was reached in the treatment were group feeding took place and then mating. It is very likely that the consumption did not increase due to the mating process but due to the additional starvation phase after the bees had learned how to feed, as mating itself does not seem to have an effect.

Conclusions

Not only the type of food or feeder offered to Osmia can make a difference in the consumption rates, but the way the bee is treated before the test can have a large influence. This data shows that bees being exposed to a certain type of feeder in a group setting before the experiment will have better consumption rates when that same feeder is used during the experiment.

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4.8 Field exposure study: handling three different pollinator species and several matrices of residue analysis

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Introduction

The here presented study was set up to determine residues and ecotoxicologically relevant concentrations (ERCs) of a plant protection product in rapeseed (*Brassica napus*) inflorescences and their respective pollinator food matrices followed by single application after daily bee flight activity. Application was conducted under field conditions and in terms of good agricultural practice on five different trials in Northern-western Switzerland. The maximum mean concentration of residues over time was determined in different matrices collected by honey bee colonies (*Apis mellifera* L. (Hymenoptera: Apidae)), bumble bee colonies (*Bombus terrestris* (Hymenoptera: Apidae)) and solitary bee nesting cavities (*Osmia bicornis* (Hymenoptera: Megachilidae)). Sampling was conducted in a setup that the way of exposure / possible pesticide entry from field to hive could be demonstrated. The presented results and mode of action may be a significant addition and useful approach for creating further input and detailed data needed for the risk assessment on pollinators and their actual, realistic exposure to plant protection products based on the recent EFSA guidance document on the risk assessment of plant protection products for pollinator species (revised version July 2014).

Material & Methods

Content of active ingredient (analysed): 288 g active compound /L

Test species Honey bee (*Apis mellifera carnica*; ecotype: sklenar), 5 to 7 healthy honey bee colonies per field with one hive body including 14 Swiss format frames and containing between 2,350 to 12,300 bees, 4 to 8 frames with brood of all stages and at least 4 frames with stores (honey and pollen).

Bumble bee (*Bombus terrestris*) 8 healthy bumble bee colonies per field with one hive body containing between 48 to 124 bumble bees (manually counted in the lab before the transfer into the field) and a brood nest containing all developmental stages (i.e., eggs, larvae and pupae).

Solitary bee (*Osmia bicornis*) cocoons (in total 40 to 70 female and 40 to 72 male cocoons) were placed in every field at two/three different timepoints.

Five fields grown with *Brassica napus*, separated by at least 3 km were used. The test item was applied on DAT 0 on Brassica napus fully flowering at stage BBCH 63-66:

One to seven days before the test item application 5 to 7 honey bee colonies and 8 bumble bee colonies were placed at the margins of each field for residue sampling. Solitary bee cocoons were placed at 2-3 different occasions in special nesting aids on the fields during the two weeks prior to application for residue sampling. After the test item application (DAT 0), colonies remained at the field sites for 10 to 11 consecutive days.

Treatment was 0.2 L formulated product/ha, corresponding to 60 g active ingredient/ha, (based on nominal content). The test item was applied once per field at the same treatment rate. The spray treatment was carried out with a spray volume of 200 L/ha and applied on *Brassica napus* (at peak flowering) after bee flight activity and before 12 pm (midnight). To determine residues of the active compound, liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) was used.

The analytical procedure has been developed at the test facility, with sample preparation steps based on the QuECHERs method, and was validated according to SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1 within this study. For validation purposes, 2 control samples (untreated) were prepared and analyzed together with five test samples fortified with the active compound at 0.01 mg/kg (Limit of Quantification, LOQ) and five test samples fortified at ten times this limit (0.1 mg/kg, 10 x LOQ).

Fortification and Calibration solutions were prepared from stock solutions and serial dilutions of it, which were made by dissolving approx. 10 mg of the analytical standard in 10 mL of acetonitrile as well as acetonitrile/purified water (50/50, v/v), respectively. For calculation of the nominal concentrations of fortification and calibration solutions, the actual weighing's and the purity of the analytical standard (99.6 %) was taken into consideration. Fortification and calibration solutions were stored refrigerated (2 – 8°C) until required for analysis.

In order to assess possible matrix effects, which may result in either signal enhancement or signal suppression caused by interferences from matrices, matrix matched standard were prepared from extracts of untreated control samples. The signals obtained were compared with the response observed for calibration standard prepared in solvent. Matrix matched calibration standards were prepared by spiking of 0.485 mL of extracts obtained from untreated control samples with 0.015 mL of a reference solution comprising of the active compound at a nominal concentration of approx. 0.015 µg/mL (preparation of matrix matched standard at LOQ level) and 0.15 µg/mL (preparation of matrix matched standard at 10 x LOQ level). Matrix matched calibration standards were analyzed within each analytical sequence.

Specificity Testing was carried out along with each analytical sequence, blank solvent (acetonitrile/purified water; 50/50, v/v) samples were analyzed for specificity testing.

The linearity of the detector response was tested within each analytical sequence by the analysis of calibration solutions.

If required, samples were homogenized. Prior to LC-MS/MS determinations of residues, representative sub-aliquots were extracted as described. Where high residues were observed, dilution of the obtained analysis extract into the calibration range was carried out

Within each analysis batch, the efficiency of the sample workup and applicability of the analytical method was verified by procedural recovery test samples (each three untreated control samples fortified with the analytical standard at the Limit of Quantification (LOQ, 0.01 mg/kg) and at ten times this limit (10 x LOQ, 0.1 mg/kg). Due to the expected high level of residues, 1000 x LOQ spiked procedural recovery test samples (10 mg/kg) were prepared instead of 10 x LOQ spiked procedural recovery test samples and worked up and analyzed together with flower buds harvested at DAT 1.

The sugar content in selected nectar samples was determined optically by use of a digital refractometer: Kern ORD 92HM. Selected samples were analyzed in triplicate with the results expressed in Brix [%] and refractive index (nD).

Results & Discussion:

No residues were detected (below LOD; < 0.003 mg/kg) in untreated pollen, nectar and flower samples of the first samplings (DAT -2 to DAT 0 b.a.) from trials 1 to 5 with one exeption in trial 3 (two pollen samples from honey bee foragers (collected in the field), 0.014 mg/kg and 0.005 mg/kg (< LOQ)). A possible explanation for these values might be contamination due to handling of samples along the way from the field over work up and sample preparation to final measurement in the end. Since these values are very low (< LOQ and in one case slightly above LOQ) and all other measured samples of these specific timepoints from all fields were < LOD, these two values are not considered to have an impact on the outcome of the study.

The residues in pollen collected from honey bee foragers in the fields ranged from 0.938 mg/kg to 1.55 mg/kg (DAT 1) at the 1st sampling after the application. Until the last sampling a reduction of 88.9 % was visible (based on average values).

The residues in pollen collected from returning honey bee foragers at the hive entrance ranged from 0.836 mg/kg and 1.75 mg/kg at the 1st sampling after the application (DAT 1). Until the last sampling a reduction of 92.5 % was visible (based on average values).

In-hive pollen residues collected from honey bee colonies ranged from 0.01 mg/kg to 0.162 mg/kg at the 1st sampling after the application (DAT 4). Until the last sampling a reduction of 40.2 % was visible (based on average values).

In-hive residues of pollen collected from bumble bee colonies ranged from < LOD (< 0.003 mg/kg) to 0.75 mg/kg at the 1st sampling after the application (DAT 1). Until the last sampling a reduction of 78.3 % was visible (based on average values).

In-hive residues of pollen from solitary bee nesting cavities ranged between < LOD (< 0.003 mg/kg) and 0.034 mg/kg at the first samplings after the application (DAT 1 and DAT 4).

Residues of nectar samples sampled by honey bee foragers (collected in the field) ranged between < LOQ (< 0.01 mg/kg) and 0.047 mg/kg.

Residues of nectar samples sampled by honey bee foragers (collected at the hive entrance) ranged between < LOD (< 0.003 mg/kg) and 0.018 mg/kg (DAT 7 and DAT 10, values slightly over LOQ (0.01 mg/kg)). No residues were found in samples from DAT 1 to DAT 4.

No residues were found in in-hive nectar specimens collected from honey bees and bumble bees (< LOD; < 0.003 mg/kg).

For flowers residues between 0.182 mg/kg and 4.78 mg/kg were measured at the 1st sampling after application (DAT 0 a.a. and DAT 1). A reduction of 96.8 % was visible until the last sampling (DAT 10) (based on average values).

For an overview of the range of analysed residues and their reduction from the 1st to the 4th sampling, after application calculated from the average residue values from all 5 trials see the following table:

Matrix			Treatment	Timing [DAT]	Range of residues [mg/kg][1)]	90th percentile [mg/kg][1)]	Average [mg/kg][1)]	Reduction [%]
Nectar	Honey bees	Foragers in the field	C	-1	< LOD	-	-	-
			T	1	< LOQ – 0.047	0.038	0.021	-
				4	< LOD	< LOD	< LOD	100
				7	< LOD - < LOQ	< LOQ	< LOD	100
				10	< LOD	< LOD	< LOD	100
		Foragers at the hive	C	-1	< LOQ	-	-	-
			T	1	< LOD –	< LOQ	< LOQ	-

Matrix			Treatment	Timing [DAT]	Range of residues [mg/kg][1)]	90th percentile [mg/kg][1)]	Average [mg/kg][1)]	Reduction [%]
		entrance			< LOQ			
				4	< LOD	< LOD	< LOD	-
				7	< LOD – 0.024	0.018	< LOQ	-
				10	< LOD – 0.018	0.017	0.012	-
		In-hive	C	-1	< LOD	-	-	-
			T	4	< LOD	< LOD	< LOD	-
				7	< LOD	< LOD	< LOD	-
				10	< LOD	< LOD	< LOD	-
	Bumble bees	In-hive	T	1	< LOD	< LOD	< LOD	-
				4	< LOD [2)]	< LOD	< LOD	-
				7	< LOD [3)]	< LOD	< LOD	-
				10	< LOD [4)]	< LOD	< LOD	-
Pollen	Honey bees	Foragers in the field	C	-1	< LOD - LOQ	-	-	-
			T	1	0.938 – 1.55	1.46	1.26	-
				4	0.046 – 0.159	0.15	0.12	90.5
				7	0.056 – 0.157	0.12	0.081	93.6
				10	0.02 – 0.474	0.31	0.14	88.9
		Foragers at the hive entrance	C	-1	< LOD	-	-	-
			T	1	0.836 – 1.75	1.61	1.15	-
				4	0.066 – 0.197	0.18	0.13	88.7
				7	0.049 – 0.12	0.11	0.084	92.7
				10	0.058 – 0.109	0.11	0.086	92.5
		In-hive	C	-1	< LOD	-	-	-
			T	4	0.01 – 0.162	0.14	0.082	-
				7	0.026 – 0.092	0.086	0.053	35.4
				10	< LOQ – 0.132	0.094	0.05	40.2
	Bumble bees	In-hive	T	1	< LOD – 0.75	0.53	0.18	-
				4	0.009 - 0.504 [5)]	0.38	0.17	5.6
				7	< LOD – 0.137	0.1	0.036	80.0
				10	< LOD – 0.083 [6)]	0.068	0.039	78.3
	Solitary bees	In-hive	T	1	< LOD [2)]	< LOD	< LOD	-
				4	< LOD – 0.034 [7)]	0.024	0.01	-
				7	< LOD [7)]	< LOD	< LOD	70.0
				10	< LOD [7)]	< LOD	< LOD	70.0
Flowers	Hand sampling		C	-2 to 0 b.a.	< LOQ	-	-	-
			T	1	0.182 – 4.78	3.06	1.61	-
				3 to 5	0.011 – 0.508	0.41	0.23	85.7
				7 to 8	0.011 – 0.179	0.13	0.083	94.8
				10	< LOD – 0.153	0.11	0.051	96.9

DAT: days after application; C: Control; T: Test item;
LOQ = 0.01mg/kg, LOD = 0.003 mg/kg
1) for the calculation of the 90th percentile and mean all values < LOD were set to ½ of LOD (0.0015mg/kg) and values < LOQ were set to 0.01mg/kg according to FOCUS 2006
2) values from trial 3 and 4
3) values from trial 1, 2 and 4
4) values from trial 2 and 3
5) values from trial 2 – 5
6) values from trial 3 – 5
7) values from trial 1, 3, 4 and 5

Conclusions:

Comparing all data a dilution of residues via the respective route of entry can be shown, starting with high residue values from the applied flower buds over reduced residue values sampled from

honey bee foragers in the field and at the hive entrance (pollen and nectar) and stored food items (bee bread and nectar) with significant lower in-hive residues (sampled from honey bee and bumble bee colonies).

Residues on pollen sampled from solitary bee hives are difficult to be interpreted since results are based on only four of five study fields and on a limited number of samples due to methodological limitations in this test system. The residues on pollen were < LOD in three study fields at all samplings dates and very low at DAT 4 in one study field in comparison with honey bees and bumbles bees at the respective sampling date.

The highest residues in bee-relevant matrices were found in pollen (maximum 1.75 mg/kg). Decline of residues in pollen was observed for all samples. Dissipation time (DT50) was < 4 days. No residues or residues close to the LOQ (0.01 mg/kg) were found in nectar samples. The sugar content was determined to be 81.5 %.

No other attractive crops that flowered during the course of the study were detected. Therefore, the obtained data reflect a worst-case scenario under realistic conditions (trials conducted in agricultural landscapes).

The selected application rate (60 g a.i./ha) covers the maximum single application rate according to GAP. Based on the highest residues, found in the bee-relevant matrix pollen, the 90th percentile was determined to be 1.61 mg/kg at the first sampling after application (honey bee foragers) with an average value of 1.15 mg/kg.

4.9 Exposure by nesting material? – Investigation of potentially suitable methods for higher tier studies with solitary bees

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The registration processes and risk assessment of plant protection products (PPPs) on bees resulted in an increasing need for experiments with non-apis pollinators to assess potential side effects of PPPs on this relatively new group of test organisms. Recently, numerous studies have been performed but there is still a wide range of ongoing challenges. One of the challenges is the risk from insecticide exposure to solitary bees (especially at larval stages) by contaminated nesting material (e.g. mud partitions – mason bees). In 2017, an experiment was performed with the horn-faced mason bee *Osmia cornuta* (Hymenoptera, Megachilidae) under modified field conditions. The aim of the experiment was to develop a suitable test method for higher tier risk assessments with solitary wild bees exposed to treated nesting material. The potential effect of an insect growth regulator (IGR) to bees and their brood was examined. The reproduction capacity and brood termination rate were observed in the study as endpoints. Furthermore, hatching success and flight activity were recorded as additional information at several occasions.The present results provide no evidence that the exposure has an effect on the development during the larval stages of *Osmia cornuta*, neither in pollen mass nor in the nesting material.

Introduction

Pollination plays as ecosystem service[1] an important role in maintaining the global biodiversity and food production[2,3]. Over the last decades the global pollinator diversity decreased[4] and consequently the status of the bees moved in the focus of public interest. As a result, the registration processes and risk assessment of plant protection products (PPPs) on bees proposed