Literature review

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Title:

Review on the available scientific literature and industry studies on guttation as potential exposure route for honey bees

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1 Summary

With the increased awareness of the importance of pollinating insect species in light of potential risks caused by plant protection products (PPP) EFSA proposed a new guidance on honey bees, bumblebees and solitary bees in 2013. While this risk assessment scheme is not yet in force, additional studies and endpoints, as well as the assessment of additional exposure routes, are already required for the registration of PPP. One of these exposure routes is via the foraging on guttation droplets potentially contaminated with PPP residues.

Honey bees, which often serve as model species in the regulatory context, need water and are known to exploit different water sources. A potential risk exists when e.g. residues of water soluble (systemic) substances that the plant has been exposed to are present in the guttation water on which honey bees in turn forage to satisfy the water demand of the colony.

A standard procedure to assess the risk in the regulatory context of PPP is the comparison of toxicity and exposure. While the toxicity is inherent to a certain substance and can be measured in standardized laboratory tests, the exposure part in connection with guttation is more complex. Three elements need to be considered in order to assess exposure via guttation to foraging honey bees:

1. The amount of residues in guttation water after PPP application.

2. The occurrence of guttation on a certain plant species.

3. The extent to which honey bees are actually collecting guttation droplets.

In this review we evaluated field studies on guttation, which were conducted by the industry for registration purposes between 2010 and 2017. The aim was to find a realistic estimate (90^{th} percentile) for the occurrence of guttation in a certain crop species as well as an estimate for the number of honey bees foraging on guttation water (90^{th} percentile) on a certain crop. Residue contents in guttation water were also briefly summarised. Furthermore, data from peer-reviewed publications (open literature) with relevance to guttation and the exposure of honey bees were evaluated with the same aims.

Data from open literature was less comprehensive than in the industry studies that were specifically designed for regulatory purposes. Industry studies followed a more extensive protocol, presented larger datasets and more detailed results. While the focus of industry studies was clearly on the risk to honey bee colonies from the application of PPPs, open literature studies included a range of questions and approaches at different levels. Furthermore, open literature studies often lacked the level of detail in the description of the methodology or the reporting of the results presented in the industry studies. A comparison between the data derived from industry studies and data from open literature proved therefore to be difficult. Overall results show that residues to a certain extent can be expected in guttation fluid from the application of PPPs. Guttation was frequently observed for numerous crops. However, the number of honey bees actually foraging on guttation droplets was low for all crops. These findings were further discussed and their relevance for the risk assessment for PPPs was evaluated.

2 Introduction

2.1 Purpose of this review

In this review we evaluated field studies on guttation, which were conducted by the industry for registration purposes between 2010 and 2017. The aim was to find a realistic estimate (90th percentile) for the occurrence of guttation in a certain crop species as well as an estimate for the number of honey bees foraging on guttation water (90th percentile) on a certain crop. Furthermore, all available residue concentrations from PPPs in guttation water are briefly summarized.

Reviews on this subject have been conducted by e.g. Pistorius et al. 2012 and Schmolke et al. 2018, however the focus of these evaluations were more on the amount of residues and the effects on honey bee colonies that could be measured.

Additionally, data from peer-reviewed publications (open literature) with relevance to guttation and the exposure of honey bees were evaluated with the same aims.

2.2 Water foraging of honey bee colonies

Honey bees as domesticated animals do not only have a long history with humans, they produce honey, are valued for their pollination services and due to their unique biology have been and still are the object of scientific research.

Honey bees thrive under different climatic conditions, in fact due to human proliferation they are present in an area well beyond their original distribution range (Michener 2007). Beekeeping is possible in temperate, sub-tropic and tropic regions of the world where flowering plants provide pollen and nectar. The seasonality of influx of pollen and nectar is compensated by the colonies by creating stores of these two essential resources. In temperate regions this adaption to the seasons can be observed in the typical colony lifecycle with activity during the months that floral resources are available and a consecutive build-up of in-hive stores and a phase with restricted activity during the time of overwintering. However, beside the essential resources provided by flowering plants, honey bee colonies need water to survive. Water is either used to dilute stored honey or for the cooling of the hive via evaporation (Lindauer 1951). Water used for dilution of honey is mainly important in spring when the stored honey resources are needed for the rearing of new larvae. The evaporation of water in order to cool the hive is part of an elaborate behavioural pattern that allows thermoregulation of the hive by the colonies under different environmental conditions (e.g. Gates 1914, Himmer 1932, Free and Spencer-Booth 1958). In order to maintain the optimal temperature for brood rearing (34-35°C) water is an essential resource for honey bee colonies in hot summer days. However, water is, in contrast to nectar and pollen, not stored in the hive and is collected depending on the immediate needs of the colony. Adaptive behavioural mechanisms enable honey bees to respond to the water demand of the colony and control water foraging (Lindauer 1955, Seeley 1995, Kühnholz and Seeley 1997). These mechanisms are important as it can be said in general, that water foraging stands in competition with the foraging for pollen and nectar. The foraging for nectar will in most cases lead to a positive energy balance for the

colony as foragers will bring more of this energy-rich resource to the colony than they spend for their foraging flight. Foraging for pollen follows basically the same principle, although it is a protein source used for rearing of brood and not necessarily an energy source for adult honey bees. Additionally, foragers can take up nectar either from the same plant or from flowering plants on their way and store it in their honey sac (crop) as pollen is transported in the bee's corbiculae. The collection of water, in contrast, causes the colonies to spend energy. Vischer et al. (1996) showed that honey bees foraging for water stored only little nectar in their crop when they started out and used the sugar reserves of their bodies for the return flight. A "re-fuelling" as during pollen foraging is not possible, because the crop will hold the collected water and its content will have to be passed to the bees midgut with an subsequent excretion of the water in order assimilate the dissolved sugars by the bee (Vischer et al. 1996). This effectively limits the range of honey bee water foraging. It has to be pointed out that the most energy efficient way to satisfy the colony's water demand is the uptake of diluted nectar, which will be further condensed by the honey bees effectively delivering water and sugar at the same time. In fact much of the water demand of a colony is met by the incoming nectar (Seeley 1995). Nevertheless, active water foraging is a common phenomenon in honey bee colonies as the environmental condition required to induce this behaviour regularly occurs even in temperate regions.

Furthermore, water foraging differs from nectar and pollen foraging as the quality of the resource may be less important for the foraging decisions of the colony. While it may be worth to explore an abundant source of nectar with high sugar content which requires a longer flight distance from the hive, spending more energy to reach more distant water sources may not be of an advantage for the colony. However, there is some evidence (Kiechle 1961, Bonoan et al. 2017) that honey bees prefer compound rich "dirty water" over water with low mineral concentrations. The authors explain this preference by the demand of bees for micro-nutrients (especially sodium). This may play a role for the foraging choice when water is needed for the dilution of honey and the feeding of larvae. When water is needed for the cooling of the hive via evaporation nutrient content of the water seems unlikely to be relevant. Honey bees are known to exploit several different water sources ranging from streams and ponds to small puddles up to dew, raindrops and guttation droplets of plants. A study by Joachimsmeier et al. (2012a) suggests that honey bee do not distinguish between the latter three.

2.3 Guttation as a phenomenon in vascular plants

Guttation is a phenomenon in vascular plants where small droplets of water are exuded. Droplets will usually occur at the tip or the edges of a leaf where specialized structures called hydrathodes, which act as water pores, are located. Due to these structures guttation droplets have a distinct almost round form by which they can be distinguished from dew which consists of atmospheric water condensed on the leaf surfaces. Guttation occurs under conditions of high humidity and low transpiration for instance at night or in the early morning. Under these conditions water will enter the root due to the difference in water potential to the surrounding soil. This accumulation is responsible for the root pressure which causes water to be pushed upwards the stem of the plant. This is in contrast to the transpirational pull which provides xylem flow during the daytime when stomata are open for the gas exchange during photosynthesis. For a more detailed review on guttation please refer to Singh (2014). Guttation can be seen as an adaption in plants to maintain xylem flow and thus the supply of water and nutrients to the

above ground plant tissue. However, the occurrence is not only dependent on external conditions, but also on plant species and growth stage (Joachimsmeier et al. 2012b). Part of this review was an evaluation of the available data to estimate how often guttation occurs for a given plant species (crop).

2.4 Residues of systemic plant protection products in guttation water

Several ways exist to deliver PPPs to the crop to be protected or the targeted pest. However, in agricultural practice the application as spray solution and the coating of crop seeds (seed treatment) are the most commonly used methods. For both methods of application it has to be differentiated between systemic and non-systemic compounds. While the latter stay on the surface of the leaves or around the treated seed, systemic substances are taken up by the plants and are present in the plant tissue. Typically, systemic PPPs are applied as seed treatment and provide protection for the growing plant in the seedling stage and beyond depending on the degradation characteristics of the substance used. An advantage of this method is that plants are protected “internally” for a longer period of time in contrast to a foliar spray application of a non-systemic PPP, where e.g. rain events can lead to a wash-off of the applied substance causing a reduced effectiveness of the treatment. The downside of a seed treatment with a systemic PPP is that there is a potential for a comparably high amount of residues in plant tissues or fluids. Depending on the toxicological profile of the substance this can be of ecotoxicological relevance. When for instance residues of a systemic insecticide can be detected in nectar of a flowering crop (e.g. oilseed rape) this needs to be considered in the risk assessment during the registration process of the PPP for bee species foraging on this crop. As several studies imply that residues of systemic PPP can also be found in guttation fluid of treated plants (reviewed in Pistorius et al. 2012, Schmolke et al. 2018 and this report) also this potential route of exposure warrants further attention.

A more detailed consideration of the exposure and risk from guttation fluid for honey bees is presented in the following chapter.

2.5 Exposure and risk for water foraging honey bees

A standard procedure to assess the risk in the regulatory context of PPP is the comparison of toxicity and exposure. While the toxicity is inherent to a certain substance and can be measured in standardized laboratory tests and expressed as standard value (e.g. LD_{50}), the exposure part in connection with guttation is more complex. Three elements need to be considered in order to assess exposure via guttation to foraging honey bees:

1. The amount of residues in guttation water after PPP application.

2. The occurrence of guttation on a certain plant species.

3. The extent to which honey bees are actually collecting guttation droplets.

It has to be pointed out that consideration of guttation water as a potential exposure route has only been recently considered in the risk assessment process of PPPs in the EU. It is included in the new Guidance Document proposed by the European Food Safety Authority (EFSA) in 2013, however, it

contains some precautionary assumptions, which are likely to lead to the need of further higher tier data. While it is possible to conduct higher tier studies e.g. in a residue trial or field effect study for the specific substance, the data that is already available should also be considered, especially for the more generic, i.e. not substance specific, part (points 2 and 3 mentioned above).

3 Methods

3.1 Evaluation of available industry studies

Twenty-five studies provided by different manufacturers of PPP were evaluated. If a study was conducted at two explicitly different seasons (e.g. with a gap of assessments in winter) results for the different seasons (i.e. autumn and spring) were reported separately, this yielded 31 datasets. A summary of the study protocols for the studies can be found Table 1.

The compilation of results followed the three elements to be assessed as laid out under 2.5:

1. The amount of residues in guttation water for each plant (crop) species, which included the context of test design, active substance and timescale of sampling. 2. The occurrence of guttation on a certain plant species, which was recorded (or re-calculated where possible) as the fraction of days that guttation was observed. A value of 1 (equivalent to 100%) means guttation occurred on all observation days of a study. As contextual data information on the test design, growth stage of the plants and season were recorded. 3. The number of honey bees taking up guttation fluid: In order to have a comparable value for the number of honey bees taking up guttation fluid the reported values were standardized to 1 square meter of observed crop for 1 minute. As methodology varied between the studies, the length of the observation units, the area observed and the total number of observation units were recorded in order to make the re-calculation more transparent. Table 2 gives an overview of the observation effort for each crop evaluated in the industry studies. Mean and maximum values were recorded where possible. For points 2 and 3 data for the treated field as well as for the control were assessed.

In cases where more than one datapoint per crop and season was available the overall minimum, maximum and the 90^{th} percentile were derived. For the residue dataset additionally a Residue Unit Dose (RUD) was calculated, which is the amount of residues based on a theoretical application rate of 1 mg a.s./seed. This was possible where the application rate was stated accordingly or could be calculated from the given data.

Additionally, possible effects of the test item in terms of colony strength and mortality were summarized, if available. For this purpose, mean daily mortality of honey bees during and after the potential exposure to guttation water containing residues of PPP was recorded for the test item and control group for each study. From these values, overall means for the respective groups and each crop were calculated, if more than one datapoint was available. The same procedure was applied to the data for the estimated number of honey bees in a colony (colony strength). If the respective data was available, overall means for the test item and control groups per crop were calculated for the following assessments: the last before exposure, the first post-exposure, the last before and the first after overwintering.

Table 1: Protocol summaries of evaluated industry studies

No.	Crop	Test design	Protocol summary
1	Maize	1 control field 1 treatment field	Six colonies per field. Bee hives were placed in a distance of about 1 to 2 m to the sowing area. Assessments: - Mortality (dead-bee traps and linen sheets) - Occurrence and duration of guttation - Flight activity and behaviour of the bees at the hive entrances and in the field plots - Flight activity in defined areas within the assessment zones - Bees collecting guttation liquid as water supply - Colony development
2	Maize	1 control field 1 treatment field	Six colonies per field. The bee colonies were placed at the edge of the test item treatment plot in a distance of 1-2 m and in the control of less than 1 m. Assessments: - Mortality (dead-bee traps and linen sheets) - Occurrence and duration of guttation - Flight activity and behaviour of the bees at the hive entrances and in the field plots - Flight activity in defined areas within the assessment zones - Bees collecting guttation liquid as water supply - Colony development
3	Maize	1 control field 1 treatment field	Six colonies per field. The bee colonies were placed at the edge of each field plot in a distance of 1-2 m to the sowing area. Assessments: - Mortality (dead-bee traps and linen sheets) - Occurrence and duration of guttation - Flight activity and behaviour of the bees at the hive entrances and in the field plots - Flight activity in defined areas within the assessment zones - Bees collecting guttation liquid as water supply - Colony development
4a,b	W-OSR	2 fields with 3 study plots for control and treatment (1 field with 1 and 1 field with 2 study plots)	Five honey bee colonies per study plot were placed along a line shortly before or shortly after sowing and in any case well before seedling emergence, either directly adjacent or within a maximum distance of 0.5 m to the W-OSR crop. Assessments: - Mortality (dead bee traps) - Occurrence of guttation - Honey bee activity in guttating crop - Sampling of guttation fluid and residue analysis - Colony strength and health
5a,b	W-Barley	2 test locations (Northern and Southern Germany) with 3 fields each (control, 2 test items)	Five honey bee colonies per field, directly adjacent or within a maximum distance of 0.5 m to the crop. Assessments: - Mortality (dead bee traps) - Occurrence of guttation - Honey bee activity in guttating crop - Sampling of guttation fluid and residue analysis - Colony strength and health

No.	Crop	Test design	Protocol summary
6a,b	W-Wheat	2 test locations (Northern and Southern Germany) with 3 fields each (control, 2 test items)	Five honey bee colonies per field, directly adjacent or within a maximum distance of 0.5 m to the crop. Assessments: - Mortality (dead bee traps) - Occurrence of guttation - Honey bee activity in guttating crop - Sampling of guttation fluid and residue analysis - Colony strength and health No assessments of guttation and honey bee activity in autumn in Northern Germany due to late germination of crop.
7a,b	Sugar beet	1 control field 1 treatment field	Eight colonies per field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Observation of honeybees visiting sugar beet plants displaying guttation - Behaviour of the bees in the crop and around the hive - Residue analysis - Colony strength and health (incl. overwintering performance)
8a,b	Sugar beet	1 control field 1 treatment field	Eight colonies per field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Observation of honeybees visiting sugar beet plants displaying guttation - Behaviour of the bees in the crop and around the hive - Residue analysis - Colony strength and health (incl. overwintering performance)
9a,b	W-Barley	4 control fields (3 fields 1 study plot, 1 field 2 study plots) 4 treatment fields (3 fields 1 study plot, 1 field 2 study plots)	Five colonies per study plot. Honey bee colonies were set up at the study fields either directly adjacent to the crop or in a distance of approximately 4.5 m to the crop margin. Assessments: - Mortality (dead bee traps) - Occurrence of guttation - Honey bee activity in guttating crop - Sampling of guttation fluid and residue analysis - Colony strength and health (incl. overwintering performance)
10	Potatoes	1 control field 1 treatment field	Eight colonies per field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Observation of honey bees visiting potato plants displaying guttation - Behaviour of the bees in the crop and around the hive - Condition of the colonies
11	Potatoes	1 control field 1 treatment field	Eight colonies per field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Observation of honey bees visiting potato plants displaying guttation - Behaviour of the bees in the crop and around the hive - Condition of the colonies

No.	Crop	Test design	Protocol summary
12	Maize	1 control field 1 treatment field	Six honey bee colonies per field. Hives were set facing the maize fields within 5.7 m from the crop in the test item treated field and within 10.4 m in the control field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence of guttation - Honey bee activity and behaviour - Colony health, strength and brood development (incl. overwintering performance) - Samples of guttation liquid, soil, maize plants, pollen taken directly from maize plants and forager bees, dead bees from dead bee traps were collected for residue analysis
13	Maize	1 control field 1 treatment field	Six honey bee colonies per field. Hives were set facing the fields within 7 m distance from the crop in the test item treated and approximately 3 m in the control field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence of guttation - Honey bee activity and behaviour - Colony health, strength and brood development (incl. overwintering performance) - Samples of guttation liquid, soil, maize plants, pollen taken directly from maize plants and forager bees, dead bees from dead bee traps were collected for residue analysis
14	Maize	1 control field 1 treatment field	Six honey bee colonies per field. Hives were set facing the fields within 4.5 m from the crop in the test item treated and 5 m in the control field. Six honey bee colonies per field. Hives were set facing the fields within 7 m distance from the crop in the test item treated and approximately 3 m in the control field. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence of guttation - Honey bee activity and behaviour - Colony health, strength and brood development (incl. overwintering performance) - Samples of guttation liquid, soil, maize plants, pollen taken directly from maize plants and forager bees, dead bees from dead bee traps were collected for residue analysis
15	Maize	1 control field 1 treatment field	Six colonies per field. The shortest distance from the colonies to the maize crop was 5 m. Assessments: -Dust samples during drilling - Mortality (dead bee traps and linen sheets) - Occurrence of guttation - Honey bee activity and behaviour - Colony health, strength and brood development - Forager bee samples (pollen loads and nectar stomachs), pollen from the hive, soil, guttation liquid for residue analysis

No.	Crop	Test design	Protocol summary
16	Sugar beet	1 control field 1 treatment field	Six colonies per field. Hives placed directly in the sugar beets fields. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting sugar beet plants displaying guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis
17	Sugar beet	1 control field 1 treatment field	Six colonies per field. Hives placed directly in the sugar beets fields. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting sugar beet plants displaying guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis
18	Sugar beet	1 control field 1 treatment field	Six colonies per field. Hives placed directly in the sugar beets fields. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting sugar beet plants displaying guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis
19a,b	W-OSR	1 control field 1 treatment field	Six colonies per field. Hives placed directly in OSR fields. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting OSR plants displaying guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis

No.	Crop	Test design	Protocol summary
20a,b	W-OSR	1 control field 1 treatment field	Six colonies per field. Hives placed directly in OSR fields. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and proportion of guttation - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting OSR plants displaying guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis
21	W-OSR	1 control field 1 treatment field	Six colonies per field. Hives placed directly in OSR fields. Assessments: - Dead bees on the linen sheets and in the dead bee traps; - Flight intensity in the field - Flight intensity at the hive entrances - Observation of honey bees visiting OSR plants displaying guttation - Occurrence and proportion of guttation - Behaviour of the honey bees - Condition and health of the colonies (incl. overwintering performance) - Samples of nectar, pollen and wax from combs, plant material, guttation liquid for residue analysis
22	Maize	2 test locations (Southern and Northern Alsace) 11 treatment and 2 control fields in the South, 8 treatment and 1 control field in the North	Four colonies per field. Hives were placed at the edge of each field within 0 to 6.4 m distance to the drilled area facing the maize. Assessments: - Mortality (dead bee traps, linen sheets) - Occurrence of guttation - Flight activity in the fields and behaviour of the honey bees - Colony strength and development - Samples of dead honey bees for residue analysis
23	Onion	2 test locations in NL (Limburg, Zuid-Holland), 10 treatment fields in Limburg, 5 treatment and 5 control in Zuid-Holland.	Monitoring study. One hive per field (except for one field which was close to apiary). Distance to the field was averagely 10 m. Assessments: - Occurrence of guttation - Occurrence of honey dew - Flight activity of honey bee colonies - Presence of bees (solitary bees, bumble bees, honey bees) in the onion fields and off-crop
24	Brassica	7 control fields 7 treatment fields	Monitoring study. One hive per field. Distance to the field was averagely 24 m. Assessments: - Occurrence of guttation - Occurrence of honey dew - Flight activity of honey bee colonies - Presence of bees (solitary bees, bumble bees, honey bees) in the brassica fields and off-crop

No.	Crop	Test design	Protocol summary
25	S-OSR	1 control field 3 treatment fields	Four colonies per field placed at the border of each field plot. Assessments: - Mortality (dead bee traps and linen sheets) - Occurrence and percentage of guttation - Flight intensity, behaviour and honey bees visiting guttating OSR plants - Condition and health of the colonies - Samples of guttation liquid, plant material, hive products (pollen, nectar, worker jelly) as well as nectar from flowers and pollen from flowers for residue analysis

Table 2: Range of observation periods for the occurrence of guttation and total observation time of honey bee activity in the fields with guttating plants in the evaluated industry studies.

Crop	No of studies	Observation period guttation Min - Max [d]	Total observation time honey bees Min - Max [h]
Brassica	1	57	114.5
Maize	8	15-53	5-98
Onion	1	30	204
Potatoes	2	58-59	13-14
Sugar beet	5	29-42	5-10
W-Barley	2	Autumn: 11-27 Spring: 12-32	Autumn: 20-60 Spring: 19-48
S-OSR	1	30	10
W-OSR	4	Autumn: 29-52 Spring: 10-31	Autumn: 27-72 Spring: 11-21
W-Wheat	1	Autumn: 2-12 Spring: 15-31	Autumn: 5 Spring: 28

3.2 Open literature search and evaluation

The search for peer-reviewed publications (open literature) with relevance to guttation and the exposure of honey bees was conducted with specific keyword combinations using Google Scholar (https://scholar.google.com/) and Web Of Knowledge. The keyword combinations for the search are listed in Table 3. For each keyword combination, the number of publications which were found (hits) was recorded. All publications were evaluated for their relevance following a step-wise procedure: 1. All publications were screened by their title. 2. The abstract of all publications with a relevant title was assessed. 3. The full-text of all publications with a relevant abstract was obtained and was checked for relevant information, which meant that it contained data on residues in guttation water, occurrence of guttation in the field and/or observations on honey bees collecting guttation water. 4. All relevant information was recorded in a tabular form.

Table 3: Terms used in the literature search and the results obtained by different search tools

Keyword combination	Search tool					
	Google scholar			Web of Knowledge		
	No. of hits	Potentially relevant	Relevant by Abstract	No. of hits	Potentially relevant	Relevant by Abstract
Guttation AND ”Apis mellifera”	515	63	13	12	2	2
Guttation AND “honey bees”	628	1	1	12	7	6
Guttation AND residues	14200	*	*	17	2	2
Neonicotinoid AND ”Apis mellifera” AND guttation	453	66	7	10	0	0
Neonicotinoid AND “honey bees” AND guttation	539	24	5	11	1	1
Pesticide AND “honey bees” AND guttation	598	15	4	7	0	0
Pesticide AND “Apis mellifera” AND guttation	510	2	0	8	0	0
“Plant protection product” AND “honey bees” AND guttation	43	6	0	0	0	0
“Plant protection product” AND “Apis mellifera” AND guttation	44	0	0	0	0	0
“Seed coating” AND “honey bees”	724	6	0	19	1	0
“Seed coating” AND “Apis mellifera”	602	4	0	16	1	0
“Seed coating” AND guttation	246	2	1	8	1	0
“Water foraging” AND “honey bees”	237	2	0	8	0	0
“Water foraging” AND “Apis mellifera”	221	1	0	11	3	0
“Water foraging” AND guttation	63	2	0	3	0	0

*no further evaluation was conducted as the effort would have been disproportionate to the results, however it is almost certain that no publications have been overlooked due to the use of the additional keyword combinations

Some papers could appear in several searches of different keywords, if possible an additional double count was avoided. This means that once a publication was deemed to be relevant and it appeared for another set of keywords it was not additionally counted as “potentially relevant” or “relevant by abstract”.

The search was not limited to a specific period, all published data that could be obtained was included in this review.

While only publications of original data were considered, review publications found in the search were assessed to make sure that no article or data were missed.

In parallel to the evaluation of the industry studies the compilation of results followed the three elements to be assessed as laid out under 2.5:

1. The amount of residues in guttation water for each plant (crop) species, which included the context of test design, active substance and timescale of sampling. 2. The occurrence of guttation on a certain plant species, which was recorded (or re-calculated where possible) as the fraction of days that guttation was observed (i.e. the number of days with guttation in relation to the number of days that observations were made). As contextual data information on the test design, growth stage of the plants and season

were recorded. 3. The number of honey bees taking up guttation fluid: As it turned out that only a few publications recorded data on foraging honey bees this point was addressed without any attempt to calculate a standard value, but added as text entry to the table addressing point 2 (occurrence of guttation).

4 Results

4.1 Industry studies

A summary of the residues found in guttation water for each crop is presented in Table 4. The most datapoints are available for sugar beets, followed by maize and winter oilseed rape. Not all studies included the information on the application rate as the amount active substance per seed (or an equivalent which allowed a re-calculation). Therefore, the dataset for which a calculation of a RUD could be made was consequently smaller than the original dataset. There is a tendency that for crops sown in autumn (such as winter oilseed rape and winter cereals) the amount of residues in guttation fluid (expressed as 90th percentile RUD) are higher in comparison to crops sown in spring (maize, sugar beets). It has to be pointed out, that the uptake of a systemic substance depends on several factors: The crop itself, the properties of the substance and environmental parameters. Furthermore, the timing of the occurrence of guttation plays a role. If guttation occurs in the very early growth stages then residues are likely to be higher compared to guttation fluid occurring in later growth stages. At this point, the existing data does not allow to distinguish between the different factors potentially determining the amount of residues in guttation fluid. Figure 1 shows the distribution of the RUD (as logarithmic values) for the maximum residues for the three substances clothianidin, imidacloprid and thiamethoxam in the guttation fluid of sugar beets..

Table 4: Minimum, maximum and 90th percentile of residues of active substance, following the application of PPP, found in different crops based on maximum reported (worst case) values in industry studies. Additionally, values were recalculated for a theoretical application rate of 1 mg a.s./seed in order to have a comparable value (Residue Unit Dose, RUD)

Crop	Total residues				RUD [1 mg a.s./seed]			
	Min [mg/L]	Max [mg/L]	90th percentile [mg/L]	Datapoints (n)	Min [mg/L]	Max [mg/L]	90th percentile [mg/L]	Datapoints (n)
Maize	15.7	28.6	28.36	4	23.09	41.45	41.34	3
Sugar beet	0.01	123	82.68	7	0.03	279.55	187.91	7
S-OSR[a]	0.837	0.837	0.837	1	NA	NA	NA	NA
W-OSR	0.0021	11.14	9.32	6	0.11	557.00	466.00	6
W-Barley	2.3	15	13.053	4	109.52	535.71	493.10	2
W-Wheat	6.9	13	12.39	2	168.29	448.28	420.28	2

[a] spray application

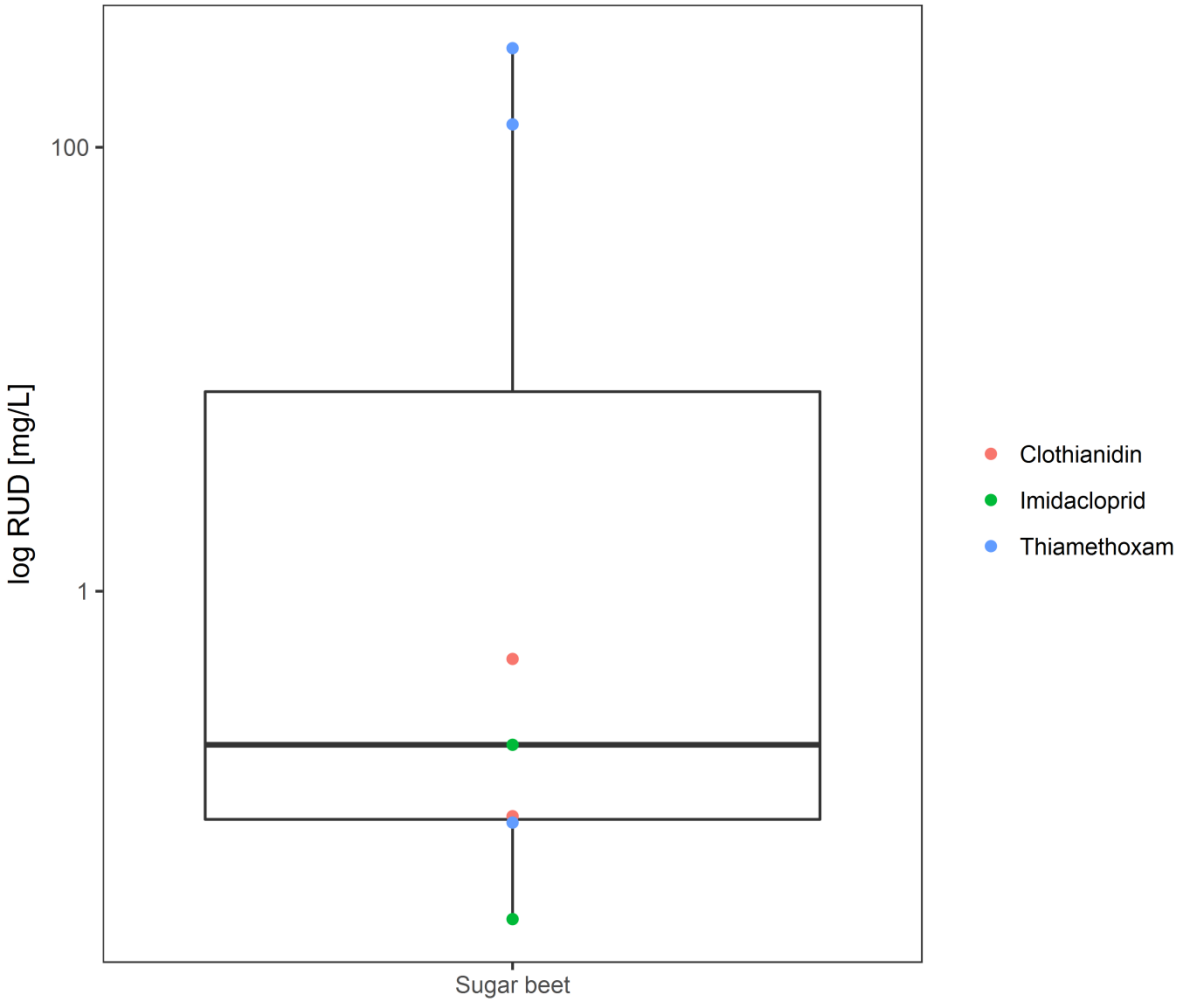

Figure 1: Boxplot for the dataset of RUDs for residues in guttation water of sugar beets as derived from industry studies. The upper and lower hinges of the boxplot correspond to the first and third quartile (25th and 75th percentile). Whiskers extend from the highest value to the lowest value within 1.5 times the interquartile range. Points show values according to active substance

Table 5: 90th percentile of the occurrence of guttation for the evaluated crop species and the 90th percentile of honey bees counted on 1 m^2 of the crop in 1 minute derived from maximum reported (worst case) values in industry studies. Datapoints refer to the number of studies for a specific crop, if a study was conducted during two different seasons (i.e. autumn and spring, with a gap for overwintering) then the findings were considered as separate datapoints.

Crop	Season	Growth stage during which guttation occurred (Min, Max)	90th percentile of fraction of days with guttation (days with guttation/observation days) based on maximum number of days with guttation	90th percentile of bees/min/m^2 based on maximum number of bees observed taking up guttation fluid	Datapoints (n)
Brassica	Spring/Summer	BBCH 13-49	1	0	1
Maize	Spring/Summer	0-53 DAE	1	0.0041	8
Onion	Spring/Summer	BBCH 13-49	0.43	0	1
Potatoes	Spring/Summer	0-57 DAE	0.61	0	2
Sugar beet	Spring	7-29 DAE BBCH 10-19	0.34	0	5
W-Barley	Autumn	BBCH 9-22	1	0.0010	2
W-Barley	Spring	BBCH 21-33	0.99	0.0079	2
S-OSR	Spring/Summer	BBCH 11-65	0.77	0	1
W-OSR	Autumn	BBCH 10-19	0.93	0.0021	4
W-OSR	Spring	BBCH 21-57	0.85	0.0051	3
W-Wheat	Autumn	NA	1	0.0005	1
W-Wheat	Spring	NA	1	0.0112	1

DAE = Days After Emergence

Guttation was frequently observed for numerous crops. In maize, which was also the crop for which the highest number of studies was conducted; guttation seemed to be a very common phenomenon. In sugar beets on the other hand the 90th percentile of days when guttation was observed was only in roughly one third of the observation days. The 90th percentiles for OSR and cereals (barley and wheat) range between 0.77 and 1, so that it can be concluded that guttation frequently occurs in important agricultural crops. The dataset for crops like onion, potatoes or brassica is too small to make a definite statement here. In consideration of an agricultural landscape as a whole composed of numerous crops it can be concluded that under certain climatic conditions guttation will most likely be occurring.

The number of honey bees actually foraging on guttation droplets was low for all crops. The numbers presented here (Table 5) are based on a re-calculation, which was necessary as the methodology differed between studies. In the raw data of the respective studies, only few individuals were recorded taking up guttation fluid and the dataset consisted mainly of zeroes. Therefore, the re-calculated values are all significantly smaller than 1. The number for maize could also be interpreted in a way that in ca. 244 observation units of 1 m^2 crop and 1 min duration one water foraging honey bee was encountered. Where data were available for two seasons (autumn and spring) from the same crop there was a tendency that more honey bees were observed taking up guttation fluid in the spring. No honeybees foraging on guttation droplets at all were recorded for brassica, onion, potatoes, S-OSR and sugar beet.

While for the former four crops only a few datapoints are available, the dataset for sugar beet comprised five studies.

Furthermore, all studies included a placement of honey bees near the fields, which can be considered a worst-case scenario. 23 out of the 25 studies were designed as effect studies (see Table 1 for information on the methodology and placement of the hives). However, none of these studies reported an effect of the test item. An overview for the overall means for treatment and control is given in Table 6 and Figure 2. While there was variability in the data, as it can be expected given that studies have been conducted in different years at different locations, there was no evidence that an increase of mortality occurred in the test item groups after the drilling of the crop.

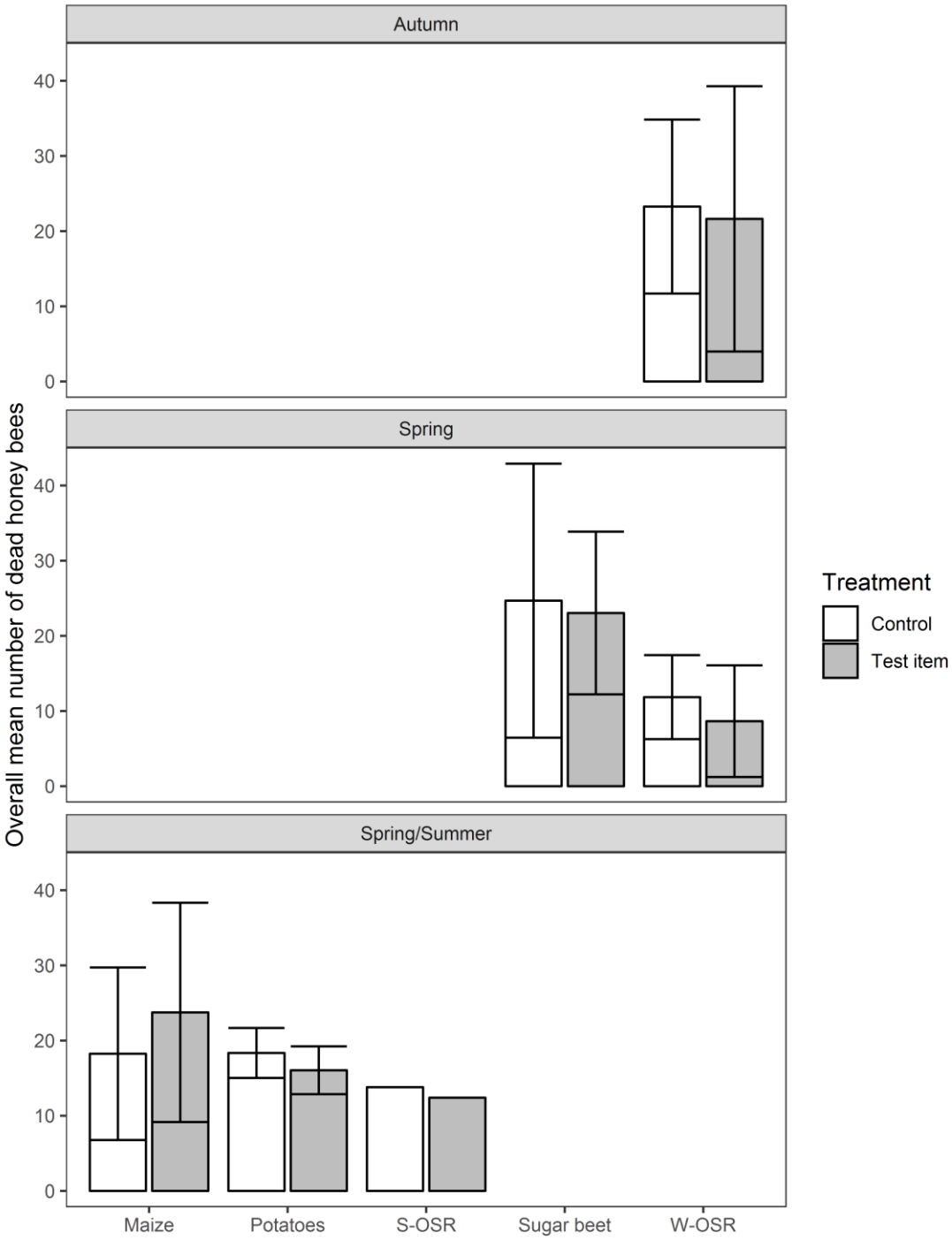

Figure 2: Overall mean (± standard deviation) for mortality for test item and control treatment per crop.

Table 6: Overall mean mortality post-exposure / post drilling) based on the data of the industry studies. Further details are given in the Appendix in Table 10

Crop	Season	Test item		Control		Datapoints (n)
		Overall mean	±SD	Overall mean	±SD	
Maize	Spring/Summer	23.7	14.6	18.2	11.5	8
Potatoes	Spring/Summer	16.1	3.2	18.4	3.3	2
Sugar beet	Spring	23	10.8	24.7	18.2	5
S-OSR	Spring/Summer	12.4	NA	13.8	NA	1
W-OSR	Autumn	21.6	17.6	23.3	11.6	3
W-OSR	Spring *	8.7	7.4	11.9	5.6	2

* after overwintering

Throughout the entire observation period colony strength in control and test item groups fluctuated within the range of natural variability of this endpoint and was in the same order of magnitude in control and treatment. The dynamic in both groups followed the natural dynamic of increase during spring time and summer and decline toward the end of the year. An overview of these data is given in Table 7 Colony losses after overwintering were in the same order of magnitude in both groups. Swarming, predation and heavy infestations with Varroa mites were reported to be the main causes for the losses, all of which could not be attributed to the test items.

Table 7: Overall means colony strength (estimated number of honey bees per colony) for test item (T) and control groups (C) based on the data of the industry studies. Further details are given in the Appendix in Table 11.

Crop	Season	Last pre-exposure assessment		1st post exposure / post-drilling assessment		Last assessment in the year		After overwintering		Datapoints (n)
		T	C	T	C	T	C	T	C	
Maize	Spring/Summer	11460	11895	19471	20385	14435	14125	8752	7464	4 / 4 / 4 / 3
Potatoes	Spring/Summer	15839	15494	15917	16416	12894	14682	NA	NA	2 / 2 / 2 / 0
Sugar beet	Spring	NA	NA	20428	17503	10950	10652	6853	5963	0 / 4 / 4 / 4
S-OSR	Spring/Summer	10303	10173	14121	13374	9815	13000	NA	NA	1 / 1 / 1 / 0
W-OSR	Autumn	NA	NA	5539	4742	6485	4750	No colony survived	188*	0 / 3 / 1 / 1
W-OSR	Spring**	NA	NA	NA	NA	NA	NA	9177	7749	0 / 0 / 0 / 2

* not counted as survival
** after overwintering

In conclusion, the results of the methodology for the observation of honey bees foraging on guttation droplets are corroborated by the data for colony strength and mortality and confirm that the exposure was negligible. Any unobserved, significant foraging activity on guttation droplets otherwise would have led to detectable effects at the colony level.

4.2 Open literature

Sixteen papers were recognized for their relevant data for the topics of this review (Shawki et al. 2006, Girolami et al. 2009, Marzaro et al. 2011, Reetz et al. 2011, Tapparo et al. 2011, Frommberger et al.

2012, Joachimsmeier et al. 2012a,c, Hoffmann and Castle 2012, Larson et al. 2014, Nikolakis et al. 2014, Reetz et al. 2016, Mörtl et al. 2017, Mörtl et al. 2018, Schenke et al. 2018, Wirtz et al. 2018). In addition eight reviews were assessed for their references.

Some of the publications namely Joachimsmeier et al. 2012a,c and Frommberger et al. 2012 were extended abstracts of presentations at the "Hazards of pesticides to bees: 11th International Symposium of the ICP-BR Bee Protection Group" held in Wageningen in November 2011. While these are available in the Julius Kühn Archive the information there is limited. However, even after an inquiry at the Julius-Kühn-Institute no further data could be retrieved, so that only the abbreviated data could be evaluated.

All papers except Joachimsmeier et al. 2012 measured the residue concentration in guttation droplets. The paper of Girolami et al. (2009) can be regarded as a benchmark in this context as almost all later studies refer to this publication. Average residue concentration for four different active substances are reported here: clothianidin: 23.3 +/- 4.2 mg/L, imidacloprid: 47 +/- 9.96 mg/L, thiamethoxam: 11.9 +/- 3.32 mg/L and fipronil: below the detection limit.

Most of the studies were conducted in spring and some in autumn. Different crops such as maize, winter oil seed rape, wheat, sugar beet, potato, winter barley, cereal and cantaloupe were used. Maize was the crop that was mainly in focus. The neonicotinoids clothianidin, imidacloprid and thiamethoxam were the compounds assessed in most of the publications, further data exists for fipronil and one study (Shawki et al. 2006) used chlorpyrifos. Two publications measured the residues in guttation droplets from untreated plants planted in the proximity of the treated coated seeds crops: Mörtl et al. (2017) and Mörtl et al. (2018). Treated seeds were mostly used as the application method except for Hoffmann and Castle (2012), Mörtl et al. (2017) and Shawki et al. (2006), where spray applications were conducted. Sampling was mostly conducted from the emergence of the crop or short time after application, but the sampling duration varies from study to study. Furthermore, there was a wide range of different experimental set-ups depending on the aims of the experiment and the questions to be answered. Some studies used plants in pots for their experiment and included different soil types and/or different soil moisture contents (Tapparo et al. 2011, Girolami et al. 2009, Mörtl et al. 2017 and Mörtl et al. 2018). While this data presents valuable information on the basic mechanisms behind the uptake of compounds of PPPs, it is also difficult to compare to the data obtained from studies for regulatory purposes, where the focus was on a more realistic approach based on actual agricultural uses.

Table 8: 90th percentile of the occurrence of guttation for the evaluated crop species from maximum reported (worst case) values in open literature

Crop	Season	90th percentile of fraction of days with guttation (days with guttation/observation days) based on maximum number of days with guttation	Datapoints (n)
Cantaloupe	Summer	0.25	1
Cereals	Spring	0.56	1
Maize	Spring	0.83	4
Maize	All year	0.39	7*
Maize	Greenhouse study	1	1
Maize	Not stated	1	1
Sugar beet	Spring	0.08	1
Wheat	Spring	0.6	1
W-OSR	Spring	1	1

*Dataset from one publication (Schenke et al. 2018)

Table 8 gives an overview of data on the occurrence of guttation as found in the open literature. As pointed out above these studies are much more heterogeneous in terms of methodology and reported results due to the fact that they were conceived as scientific experiments and therefore had a much broader range of questions to be answered in contrast to the industry studies that were conducted for regulatory purposes. Similar, however, to industry studies is that the dataset for maize is the largest, while for all other crops much less data is available, which makes a comparison to data derived from industry studies difficult. In case of maize different experimental setups were used: Schenke et al. (2018) presented a dataset from a randomized block design experiment comparing the residues in guttation fluid from three different neonicotinoids and two different maize cultivars, which was conducted over two years. In this case the 90th percentile of the dataset for the occurrence of guttation was much lower than the value derived from industry, however this is likely due to the fact that measurements were taken throughout the whole year and not just in the critical crop stages under which guttation most likely occurs as in the industry studies. The 90th percentile for studies conducted on maize in spring is 0.83 which considering natural fluctuations is within the range of the value found in industry studies (1).

In contrast to the available industry studies almost no observations on honey bees foraging on guttation fluid are reported. The only study where the test design included systematic observations of honey bee foraging behaviour was Joachimsmeier et al. 2012a. However, the authors only state the total numbers of bees taking up guttation fluid, so it is not possible to place this finding in the context. Furthermore, Reetz et al. (2011) state that honey bees were observed taking up fluid on weeds at the edge of the plot, but not on the treated crop. No systematic observations or quantifications were undertaken. In order to show that exposure to guttation containing residues of PPPs occurred Reetz et al. (2016) used an indirect sampling method by sampling returning foragers and subsequently analysing their honey sac contents. The honey bee colonies from which foragers were sampled were located near fields with winter oilseed rape grown from seeds treated with neonicotinoids. On one of their field sites (a small structured habitat in Southern Germany) no residues of the active substance were detected, while on another site (in a landscape with intensive agriculture) ca. 19% of honey sac contents of collected foragers showed detectable residues of PPPs. The quantifiable amount of residues in honey sacs was much lower than the

residues detected in guttation fluid in the same study. Nevertheless, these findings only show that exposure occurs under certain conditions, it is plausible but not certain that it stems from the uptake guttation fluid. In conclusion, the search in the open literature did not reveal any further data on the uptake of guttation fluid by honey bees based on direct observations.

5 Discussion

In terms of residues most studies, industry studies as well as the open literature, were conducted with imidacloprid, clothianidin and thiamethoxam. As these are systemic substances it makes sense that they are subject to respective studies and are detectable. A comparison of the measured residues between industry data and open literature data is difficult as measured results are dependent on climatic conditions, application method and rate as well as the used analytical methodology. Not all this information can be found in the studies from open literature. While this evaluation confirms that systemic substances will be most likely present as residues in guttation fluid, their relevance in terms of an exposure route needs further description in regard of occurrence in crops and actual consumption by bees. Nevertheless, the data also show that an exposure assessment based on the maximum water solubility of a given substance (EFSA 2013), will lead to an overestimation of the concentration in guttation fluid. The overall maximum residue value based on industry data (without accounting for the application rate) was 123 mg/L[1]. This value was measured in a study with for sugar beets treated with thiamethoxam. The 90th percentile for this crop (which is also the highest in the entire dataset) was 82.68 mg/L. The maximum water solubility of thiamethoxam is 4100 mg/L (EFSA 2015a); it is 610 mg/L for imidacloprid (EFSA 2015b) and 330 mg/L for clothianidin (SANCO/10533/05). It can be concluded, at least for these three substances, but plausibly for all other substances as well, that measured residues in guttation fluid can be expected to be well below the water solubility of the applied substance.

The occurrence of guttation is dependent on the specific crop. It can be said that guttation in maize during spring and summer occurs very often, while it seldom occurs in sugar beets in the same period. While it is possible to assume as a conservative approach for the risk assessment that guttation occurs on any given crop, it also is useful to consider the crop specific data if it is available.

Almost no data is available from open literature on foraging honey bees while data from industry studies suggest that foraging on guttation droplets is low and also crop dependent. Residues will find their way into the hive occasionally (Reetz et al. 2016), but no indication was found that honey bees exploit guttation water on a regular basis. The data from the industry studies show, that even if residues in guttation water were detected in potentially toxic levels, no negative effects on mortality or colony strength could be detected This would have been very likely the case, if any significant foraging on guttation fluid had occurred. Further reference is made to Table 10 in the Appendix to see the level of maximum residues detected in connection with the overall effects on mortality.

In consideration of the three elements which in combination can be used to assess the risk of exposure for honey bee colonies: residues, occurrence of guttation and honey bees collecting water, it is important to see the latter two phenomena in their respective context. Any risk from contaminated water arises only when they appear at the same time. Seeley (1995) describes two opposite weather conditions under which the need arises to collect water: On hot days where the temperatures rise above a critical level and cooling is required and on cold days when honey stored in the comb needs to be

[1] Please note, that the RUDs in Table 4 are calculated values in order to create comparable values for the uptake in different crops based on a theoretical application rate of 1 mg a.s./seed. Actual application rates in the industry studies were always below 1 mg a.s./seed.

diluted for larval food. As guttation occurs typically under cold and humid conditions with low or no photosynthetic activity (e.g. in the morning) it is very unlikely that guttation drops on plants are formed and honey bee colonies need water for cooling at the same time. The second case, however, is more likely as cold temperatures can induce the colonies' need for water (Seeley 1995), although cold temperatures are not favourable for honey bee foraging as they are prevented from flying below certain temperatures. Nevertheless, Lindauer (1955) describes a case where the need for water of a colony during a cold spell was so urgent that water foragers risked flying on sub-optimal temperatures. In this case, however, bees will fly to the nearest available water source. Considering that the industry studies included a worst-case setup in which colonies were closely placed to the treated crop and still only low numbers of bees were taking up guttation fluid, it is not clear how often water foraging under sub-optimal temperature conditions actually occurs. Furthermore, it is plausible that multiple water sources are available under realistic field conditions, so that colonies do not rely on guttation as a water source. Seeley (1995) describes the water collection as *demand driven* meaning it may change hourly as the colonies' need for water changes, which is in contrast to the *supply driven* nectar collection where the demand is constant and any fluctuations are caused by the changes in supply. As guttation is a transient phenomenon, honey bees will need additional water sources as there is not necessarily a coincidence of guttation and the hive's water demand.

From a regulatory point of view, where the risk assessment follows a simplified worst-case approach, the exposure from guttation, especially at the colony level, can be considered as negligible. This is illustrated by the comparison of numbers of foraging bees on a bee-attractive crop (OSR). Data[2] from additional industry studies showed maximum numbers of 3-8 bees/min/m^2 foraging on flowering OSR. In comparison to the numbers of foraging bees on guttation droplets as derived from the industry studies (e.g. the 90th percentile for maize was 0.0041 bees/min/m^2) this reveals several orders of magnitude in the risk of exposure related to honey bee colonies.

In conclusion the available data show, that neither the detection of residues in guttation droplets nor the occurrence of guttation alone are sufficient to conclude an exposure (and hence a risk) for honey bees. The numbers of foraging honey bees were low to virtually zero for all evaluated crops. Thus, in a regulatory context exposure from guttation droplets can be considered not more than a minor route in comparison to exposure from nectar and pollen. As mostly systemic substances will be in focus in the risk assessment, this will include a risk assessment from nectar or pollen foraging, either on the treated crop or the (potentially) flowering weeds in the field. As honey bee foraging behaviour (*demand driven* vs. *supply driven*) on flowering plants will constitute a worst-case exposure, the respective risk assessment should cover the risk from exposure to guttation water. In cases were no such risk assessment is available or necessary, crop specific data should be considered. The evaluated data show e.g. for sugar beets that the respective risk can be considered negligible. Additionally, modelling approaches could give information on predicted climatic conditions in which guttation and honey bee water demand are likely to coincidence. If adequate models become available, also the foraging behaviour on guttation droplets in a landscape could be predicted.

[2] Data taken from Bayer studies M-53385901-01-1 (Jaekel 2015) and M-595462-01-1 (Woodcock et al. 2017a). The latter was partly published as Woodcock et al. (2017b). Further details can be found in Table 15 in the Appendix.

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7 Appendix

Table 9: Results for residues from industry studies

No.	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
1	2010	Maize	1 control field 1 treatment field	Clothianidin	0.500 mg a.s./seed (nominal)	41 d	NA
2	2010	Maize	1 control field 1 treatment field	Clothianidin	0.500 mg a.s./seed (nominal)	24 d	NA
3	2010	Maize	1 control field 1 treatment field	Clothianidin	0.500 mg a.s./seed (nominal)	27 d	NA
4a	2010	W-OSR	2 fields with 3 study plots for control and treatment (1 field with 1 and 1 field with 2 study plots)	Clothianidin	7.28 g a.s./kg seeds	ca. 8 months	0.41
4b	2010	W-OSR	2 fields with 3 study plots for control and treatment (1 field with 1 and 1 field with 2 study plots)	Clothianidin	7.28 g a.s./kg seeds	ca. 8 months	0.41
5a	2012	W-Barley	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	Clothianidin	50 g a.s./100 kg seeds (nominal) 52.24 g a.s./100 kg seeds (analytical, Northern Germany) 50.03 g a.s./100 kg seeds (analytical, Southern Germany)	15 September 2009 to 02 November 2009, 22 March 2010 to 17 May 2010	2.3
5b	2012	W-Barley	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	Imidacloprid	70 g a.s./100 kg seeds (nominal) 60.01 g a.s./100 kg seeds (analytical, Northern Germany) 68.82 g a.s./100 kg seeds (analytical, Southern Germany)	16 September 2009 to 02 November 2009, 22 March 2010 to 17 May 2010	15
6a	2014	W-Wheat	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid) No assessments of guttation and honey bee activity in Northern Germany in autumn due to late germination of crop.	Clothianidin	50 g a.s./100 kg seeds (nominal) 52.05 g a.s./100 kg seeds (analytical, Northern Germany) 53.55 g a.s./100 kg seeds (analytical, Southern Germany)	02 October 2009 to 02 November 2009, 23 March 2010 to 18 May 2010	13
6b	2014	W-Wheat	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	Imidacloprid	70 g a.s./100 kg seeds (nominal) 62.30 g a.s./100 kg seeds (analytical, Northern Germany) 74.97 g a.s./100 kg seeds (analytical, Southern Germany)	3 October 2009 to 02 November 2009, 23 March 2010 to 18 May 2010	6.9
7a	2014	Sugar beet	1 control field 1 treatment field	Clothianidin	0.6 mg a.s./seed (nominal) 0.6612 mg a.s./seed (analytical)	42 d	0.327
7b	2014	Sugar beet	1 control field 1 treatment field	Imidacloprid	0.3 mg a.s./seed (nominal) 0.2994 mg a.s./seed (analytical)	42 d	0.061
8a	2014	Sugar beet	1 control field 1 treatment field	Clothianidin	0.6 mg a.s./seed (nominal) 0.6612 mg a.s./seed (analytical)	40 d	0.064
8b	2014	Sugar beet	1 control field 1 treatment field	Imidacloprid	0.3 mg a.s./seed (nominal) 0.2994 mg a.s./seed (analytical)	40 d	0.01
9a	2014	W-Barley	4 control fields (3 fields 1 study plot, 1 field 2 study plots) 4 treatment fields (3 fields 1 study plot, 1 field 2 study plots)	Clothianidin	Not stated	28 September 2011 to 26 October 2011, 16 March 2012 to 17 April 2012	8.51

No.	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
9b	2014	W-Barley	4 control fields (3 fields 1 study plot, 1 field 2 study plots) 4 treatment fields (3 fields 1 study plot, 1 field 2 study plots)	Imidacloprid	Not stated	29 September 2011 to 26 October 2011, 16 March 2012 to 17 April 2012	6.65
10	2014	Potatoes	1 control field 1 treatment field	Imidacloprid	180 g/ha	58 d	NA
11	2014	Potatoes	1 control field 1 treatment field	Imidacloprid	180 g/ha	59 d	NA
12	2012	Maize	1 control field 1 treatment field	Thiamethoxam	0.63 mg a.s./seed (nominal) 0.69 mg a.s./seed (analytical) 111026 seeds/ha	31 May 2010 to 08 July 2010 (Guttation liquid was sampled daily in the first week, twice in the second week and once in each following week after emergence of maize seedlings)	28.6
13	2012	Maize	1 control field 1 treatment field	Thiamethoxam	0.63 mg a.s./seed (nominal) 0.68 mg a.s./seed (analytical) 101684 seeds/ha	29 May 2010 to 06 July 2010 (Guttation liquid was sampled daily in the first week, twice in the second week and once in each following week after emergence of maize seedlings)	27.8
14	2012	Maize	1 control field 1 treatment field	Thiamethoxam	0.63 mg a.s./seed (nominal) 0.68 mg a.s./seed (analytical) 109845 seeds/ha	01 June 2010 to 07 July 2010 (Guttation liquid was sampled daily in the first week, twice in the second week and once in each following week after emergence of maize seedlings)	15.7
15	2010	Maize	1 control field 1 treatment field	Thiamethoxam	78 g/ha (actually applied)	14 May 2009 to 22 June 2009 (Guttation fluid was sampled on 1, 2, 4, 10, 16, 32 and 40 days after maize emergence)	27.7
16	2016	Sugar beet	1 control field 1 treatment field	Thiamethoxam	0.45 mg a.s./seed (nominal) 0.44 mg a.s./seed (analytical)	28 April 2015 (1DAE) to 9 May 2015 (12DAE)	123
17	2016	Sugar beet	1 control field 1 treatment field	Thiamethoxam	0.45 mg a.s./seed (nominal) 0.44 mg a.s./seed (analytical)	6 May 2015 to 3 June 2015	0.03994
18	2016	Sugar beet	1 control field 1 treatment field	Thiamethoxam	0.45 mg a.s./seed (nominal) 0.44 mg a.s./seed (analytical)	15 April 2015 (0DAE) to 21 May 2015 (36DAE)	55.8
19a	2015	W-OSR	1 control field 1 treatment field	Thiamethoxam	21 µg a.s./seed (nominal) 20.1 µg a.s./seed (analytical)	11 Sep 2014 (0DAE) to 14 Nov 2014 (64DAE)	11.14
19b	2015	W-OSR	1 control field 1 treatment field	Thiamethoxam	21 µg a.s./seed (nominal) 20.1 µg a.s./seed (analytical)	20 Mar 2015 to 17 Apr 2015	0.0021

No.	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
20a	2015	W-OSR	1 control field 1 treatment field	Thiamethoxam	21 µg a.s./seed (nominal) 20.1 µg a.s./seed (analytical)	13 Sep 2014 (0DAE) to 14 Nov 2014 (62DAE)	7.5
20b	2015	W-OSR	1 control field 1 treatment field	Thiamethoxam	21 µg a.s./seed (nominal) 20.1 µg a.s./seed (analytical)	25 Mar 2015 to 16 Apr 2015	0.004
21	2015	W-OSR	1 control field 1 treatment field	Thiamethoxam	21 µg a.s./seed (nominal) 20.1 µg a.s./seed (analytical)	23 Sep 2014 (0DAE) to 14 Nov 2014 (52DAE)	0.274
22	2013	Maize	2 test locations (Southern and Northern Alsace) 11 treatment and 2 control fields in the South, 8 treatment and 1 control field in the North	Thiamethoxam	Not stated	NA	NA
23	2015	Onion	2 test locations in NL (Limburg, Zuid-Holland), 10 treatment fields in Limburg, 5 treatment and 5 control in Zuid-Holland.	NA	NA	09 April 2014 to 29 August 2014	NA
24	2015	Brassica	7 control fields 7 treatment fields	NA	NA	28 April 2014 to 11 August 2014	NA
25	2017	S-OSR	1 control field 3 treatment fields	Sulfoxaflor	48 g a.s./ha (spray application, nominal)	24 May 2017 (0 DAE) to 22 Jun (29 DAE) Note: DAE means Day After Exposure here	0.837

Table 10: Maximum residues and mean daily mortality for control and test item groups after the exposure to the test item for each crop in the industry studies

No.	Crop	Season	Max residue concentration [mg/L]	Mean daily mortality post-exposure / post drilling	
				Test item groups	Control groups
1	Maize	Spring/Summer	NA	54.5	32
2	Maize	Spring/Summer	NA	11.1	12.7
3	Maize	Spring/Summer	NA	35.7	39.6
12	Maize	Spring/Summer	28.6	13.4	12.8
13	Maize	Spring/Summer	27.8	16.9	11
14	Maize	Spring/Summer	15.7	22.7	9.6
15	Maize	Spring/Summer	27.7	15.1	8.9
22	Maize	Spring/Summer	NA	20.6	19.4
10	Potatoes	Spring/Summer	NA	18.3	20.7
11	Potatoes	Spring/Summer	NA	13.8	16
7	Sugar beet	Spring	0.3 / 0.1 *	16.6	12.9
8	Sugar beet	Spring	0.1 / 0.01 *	14.1	13.1
16	Sugar beet	Spring	123	29.7	32.8
17	Sugar beet	Spring	0.04	15.9	11.4
18	Sugar beet	Spring	55.8	38.9	53.2
25	S-OSR	Spring/Summer	0.8	12.4	13.8
19a	W-OSR	Autumn	11.1	11.9	14.2
20a	W-OSR	Autumn	7.5	11	19.3
21	W-OSR	Autumn	0.3	42	36.3
19b	W-OSR	Spring	0.0021	13.9	15.8
20b	W-OSR	Spring	0.004	3.4	7.9

* formulation containing two active substances

Table 11: Colony strength (mean number of honey bees) as recorded on each assessment date for each crop in the industry studies

No.	Crop	Season	Last pre-exposure assessment		First post-exposure assessment		Before overwintering / last assessment		First assessment after overwintering	
			Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
12	Maize	Spring/Summer	14041	11603	22407	18322	10557	13451	6676	8206
13	Maize	Spring/Summer	8277	8161	12957	13989	13362	12560	9082	8659
14	Maize	Spring/Summer	10890	12276	18075	23575	20309	16248	10499	5526
15	Maize	Spring/Summer	12633	15540	24447	25656	13513	14240	NA	NA
10	Potatoes	Spring/Summer	17704	17184	14121	14365	15836	15633	NA	NA
11	Potatoes	Spring/Summer	13975	13804	17713	18468	9953	13731	NA	NA
8	Sugar beet	Spring	NA	NA	24651	18428	11594	11723	9815	7670
16	Sugar beet	Spring	NA	NA	27141	21403	13172	12338	4641	4603
17	Sugar beet	Spring	NA	NA	17648	17388	11462	12383	7497	6923
18	Sugar beet	Spring	NA	NA	12272	12795	7573	6164	5460	4658
25	S-OSR	Spring/Summer	10303	10173	14121	13374	9815	13000	NA	NA
19a	W-OSR	Autumn	NA	NA	8021	7448	6485	4750	NA	NA
20a	W-OSR	Autumn	NA	NA	5063	5372	NA	NA	NA	NA
21	W-OSR	Autumn	NA	NA	3532	1407	NA	NA	No colony survived	188
19b	W-OSR	Spring	NA	NA	NA	NA	NA	NA	12907	10163
20b	W-OSR	Spring	NA	NA	NA	NA	NA	NA	5447	5335

Table 12: Results for residues from open literature

No.	Authors	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
26a	Frommberger et al.	2012	Maize	4 plots on the treated field and 2 plots on the control field. 2 treated and 1 control with and the 3 others without artificial water source containing uncontaminated tap water (semi-field conditions)	Clothianidin	0.5 mg/kernel	11 days	46.55
26b	Frommberger et al.	2012	Maize	4 plots on the treated field and 2 plots on the control field with additional uncontaminated water source (semi-field conditions)	Clothianidin	0.5 mg/kernel	10 days	1.71
28a	Girolami et al.	2009	Maize	Corn grown in open field and in pots in the lab. Collection of guttation drops from corn emergence up to the first 3 weeks.	Imidacloprid	0.5 mg/seed	3 weeks	47 +/- 9.96
28b	Girolami et al.	2009	Maize	Corn grown in open field and in pots in the lab. Collection of guttation drops from corn emergence up to the first 3 weeks.	Clothianidin	1.25 mg/seed	3 weeks	23.3 +/- 4.2
28c	Girolami et al.	2009	Maize	Corn grown in open field and in pots in the lab. Collection of guttation drops from corn emergence up to the first 3 weeks.	Thiamethoxam	1 mg/seed	3 weeks	11.9 +/- 3.32
28d	Girolami et al.	2009	Maize	Corn grown in open field and in pots in the lab. Collection of guttation drops from corn emergence up to the first 3 weeks.	Fipronil	1 mg/seed	3 weeks	< LOD
29a	Hoffmann and Castle	2012	Cantaloupe	2 fields planted, one treated with a drip application of Admire Pro when the cantaloupe were at sixth node stage and had begun to bloom and one field non-treated	Imidacloprid	767.3 ml/ha	4 days	4.115
29b	Hoffmann and Castle	2012	Cantaloupe	1 treated field. Row irrigation applied immediately after treatment to disperse the application into the root zones of plants.	Imidacloprid	282 g/ha	5 days	37.35
29c	Hoffmann and Castle	2012	Cantaloupe	1 treated field. Row irrigation applied immediately after treatment to disperse the application into the root zones of plants.	Imidacloprid	422g/ha	5 days	37.35
30	Joachimsmeier et al.	2012c	Maize	4 locations, 6 km away from the experimental field, used to place the colonies. Placing the bees on the maize field when guttation events started, 4 weeks after emergence, with 1 plot planted with seed-treated maize and 1 plot with untreated maize	Clothianidin	1.25 mg/kernel	41 days	4
31	Marzaro et al.	2011	Maize	Sowing of 3 different types of coated seeds. Guttation drops samples were collected on the margins of the sown area.	Clothianidin	1.25 mg/seed	2 days	0.027
32a	Mörtl et al.	2018	Maize	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Thiamethoxam	0.61 +/- 0.07 mg per seed	35 days, sampling every day but only high peak concentrations reported	150
32b	Mörtl et al.	2018	Maize	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Clothianidin	1.22 +/- 0.66 mg per seed	35 days, sampling every day but only high peak concentrations reported	73
32c	Mörtl et al.	2018	Creeping thistle	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Thiamethoxam	no direct application	35 days, sampling every day but only high peak concentrations reported	20.7
32c	Mörtl et al.	2018	Creeping thistle	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Clothianidin	no direct application	35 days, sampling every day but only high peak concentrations reported	22.3
32d	Mörtl et al.	2018	Red poppy	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Thiamethoxam	no direct application	35 days, sampling every day but only high peak concentrations reported	0.63
32e	Mörtl et al.	2018	Red poppy	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	Clothianidin	no direct application	35 days	0.74

No.	Authors	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
33a	Mörtl et al.	2017	Maize	Guttation drops sampled from non-coated maize seeds planted in pots in three different soil types after pre-emergence spray application of CLO and TMX.	Clothianidin	4.12 mg/L	32 days after sowing	1.63
33b	Mörtl et al.	2017	Maize	Guttation drops sampled from non-coated maize seeds planted in pots in three different soil types after pre-emergence spray application of CLO and TMX.	Thiamethoxam	4.8 mg/L	32 days after sowing	1.82
33c	Mörtl et al.	2017	Maize	Guttation drops sampled from plants (in pots with different soil types) closely planted to plants emerged from CLO- or TMX- coated maize seeds.	Clothianidin	1.22 +/- 0.66 mg/seed	32 days after sowing	19.6
33d	Mörtl et al.	2017	Maize	Guttation drops sampled from plants (in pots with different soil types) closely planted to plants emerged from CLO- or TMX- coated maize seeds.	Thiamethoxam	0.61 +/- 0.07 mg/seed	32 days after sowing	50.9
33e	Mörtl et al.	2017	Maize	Guttation drops sampled from coated maize seeds and non-coated maize seeds planted in close proximity in pots	Clothianidin	1.22 +/- 0.66 mg/seed	32 days after sowing	coated seeds: 234.7 and non-coated seeds: 53.1
33f	Mörtl et al.	2017	Maize	Guttation drops sampled from coated maize seeds and non-coated maize seeds planted in close proximity in pots	Thiamethoxam	0.61 +/- 0.07 mg/seed	32 days after sowing	Not reported
34	Nikolakis et al.	2014	Please note that the data presented in this publication are based on study reports already evaluated as industry studies. Please refer to the corresponding table for further information.					
35a	Reetz et al.	2011	Wheat	Sowing of wheat and maize treated seeds and collection of guttation droplets for residue determination. 4 colonies of bees placed at the field site to observe if they forage on guttation water.	Imidacloprid	1.75 mg/seed	from April to July 2009	0.013
35b	Reetz et al.	2011	Maize	Sowing of wheat and maize treated seeds and collection of guttation droplets for residue determination. 4 colonies of bees placed at the field site to observe if they forage on guttation water.	Clothianidin	4 different treatments	from April to July 2009	0.008
35c	Reetz et al.	2011	Maize	Sowing of wheat and maize treated seeds and collection of guttation droplets for residue determination. 4 colonies of bees placed at the field site to observe if they forage on guttation water.	Imidacloprid	4 different treatments	from April to July 2009	0.064
36a	Reetz et al.	2016	Winter oil seed rape	Northern Germany in intensive agricultural region, sowing seed-coated winter oil seed rape. Collection of guttation droplets and measurements of neonicotinoid residues. Returning honey bees were sampled at the hive and their honey sac contents were analysed as an indirect measure of uptake of guttation fluid.	Thiamethoxam	3.6 g/kg seed	August - September 2011	0.01294
36b	Reetz et al.	2016	Winter oil seed rape	Southern Germany in small-patterned landscape, sowing seed-coated winter oil seed rape. Collection of guttation droplets and measurements of neonicotinoid residues.	Clothianidin	10.0 g/kg seed	August 2009 to May 2010	0.132
37a	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	None	NA	May 2010 - August 2011	0.11
37b	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	None	NA	May 2010 - August 2011	0.01
37c	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Clothianidin	545 µg/seed (analytical)	May 2010 - August 2011	9.98
37d	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Clothianidin	50 g a.s/ha (nominal, granules)	May 2010 - August 2011	2.66
37e	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Clothianidin	514 µg/seed (analytical)	May 2010 - August 2011	90.95
37f	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Clothianidin	245 µg/seed (analytical)	May 2010 - August 2011	9.46

No.	Authors	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
37g	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	None	NA	May 2010 - August 2011	0.24
37h	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Thiamethoxam	547 µg/seed (analytical)	May 2010 - August 2011	9.97
37i	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Thiamethoxam	229 µg/seed (analytical)	May 2010 - August 2011	8.67
37j	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Imidacloprid	501 µg/seed (analytical)	May 2010 - August 2011	9.92
37k	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Imidacloprid	264 µg/seed (analytical)	May 2010 - August 2011	9.01
37l	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	Thiamethoxam	227 µg/seed (analytical)	May 2010 - August 2011	9.91
38a	Shawki et al.	2006	Winter rape	Spray application of product on three different plots 7-9 days before flowering. Samples of guttation were collected daily until 10 days after treatment.	Chlorpyrifos	500 g/L	10 days	< LOD
38b	Shawki et al.	2006	Winter rape	Spray application of product on three different plots 7-9 days before flowering. Samples of guttation were collected daily until 10 days after treatment.	Cypermethrin	50 g/L	10 days	< LOD
39a	Tapparo et al.	2011	Maize	Seeds of coated corn planted in open field and in pots in greenhouse laboratory and non-treated seeds planted as controls, guttation drops of the seedlings collected for 20 days every day after emergence for neonicotinoids concentration analysis.	Imidacloprid	0.5, 1 or 1.25 mg/seed	Open field (April 2009 and 2010) and in lab greenhouse (November 2008-October 2012)	346
39b	Tapparo et al.	2011	Maize	Seeds of coated corn planted in open field and in pots in greenhouse laboratory and non-treated seeds planted as controls, guttation drops of the seedlings collected for 20 days every day after emergence for neonicotinoids concentration analysis.	Clothianidin	1.25 mg/seed	Open field (April 2009 and 2010) and in lab greenhouse (November 2008-October 2012)	101.7
39c	Tapparo et al.	2011	Maize	Seeds of coated corn planted in open field and in pots in greenhouse laboratory and non-treated seeds planted as controls, guttation drops of the seedlings collected for 20 days every day after emergence for neonicotinoids concentration analysis.	Thiamethoxam	0.6 or 1 mg/seed	Open field (April 2009 and 2010) and in lab greenhouse (November 2008-October 2012)	1154
39e	Tapparo et al.	2011	Maize	Seeds of coated corn planted in open field and in pots in greenhouse laboratory and non-treated seeds planted as controls, guttation drops of the seedlings collected for 20 days every day after emergence for neonicotinoids concentration analysis.	Fipronil	0.5, 0.75 or 1 mg/seed	Open field (April 2009 and 2010) and in lab greenhouse (November 2008-October 2012)	< LOD
40a	Wirtz et al.	2018	Sugar beet	31 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Thiamethoxam	0.15 mg/pill	April to July 2009 and 2010	0.307
40b	Wirtz et al.	2018	Sugar beet	32 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Thiamethoxam	0.6 mg/pill	April to July 2009 and 2010	2.081
40c	Wirtz et al.	2018	Sugar beet	33 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Imidacloprid	0.3 mg/pill	April to July 2009 and 2010	0.658

No.	Authors	Year	Crop	Test design	Active substance	Application rate	Timescale of sampling	Max residue concentration [mg/L]
40d	Wirtz et al.	2018	Sugar beet	34 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Imidacloprid	0.6 mg/pill	April to July 2009 and 2010	0.152
40e	Wirtz et al.	2018	Sugar beet	35 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Clothianidin	0.6 mg/pill	April to July 2009 and 2010	1.505
40f	Wirtz et al.	2018	Sugar beet	36 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	Clothianidin	0.45 mg/pill	April to July 2009 and 2010	9.043
41	Larson et al.	2014	Creeping bentgrass *(Agrostis stolonifera*)	5 treated an non-treated replicates of plots maintained similar to a golf course including irrigation and mowing. Guttation fluid was sampled in the morning at 1 w and 3 w after treatment.	Imidacloprid	450 g a.s/ha (spray application)	3 weeks	0.088

Table 13: Results for occurrence of guttation and uptake of guttation fluid by honey bees from industry studies

No.	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Mean number of bees taking up guttation droplets [bees/min/m^2]	Max number of bees taking up guttation droplets [bees/min/m^2]	Length observation unit [min]	Area observed [m^2]	Total number of observation units (n)
1	2010	Maize	1 control field 1 treatment field	2	Spring/Summer	0.75	0.72	53	1-2 d after emergence	45 d after emergence	0	0	4	12	171
2	2010	Maize	1 control field 1 treatment field	2	Spring/Summer	0.79	0.77	24	1 d after emergence	24 d after emergence (end of observations)	0	0	4	12	137
3	2010	Maize	1 control field 1 treatment field	2	Spring/Summer	0.6	0.56	40	1 d after emergence	30 d after emergence	0	0	4	12	70
4a	2010	W-OSR	2 fields with 3 study plots for control and treatment (1 field with 1 and 1 field with 2 study plots)	6	Autumn	0.85	0.83	41-52	1 d after emergence Earliest date of sowing: 27.08.2009 (First assessment recording guttation 10.09.2009)	Not reported (Last assessment recording guttation in autumn: 31.10.2009)	0.00099	0.00301	10	31.2	433
4b	2010	W-OSR	2 fields with 3 study plots for control and treatment (1 field with 1 and 1 field with 2 study plots)	6	Spring	0.84	0.76	31	Not reported (First assessment recording guttation in spring 23.03.2010)	Not reported (Last assessment recording guttation in spring: 17.04.2010)	0.00223	0.00641	10	31.2	128
5a	2012	W-Barley	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	6	Autumn	1	0.84	11-27	Not reported Earliest date of sowing: 22.09.2009 Earliest date of germination: 02.10.2009 (First assessment recording guttation 04.10.2009)	Not reported (Last assessment recording guttation in autumn: 31.10.2009)	0.00041	0.00112	10	31.2	117
5b	2012	W-Barley	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	6	Spring	0.94	0.81	17-32	Not reported (First assessment recording guttation in spring 23.03.2010)	Not reported (Last assessment recording guttation in spring: 25.04.2010)	0.00461	0.00833	10	31.2	113

No.	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Mean number of bees taking up guttation droplets [bees/min/m^2]	Max number of bees taking up guttation droplets [bees/min/m^2]	Length observation unit [min]	Area observed [m^2]	Total number of observation units (n)
6a	2014	W-Wheat	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid) No assessments of guttation and honey bee activity in Northern Germany in autumn due to late germination of crop.	3	Autumn	1	0.86	2-12	Not reported Earliest date of sowing: 05.10.2009 Earliest date of germination: 21.10.2009 (First assessment recording guttation 24.10.2009)	Not reported (Last assessment recording guttation in autumn: 31.10.2009)	0.00018	0.00053	10	31.2	27
6b	2014	W-Wheat	2 test locations (Northern and Southern Germany) with 3 fields each (control, clothianidin, imidacloprid)	6	Spring	1	0.88	15-31	Not reported (First assessment recording guttation in spring 23.03.2010)	Not reported (Last assessment recording guttation in spring: 26.04.2010)	0.00239	0.01118	10	31.2	169
7a	2014	Sugar beet	1 control field 1 treatment field	2	Spring	0.26	0.14	42	7 d after emergence	29 d after emergence	0	0	5	10	124
8a	2014	Sugar beet	1 control field 1 treatment field	2	Spring	0.125	0.1	40	12 d after emergence	22 d after emergence	0	0	5	10	118
9a	2014	W-Barley	4 control fields (3 fields 1 study plot, 1 field 2 study plots) 4 treatment fields (3 fields 1 study plot, 1 field 2 study plots)	10	Autumn	1	0.98	13-15	BBCH 9-10	BBCH 22	0.00004	0.00019	10	29.6	358
9b	2014	W-Barley	4 control fields (3 fields 1 study plot, 1 field 2 study plots) 4 treatment fields (3 fields 1 study plot, 1 field 2 study plots)	10	Spring	1	0.88	12-17	BBCH 21	BBCH 33	0.00087	0.00351	10	29.6	290
10	2014	Potatoes	1 control field 1 treatment field	2	Spring/Summer	0.64	0.6	58	0 d after emergence	57 d after emergence	0	0	5	10	160
11	2014	Potatoes	1 control field 1 treatment field	2	Spring/Summer	0.3	0.3	59	7 d after emergence	42 d after emergence	0	0	5	10	162
12	2012	Maize	1 control field 1 treatment field	2	Spring/Summer	0.98	0.98	41	0 d after emergence	40 d after emergence	0	0	20	20	295
13	2012	Maize	1 control field 1 treatment field	2	Spring/Summer	1	0.87	41	0 d after emergence	40 d after emergence	0	0	20	20	293
14	2012	Maize	1 control field 1 treatment field	2	Spring/Summer	0.93	0.9	41	0 d after emergence	40 d after emergence	0.00001	0.0025	20	20	294
15	2010	Maize	1 control field 1 treatment field	1	Spring/Summer	0.9	NA	40	0 d after emergence	40 d after emergence	0	0	10	Not reported	127

No.	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Mean number of bees taking up guttation droplets [bees/min/m^2]	Max number of bees taking up guttation droplets [bees/min/m^2]	Length observation unit [min]	Area observed [m^2]	Total number of observation units (n)
16	2016	Sugar beet	1 control field 1 treatment field	2	Spring	0	0	30	NA	NA	0	0	5	5	60
17	2016	Sugar beet	1 control field 1 treatment field	2	Spring	0.14	0.09	29	BBCH 14	BBCH 19	0	0	5	10	58
18	2016	Sugar beet	1 control field 1 treatment field	2	Spring	0.4	0.3	37	BBCH 10	BBCH 18	0	0	5	10	61
19a	2015	W-OSR	1 control field 1 treatment field	2	Autumn	0.66	0.66	35	BBCH 10	BBCH 17	0	0	5	5	390
19b	2015	W-OSR	1 control field 1 treatment field	2	Spring	0.85	0.81	13	BBCH 21	BBCH 57	0	0	5	5	154
20a	2015	W-OSR	1 control field 1 treatment field	2	Autumn	0.94	0.92	33	BBCH 10	BBCH 19	0	0	5	5	378
20b	2015	W-OSR	1 control field 1 treatment field	2	Spring	0.7	0.65	10	BBCH 32	BBCH 51	0	0	5	5	135
21	2015	W-OSR	1 control field 1 treatment field	2	Autumn	0.9	0.84	29	BBCH 10	BBCH 19	0	0	5	5	318
22	2013	Maize	2 test locations (Southern and Northern Alsace) 11 treatment and 2 control fields in the South, 8 treatment and 1 control field in the North	22	Spring/Summer	1	0.85	15-50	0 d after emergence	53 d after emergence	NA (Total n of observation units not reported)	0.0078	16	8	Not reported
23	2015	Onion	2 test locations in NL (Limburg, Zuid-Holland), 10 treatment fields in Limburg, 5 treatment and 5 control in Zuid-Holland.	20	Spring/Summer	0.43	0.42	30	BBCH 09	BBCH 16	0	0	40	80	590
24	2015	Brassica	7 control fields 7 treatment fields	14	Spring/Summer	1	1	57	BBCH 13	BBCH 49	0	0	40	80	196
25	2017	S-OSR	1 control field 3 treatment fields	4	Spring/Summer	0.77	0.59	30	BBCH 11	BBCH 65	0	0	5	5	119

Table 14: Results for occurrence of guttation and uptake of guttation fluid by honey bees open literature

No.	Authors	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Observations on water foraging bees
26a	Frommberger et al.	2012	Maize	4 plots on the treated field and 2 plots on the control field. 2 treated and 1 control with and the 3 others without artificial water source containing uncontaminated tap water (semi-field conditions)	6	Spring	0.09	NA	11	BBCH 13	BBCH 15	Not reported
27a	Joachimsmeier et al.	2012a	Cereals	1 organic field, 1 conventional field with each two plots. Bee hives in cereal plot with distance of 0 m, 10 m, 20 m, 30 m and 50 m to the adjacent OSR plot and a distance of 5 0m from each other. Hive entrance pointed towards the OSR plot.	4	Spring	0.56	NA	25	BBCH 29	NA	Total number: 13
28a	Girolami et al.	2009	Maize	Corn grown in open field and in pots in the lab. Collection of guttation drops from corn emergence up to the first 3 weeks.	NA	Spring	1	NA	21	At emergence	3 weeks old	Not reported
29a	Hoffmann and Castle	2012	Cantaloupe	2 fields planted, one treated with a drip application of Admire Pro when the cantaloupe were at sixth node stage and had begun to bloom and one field non-treated	2	Summer	0.25	NA	4	Sixth node stage and bloom start	Sixth node stage and bloom start	Not reported
29b	Hoffmann and Castle	2012	Cantaloupe	1 treated field. Row irrigation applied immediately after treatment to disperse the application into the root zones of plants.	1	Autumn	0.4	NA	5	NA	NA	Not reported
30	Joachimsmeier et al.	2012c	Maize	4 locations, 6 km away from the experimental field, used to place the colonies. Placing the bees on the maize field when guttation events started, 4 weeks after emergence, with 1 plot planted with seed-treated maize and 1 plot with untreated maize	2	Spring	0.12	NA	41	BBCH 15	BBCH 19	Not reported
31	Marzaro et al.	2011	Maize	Sowing of 3 different types of coated seeds. Guttation drops samples were collected on the margins of the sown area.	1	NA	1	NA	2	NA	NA	Not reported

No.	Authors	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Observations on water foraging bees
32a	Mörtl et al.	2018	Maize	Two weeds, creeping thistle and red poppy, planted in pots in close proximity to coated maize seed and look at the cross-contamination by the treated neonicotinoids.	pots in and out door	NA	NA	NA	35	NA	35 days	Not reported
33a	Mörtl et al.	2017	Maize	Guttation drops sampled from non-coated maize seeds planted in pots in three different soil types after pre-emergence spray application of CLO and TMX.	NA	NA	NA	NA	32	7-10 days	32 days	Not reported
34	Nikolakis et al.	2014	Please note that the data presented in this publication are based on study reports already evaluated as industry studies. Please refer to the corresponding table for further information.									
35a	Reetz et al.	2011	Wheat	Sowing of wheat and maize treated seeds and collection of guttation droplets for residue determination. 4 colonies of bees placed at the field site to observe if they forage on guttation water.	2	Spring	0.6	NA	47	NA	NA	No systematic observations were made. During sampling of guttation fluid bees were observed taking up fluid on weeds at the edge of the plot, but not on the treated crop.
35b	Reetz et al.	2011	Maize	Sowing of wheat and maize treated seeds and collection of guttation droplets for residue determination. 4 colonies of bees placed at the field site to observe if they forage on guttation water.	6	Spring	0.44	NA	52	NA	NA	No systematic observations were made. During sampling of guttation fluid bees were observed taking up fluid on weeds at the edge of the plot, but not on the treated crop.
36a	Reetz et al.	2016	Winter oil seed rape	Northern Germany in intensive agricultural region, sowing seed-coated winter oil seed rape. Collection of guttation droplets and measurements of neonicotinoid residues. Returning honey bees were sampled at the hive and their honey sac contents were analysed as an indirect measure of uptake of guttation fluid.	1	End of summer-autumn	NA	NA	NA	BBCH 10	BBCH 65	Indirect sampling method further discussed in report

No.	Authors	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Observations on water foraging bees
37a	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	4	All year	0.36	NA	2292	BBCH 12	BBCH 87	Not reported
37b	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	4	All year	0.44	NA	1056	BBCH 12	BBCH 87	Not reported
37c	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	4	All year	0.35	NA	764	BBCH 12	BBCH 87	Not reported
37g	Schenke et al.	2018	Maize	Field test over two years, 2 maize cultivars, 3 neonicotinoids, different formulations and application rates (seed coating and granules), randomized block design, immediate residue analysis.	4	All year	0.30	NA	1236	BBCH 12	BBCH 87	Not reported
38a	Shawki et al.	2006	Winter rape	Spray application of product on three different plots 7-9 days before flowering. Samples of guttation were collected daily until 10 days after treatment.	6	Spring	1	NA	10	NA	NA	Not reported
39a	Tapparo et al.	2011	Maize	Seeds of coated corn planted in open field and in pots in greenhouse laboratory and non-treated seeds planted as controls, guttation drops of the seedlings collected for 20 days every day after emergence for neonicotinoids concentration analysis.	2 plots and 6-8 pots for each insecticide and equal number of pots for controls	Spring, autumn, winter	1	NA	20	After emergence	20 days after emergence	Not reported

No.	Authors	Year	Crop	Test design	Number of study plots (n)	Season during which observations were made	Max fraction of days guttation observed	Mean fraction of days guttation observed	Observation period [days]	Min growth stage of treated crop when guttation occurred	Max growth stage of treated crop when guttation occurred	Observations on water foraging bees
40a	Wirtz et al.	2018	Sugar beet	31 fields of sugar beet conventionally cultivated with insecticide seed coatings containing different neonicotinoids, observations of guttation fluids sampled and analysis for residues.	31	Spring	0.08	NA	758	At emergence	Canopy closure of the plants	Not reported
41	Larson et al.	2014	Creeping bentgrass (*Agrostis stolonifera*)	5 treated an non-treated replicates of plots maintained similar to a golf course including irrigation and mowing. Guttation fluid was sampled in the morning at 1 w and 3 w after treatment.	NA	NA	NA	NA	NA	NA	NA	Not reported

Table 15: Honey bees foraging on a bee-attractive crop as reported in additional industry studies

Authors	Identifier	Crop	Short description	Method	Highest number of foraging bees (as reported)	Bees/m^2/min	Notes
Jaekel	M-53385901-01-1	OSR	TMX+CTD FR+DE+HU+PL+UK	1 m^2 @ 1 min (3 patches each at 5 different distances from hive), numbers for each patch reported	7	7	BBCH 65, FR
Jaekel	M-53385901-01-1	OSR	TMX+CTD FR+DE+HU+PL+UK	1 m^2 @ 1 min (3 patches each at 5 different distances from hive), numbers for each patch reported	8	8	BBCH 65, DE
Jaekel	M-53385901-01-1	OSR	TMX+CTD FR+DE+HU+PL+UK	1 m^2 @ 1 min (3 patches each at 5 different distances from hive), numbers for each patch reported	5	5	BBCH 65, HU
Jaekel	M-53385901-01-1	OSR	TMX+CTD FR+DE+HU+PL+UK	1 m^2 @ 1 min (3 patches each at 5 different distances from hive), numbers for each patch reported	4	4	BBCH 65, PL
Jaekel	M-53385901-01-1	OSR	TMX+CTD FR+DE+HU+PL+UK	1 m^2 @ 1 min (3 patches each at 5 different distances from hive), numbers for each patch reported	5	5	BBCH 65, UK
Woodcock et al.	M-595462-01-1	OSR	TMX+CTD DE+UK OSR	1 m^2 @ 5 min (4 patches), averages reported	17.8	3.56	Pollen foragers
Woodcock et al.	M-595462-01-1	OSR	TMX+CTD DE+UK OSR	1 m^2 @ 5 min (4 patches), averages reported	18.8	3.76	Nectar foragers