 0.6	50	0.6 \pm 0.7	20	0.2 \pm 0.6	0	nd
	Bee bread	10	0	nd	90	2.2 \pm 1.3	90	1.0 \pm 0.4	30	0.8 \pm 1.4	20	0.4 \pm 0.9
	Bees	10	0	nd	0	nd	0	nd	0	nd	0	nd
E	Nectar ff*	6	0	nd	0	nd	83	1.1 \pm 0.6	0	nd	0	nd
	Nectar fc**	20	10	0.4 \pm 0.7	0	nd	100	1.5 \pm 0.3	50	2.7 \pm 0.7	60	2.1 \pm 2.3
	Honey	10	0	nd	0	nd	100	1.2 \pm 0.2	100	1.2 \pm 0.1	90	1.8 \pm 0.9
	Pollen loads	15	0	nd	0	nd	0	nd	0	nd	0	nd
	Bee bread	10	0	nd	0	nd	0	nd	50	1.9 \pm 3.1	60	7.5 \pm 15.2
	Bees	10	0	nd	0	nd	0	nd	0	nd	0	nd
K2	Nectar fc**	20	0	nd	0	nd	0	nd	30	0.5 \pm 0.8	30	0.9 \pm 1.7
	Honey	10	0	nd	0	nd	0	nd	20	0.3 \pm 0.6	50	0.8 \pm 1.0
	Pollen loads	15	0	nd	0	nd	46	0.5 \pm 0.6	87	2.5 \pm 2.2	50	2.4 \pm 2.9
	Bee bread	10	0	nd	0	nd	0	nd	80	3.4 \pm 2.5	80	3.4 \pm 3.8
	Bees	10	0	nd	0	nd	0	nd	0	nd	0	nd

*nectar collected directly from rape flowers;

** nectar flow from combs; K2- the control group; nd - not detect

- red color indicates substances used as seed dressing or as a spray

The purpose of the GLP study was to determine the residues of thiacloprid and its metabolite KKO 2254 in guttation solutions from maize plants after seed dressing with Thiacloprid FS 400 in the field.

Three field trials with maize were conducted in Germany with Thiacloprid FS 400 seed dressing, containing 1.00 mg thiacloprid per seed. Samples of guttation liquid (up to three of 1 mL per day) were taken between May 8th and May 31st (n=113), for analysis of residues of thiacloprid and its metabolite. These sampling dates correspond to 13 - 37 days after drilling in the field; BBCH growth stage 10-11 (= first leaf) until 15-17 (5-7 leaves, before stem elongation). Between 19 and 292 plants (average 59 plants) were sampled to get the 3x1 mL guttation fluid from the tip and sides of the leaves. On a few days the target was not reached (droplets were too few/small). On several days dew was present, which was avoided during sampling. Residues of thiacloprid and its metabolite KKO 2254 in maize guttation liquid samples were quantified by reversed phase HPLC with electrospray and MS/MS-detection. The individual analytical recovery values for thiacloprid (0.01 – 50 mg/L) ranged from 68 to 105% with an overall recovery of 89% and with a relative standard deviation (RSD) of 13.3% (n = 14). For KKO 2254 the individual recovery values (0.01 – 50 mg/L) ranged from 77 to 118% with an overall recovery of 107% and with a RSD of 10.1% (n = 14). The LOQ was 0.01 mg/L for thiacloprid and its metabolite KKO 2254 in guttation liquid.

Results

Highest residues of thiacloprid were 50 mg/L and declined within the next 20 days to values with the lowest concentration of 0.06 mg/L. Highest residues of KKO 2254 were 16 mg/L and declined to values of 0.03 mg/L.

Remarks

Guttation data are relevant in the 2013 EFSA Guidance (EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295).

Regarding the timing and duration of the experiment, Girolami et al (2009) Journal of Economic Entomology, 102(5):1808-1815; and Tapparo et al (2010) J. Environ. Monit., 2011, 13, 1564; also report that guttation lasts about two-three weeks after seedling emergence, both in the lab and in the field. Test timing and duration is hence adequate.

The guttation residues in the three plots followed a similar pattern. The highest peaks were 50 mg/L for thiacloprid and 16 mg/L for metabolite KKO2254 (third peak in the graph of **Error! Reference source not found.**).

The first dataset missed data between 20 and 28 days. This dataset is not used for averages. Evaluator derived, simply by averaging the values within the time window around the two other peaks, the 10-d TWAs. The highest average for thiacloprid over 10 days around the third (highest) peak was 14 mg/L. The average for KKO2254 (second peak) was 1.3 mg/L.

Looking at the three plots together as displayed in

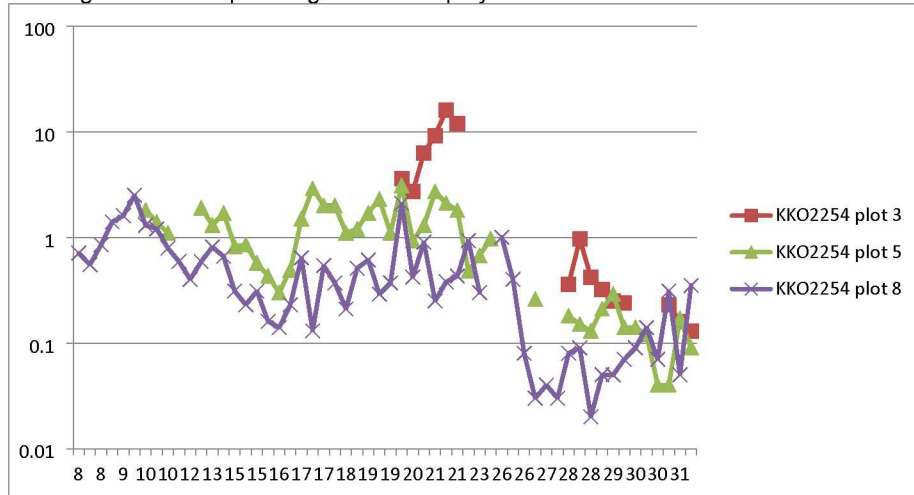
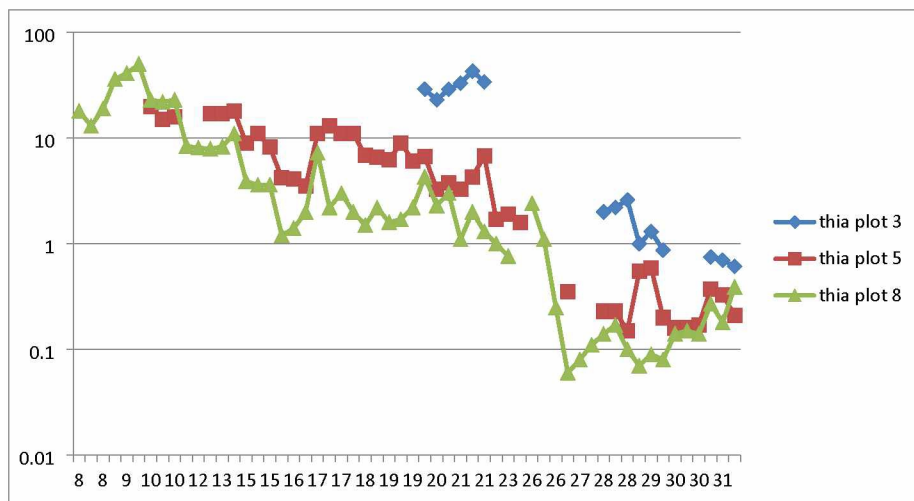


Figure 1, the highest 10-day window for thiacloprid gives an average of 14 mg/L. For KKO2254 the highest 10-day average is 1.8 mg/L.



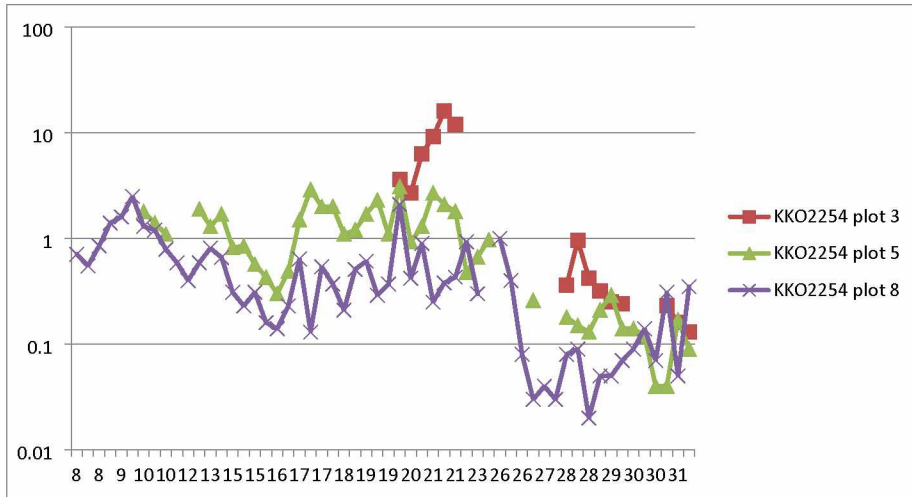


Figure 1 Concentrations of thiacloprid and KKO2254 in guttation. The X-axis shows the day (in May 2009). The Y-axis gives the concentrations in guttation for all three plots on every available timepoint.

In view of the tiered assessment approach described by EFSA (2013):
 First tier worst case approach: PEC 184 mg/L (water solubility thiacloprid)
 Refinement: peak concentration thiacloprid 50 mg/L (plus 16 mg/L KKO2254)
 2nd refinement, if peak exposure does not lead to long-term effects: 10-day TWA concentration thiacloprid 14 mg/L (plus 1.8 mg/L KKO2254).

D_2-_M-363263_0003228099_5.1.2.e 2010_residue pollen

The purpose of the study was to determine the residues of thiacloprid and its metabolite KKO 2254 (= YRC 2894-amide) in pollen from maize plants after seed dressing with Thiacloprid FS 400 in the field.

In total, six field trials were conducted in Germany in 2009. Pollen-samples were collected in the field between 76 and 84 days after drilling of the thiacloprid-dressed maize seeds. Samples of pollen were taken at BBCH 63, between July 13 and 24, for analysis of residues of thiacloprid and its metabolite. Five samples per field, of >2 g pollen (taken from 10-30 plants) per plot.

Residues of thiacloprid and its metabolite KKO 2254 (= YRC 2894-amide) in/on maize pollen were determined by reversed phase HPLC with electrospray and MS/MS-detection. The individual analytical recovery values for thiacloprid for pollen ranged from 75 to 91% with an overall recovery of 84% and with a relative standard deviation (RSD) of 5.8% (n = 7). For KKO 2254 (= YRC 2894-amide) the individual recovery values ranged from 91 to 109% with an overall recovery of 97% and with a RSD of 6.4% (n = 7). The Limit of Quantitation (LOQ) in/on pollen was 0.001 mg/kg for thiacloprid and its metabolite KKO 2254, respectively.

Results

Residues of thiacloprid were always below the LOQ (0.001 mg/kg), but never below the LOD (of 0.0001 mg/kg).

Residues of metabolite KKO 2254 were always below the LOQ, and often (19 out of 30) below the LOD of 0.0001 mg/kg.

Remarks

Data may be useful in combination with other pollen residue data. Concentrations between LOD and LOQ may be assessed as "<0.001 mg/kg" or as "half the detection limit (0.0005 mg/kg)".

Note: In the field study (5.1.2.e 2010) residues of thiacloprid and its metabolite YRC 2894-amide in/on the maize pollen samples from the test item treatment group as well as from the control were always below the LOQ of 0.001 mg/kg.

D_2-_M-368692_0003228099_bee bread residue

Text originates from the "German Bee Monitoring - Project Board". Thiacloprid was found in 47 out of 88 bee bread samples taken in 2009. No further details on origin of samples, or actual concentrations.

D_2-_M-387127_0003228099_5.1.2.e) et al 2010, levels
 Data were used in other review of (5.1.2.e) et al. 2010)

A broad survey of pesticide residues was conducted on samples from migratory and other beekeepers across 23 states (USA), one Canadian province and several agricultural cropping systems during the 2007–08 growing seasons. LC/MS-MS and GC/MS to analyze bees and hive matrices for pesticide residues utilizing a modified QuEChERS method.

Results

Acetamiprid and thiacloprid were never found in bees. Acetamiprid was not found in wax. Thiacloprid was rarely found in wax (4 out of 208 samples, with concentration between 1.9 and 7.5 ppb (LOD 1.0 ppb). Also in pollen, detects were few (19 out of 350) at rates of 1.7 – 115 ppb (LOD 1.0 ppb). Acetamiprid was likewise detected 11/350 times at 14-134 ppb (LOD 5.0 ppb) in pollen. Analysis was on mass basis: ppb equals ng/g.

Table 1. Summary of pesticide detections in wax samples from North American honey bee colonies.

Wax Pesticide*	Class*	Detects	Samples	%	Detections (ppb)							
					Analyzed	High	Low	Median	90%tile	95%tile	Mean [‡]	SEM [‡]
Thiacloprid	S NEO	4	208	1.9	7.5	1.9	5.9	7.5	7.7	5.4	1.3	1.0

Table 2. Summary of pesticide detections in pollen samples from North American honey bee colonies.

Pollen Pesticide*	Class*	Detects	Samples	%	Detections (ppb)							
					High	Low	Median	90%tile	95%tile	Mean [‡]	SEM [‡]	LOD [†]
Acetamiprid	S NEO	11	350	3.1	134.0	14.0	57.0	101.0	117.5	59.3	11.8	5.0
Thiacloprid	S NEO	19	350	5.4	115.0	1.7	14.0	42.9	108.7	23.8	7.2	1.0

D_2-_M-388231_0003228099_5.1.2.e et al. 2009, residues

=

D_2-_M-455981_0003228099_5.1.2.e et al 2009 residues

A field experiment was performed in April-May 2007 to investigate the transfer of thiacloprid residues after use in an apple orchard, into bea bread and pollen inside bee hives.

Experimental apiaries of honey bee (*Apis mellifera carnica* Pollm.) colonies were established in three sites in Slovenia: an apple orchard at Brdo near Lukovica, in the Ljubljana region; in an orchard at Cadovlje and a control apiary in Senicno, both in the Gorenjska region. Two honey bee hives at each site were fitted with pollen traps for pollen collection.

Calypso was sprayed (0.2 L/ha) at night or early in the morning when the honey bees were inside their hives. Samples of pollen were collected one, 6 and 10 days after the field treatments from colonies located at both of the treated sites and from the control colonies. Samples of bee bread were also collected from comb 16 days after the pesticide application. Pollen loads of approximately 50 g were collected from the pollen traps into plastic cups after the field application.

Thiacloprid was analysed using LC/MS/MS (recovery 100.0%) with LOD 0.01 mg/kg.

Results

Brdo: at 1DAA thiacloprid in pollen was 0.09 mg/kg; and <LOD at 6DAA.

Cadovlje: at 6DAA thiacloprid in pollen was 0.03 mg/kg. In bea bread at 6DAA, thiacloprid was <LOD.

Senicno: no residues detected at any timepoint.

Remarks

Origen of pollen was not determined; not clear what fraction of pollen could originate from the treated orchards.

D_2- M-433254_0003228099_5.1.2.e 2012 project Oostenrijk
 D_2- M-457068_0003228099_5.1.2.e 2012 meerdere stoffen

Reports on a long term monitoring study in Austria, 2009-2011; & 2012.
 The program investigated the relationship between seed treatment with thiamethoxam, imidacloprid, clothianidin and fipronil, and reports of bee damage. During spring/summer, honey was also analysed for other pesticides.

Table 1 Reported data on presence of residue in samples in Austria, 2009-2011

Substance	Matrix	Number of samples	LOQ mg/kg	LOD mg/kg	N < LOD	N < LOQ	N > LOQ	Maximum residue mg/kg	MRL mg/kg
Thiacloprid	bees	2010: 62	0.0005	0.00015		1	2		
	bread	2010: 10					5		
	Honey	2011: 13	0.0005	0.00015	5	4	4	0.044	0.2
	Fruit trees								
	Bees or bee bread	2011:				3-8	1		
Acetamiprid	bread	2010: 10	0.0002	0.00006			1	-	
	Bees or bee bread	2011:				1-3			

Table 2 Reported data on presence of residue in honey samples in Austria, 2012

Substance	Number of samples	LOQ mg/kg	LOD mg/kg	N < LOD	N < LOQ	N > LOQ	Maximum residue mg/kg	MRL mg/kg
Thiacloprid	20	0.0005	0.00015	8	1	11	0.047	0.2
Acetamiprid	20	0.0002	0.00006	20	-	-	-	

Commented [5.1.2.e]: In de jaren zijn meldingen van bijensterfte verzameld, zowel na de winter als gedurende het groeiseizoen. Vervolgens zijn bijen, bij-producten, en gewassen gemonsterd en geanalyseerd op pesticiden en bijenziekten.

Wintersterfte: in veruit de meeste gevallen van wintersterfte was varroa de enige verklarende factor. Daarnaast speelde het beheer van de imker een rol, of andere ziekten. In enkele gevallen werden ook residuen van neonico's gevonden, naast ziekten.

Zomersterfte: In veel gevallen van vermoeden van vergiftiging bleek dit in de periode van het zaaien van mais te spelen. Residuen van clothianidin, thiamethoxam, of imidacloprid werden dan vaker en hoger aangetroffen in bijen en bij-producten, dan in dergelijke matrices zonder vermoeden van vergiftiging.

Andere meldingen hingen samen met de bloei van mais of de behandeling van andere gewassen met dezelfde neonico's of met andere middelen. Diverse residuen werden aangetroffen in bij-producten.

Door de jaren was een verschuiving van clothianidin en thiamethoxam naar imidacloprid zichtbaar.

Meldingen zijn vooral geconcentreerd in regio's met kleinschalige landschappen.

•De auteurs concluderen dat een strengere naleving van de regels voor het gebruik van gecoat zaad nodig is. Gebruik met ondeugdelijke machines, of te hoge windsnelheid, wordt aangewezen.

•Gehalten van de stoffen in 2012 verschilden niet van 2011, maar het aantal meldingen van verdachte sterfte was wel maar 1/3 van die van 2011.

•Thiacloprid en acetamiprid werden in enkele honingmonsters gevonden, meestal beneden de grens van kwantificatie, en altijd beneden de MRL. Deze stoffen waren zijn in geen enkele melding aangewezen als mogelijk verdacht.

D_2-_M-329533_0003228099_5.1.2.e 2008_monitoring project

Reports on a long term monitoring study in Germany, 2004-2008. The program investigated the colony losses, honey yields, bee diseases and pesticide residues, looking for correlations between impact (colony losses, colony decline during winter) and causes (disease, residues, major crops, management).

Residue analysis of bea bread was performed for many pesticides, including thiacloprid and acetamiprid.

Table 3 Reported data on presence of residue in bea bread in Germany, 2005-2007

Substance	Number of samples	LOQ mg/kg	N <LOQ	N >LOQ	Maximum residue mg/kg
Thiacloprid, 2005-2006	105	0.001	3	6	0.199
Thiacloprid, 2007	110	0.001	10	52	Not reported
Acetamiprid 2004-2005	105	0.001	2	-	Not reported
Acetamiprid	110	0.001	-	-	-

Commented [5.1.2.e]: - Geen relatie tussen canola (oilseed rape, koolzaad) teelt en wintersterfte. Canola is een grote teelt met veel neonico's en aantrekkelijk gewas voor bijen.
-Relatie tussen varroa en wintersterfte
-Relatie tussen andere ziekten en wintersterfte
-Relatie tussen oude koningin en wintersterfte: met jonge koningin meer overleving
-Relatie tussen omvang volk en wintersterfte: hoe groter, hoe meer overleving
-Onderzoek naar correlatie tussen de achtergrondbelasting met residuen in bea bread en colony health moet nog plaatsvinden.

(Pettis, Lichtenberg et al. 2013)

Authors correlated exposure to pesticide residues in pollen to infection with *Nosema*. Pollen were collected from hives located in different crop fields in the USA, in California, Pennsylvania, Delaware and New York State. Collected pollen were identified to genus or species level. Pesticide residues in the pollen were analysed. Pollen were fed to newly emerged (*Nosema* free) bees, that were infected with *Nosema* (2^6 spores in sucrose per group of 10 bees) and fed with the pollen from the different hives, or from a control site (Arizona). After 12 days, the number of infected bees was scored (not the infection rate itself per bee). Thus, the relative risk of becoming infected with *Nosema* after exposure to a particular pesticide was calculated (vanEngelsdorp, Lengerich et al. 2013). Using a generalized linear mixed model, total pesticide diversity and fungal load were analysed in relation to *Nosema* infection.

Results

All pollen contained pesticides: mean number \pm se = 9.1 ± 1.2 (range 3-21). This number varied with the crop.

Pesticide loads mean 11760 ± 3734 $\mu\text{g}/\text{kg}$ (range 23.6-51310 $\mu\text{g}/\text{kg}$). The total load did not vary with crop or pesticide category.

147 of 630 bees fed *Nosema* spores became infected. 22 of the 35 pesticides found in pollen had a relative risk value significantly different from 1: 8 with an increased prevalence, and 14 with a decreased prevalence. The statistics correlate the *Nosema* infection rate to exposure to a pesticide per se – not to concentrations.

Acetamiprid was found 3x in Apple orchard hive pollen, mean 59.1 ± 32.2 (max 401) $\mu\text{g}/\text{kg}$.

Thiacloprid was found 2x in Apple orchard hive pollen, mean 1.1 ± 0.8 (max 12.4) $\mu\text{g}/\text{kg}$.

Both pesticides correlated significantly with a reduced risk (0.31 and 0.35, respectively) to *Nosema* infection.

With increased prevalence,

- Pyraclostrobin had the highest relative risk (2.85) with only 4 detections (2878 ± 1890 , max 27000 $\mu\text{g}/\text{kg}$)
- Chlorothalonil had the also a high relative risk (2.31) with 17 detections (4491 ± 2130 , max 29000 $\mu\text{g}/\text{kg}$)
- The varroacides DMPF (metabolite of amitraz) (2.13) and fluvalinate (2.43) were detected in 10 and 16 samples. Coumaphos had a negative correlation (0.61) and was detected in 6 samples only.
- Bifentrin had a significant relative risk of 2.08 with only 3 detections and relatively low concentrations (6.6 ± 3.8 max 53.1 $\mu\text{g}/\text{kg}$).
- Endosulfan I and II had a significant relative risk (1.60 and 1.41) with 8/6 detections at 1.5 ± 0.7 and 0.8 ± 0.3 $\mu\text{g}/\text{kg}$, respectively. However, endosulfan sulfate had a decreased relative risk.
- Pendimethalin had a significant relative risk of 1.47 with only 5 detections and relatively low concentrations (5.1 ± 3.7 max 69.5 $\mu\text{g}/\text{kg}$).

Fungicide load significantly affected *Nosema* infection (figure 5), pesticide diversity did not. Replacing fungicide load with chlorothalonil load gave same results, according to authors. Figure 5 shows a trendline for probability of infection vs. total fungicide load. The number of non-infected bees drops over 1000 ppb sharper than the number of infected bees.

Remarks

It was not presented how many control bees got infected. From the data it was deduced this was on average about 1 in 6, with a range 1 in 10 to 1 in 3. No reporting on mortality – also not in the cages that were (potentially) fed high levels of esfenvalerate and phosmet (above the LD50). Since the infection rate was presented as 147 out of 630 bees, it can be assumed that the dosages did not cause mortality, even at exposure above the LD50.

The data and the analysis do allow to conclude that higher total exposure to fungicides correlates to an

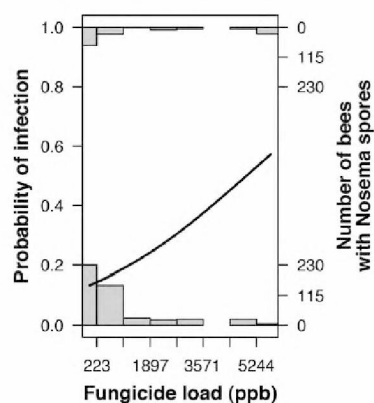


Figure 5. Probability of *Nosema* infection increased with fungicide load in consumed pollen. Histograms show the number of bees with (top) and without (bottom) *Nosema* spores as a function of the fungicide load in the pollen they were fed. The curve shows the predicted probability of *Nosema* infection. doi:10.1371/journal.pone.0070182.g005

increase in infestation risk with Nosema. Further knowledge on the mode of action here is needed, especially to incorporate this in multi-stress models.

To what fraction of actual bee hives this increased risk pertains depends on the spatial and temporal distribution crops (and associated pesticide use) in a given region. It would be interesting to investigate if distributions in established Nosema infections in bee hives correlate with fungicide in pollen and/or fungicide use in regions.

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