Assessment of risks to honey bees posed by guttation

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Abstract

Background: Besides their nectar and pollen collecting activities, honey bees also forage water. Guttation droplets may be used as a water source. Measurements of high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets triggered research activities on the potential risk for honey bees. Since 2009, a large number of studies have been conducted on the environmental conditions and factors favoring guttation, foraging of guttation, the occurrence of guttation in different crops, the frequency of guttation events and residue measurements in guttation droplets in different crops, at different growth stages and with different active ingredients. Different approaches of laboratory, semi-field and field studies were set up to address the potential risk of guttation to bees and to gain clarification whether and how this concern would need to be specifically addressed in the risk assessment for bees.

Results: Occasionally increased mortalities of worker bees were reported from single events in some trials, when colonies were placed directly next to the sown maize crop treated with a systemic insecticide. However, there were no long-term colony effects (e.g. on colony strength and brood development) reported from any of the realistic worst case exposure trials conducted by either public research institutes or industry. Conclusion: The potential risk for bees is in the first instance dependent on the distance of the colonies to treated crops. Maize is considered as the worst case crop in terms of frequency, duration and intensity of guttation and of residue level of compounds found in guttation liquid. Though increased worker bee mortality on individual days was seen in some of the field studies where hives were placed directly at guttating maize fields, adverse effects to colony vitality, colony and brood development were never observed.

Keywords: Guttation, risk assessment, pesticides, honey bees.

1. Introduction

Guttation is a physiological process by which many vascular plants can secrete water by an active process under certain environmental conditions, in contrast to transpiration which is a passive process. The secreted water forms droplets which usually occur on tips or edges of leaves. The content of dissolved substances like salts, sugars in guttation liquid is very low, usually below 1%. In recent years, attention has been focused on guttation of systemic pesticides as a possible exposure pathway for water-collecting bees to systemic pesticides, in particular soil-systemic applications (e.g. seed treatment, granular or drench applications). Measurements of high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets from different crops were reported by different researchers[4,13,14] and triggered significant interest on the possible risks posed by the presence of residues of systemic pesticides in guttation fluid to water-collecting honey bees[20].

Studies have since been conducted on the environmental conditions and factors favoring guttation, collection of guttation liquid, the occurrence of guttation in different crops, the frequency of guttation events and residue measurements in guttation droplets in different crops with different active ingredients in different growth stages. Different approaches of studies with bees in lower and higher tier tests were set up to gain clarification about collection of guttation liquids by bees and possible effects on bees and whether and how this concern would need to be specifically addressed in the risk assessment for honey bees. So far, consideration of guttation has not been specifically required in the risk assessment by SANCO/10329/2002, but it has nevertheless been addressed in the risk assessment of a few active substances. However, future European legislation could include the risk assessment for pesticides residues in water, including guttation for systemic products**.** Meanwhile, there is more information available from laboratory studies, semi-field and field studies as well as post-registration monitoring from both industry and public research institutes.

2. Results

2.1 Guttation- different factors influence the potential risk

2.1.1 Water need of bee colonies

Honey bees need water for different tasks in the hive, such as the regulation of air humidity and temperature (cooling) in the hive[8], and the production of larval food which has high water content. Water foraging activity is regulated by demand as it is not stored in the hive[9,17]. As water collecting bees will most likely choose water sources in the proximity of the hive[19] and long distance flights are avoided due to energetic reasons, the position of the bee hive in relation to the treated crop and the availability of alternative water sources, e.g. rivers, ponds, dew, condensed water in the hive, nectar flow with high water content, determine the potential risk of uptake of guttation droplets from treated crops to satisfy water requirements. Guttation may also occur in untreated plants like grasses and weeds. The possible risk from guttation water may be highly variable and is determined by, e.g. climate conditions, meteorological conditions, soil nature, time of overlapping of bee activity and guttation, the distance to treated and untreated crops and other plants, seasonal activity and seasonal water needs of colonies and the occurrence of guttation droplets with high residue levels. In general, the water need of a colony is highest during spring and summer. Plants offering nectar and pollen will attract bees from larger distances, whereas water is usually collected closer to the hive[19]. Therefore, collection of guttation liquid does not appear to be a regular exposure scenario like nectar and pollen. Usually, guttation droplets are one out of several possible water sources in the surroundings of a colony and mostly only available at a limited time period in the morning and evening and not every day.

2.1.2 Occurrence of guttation

Several crop species such as sugar beet, winter oilseed rape, maize, barley, potatoes, oat, sunflower, onions, carrots, peas and cucumber and also weeds were investigated and an assessment of the occurrence, frequency and intensity of guttation (size/number of guttation drops, number of guttating plants per culture) in the tested crop species was conducted (Fig. 1). Different crops varied in the intensity and frequency of guttation events. Some crops showed guttation more frequently than others, also the intensity of guttation varied. Some major crops like winter oilseed rape, cereals and maize showed guttation frequently. Some crops showed very low guttation probability and very small droplets, e.g. sugar beet (Fig. 1, top). Whereas some crops produced guttation throughout a large part of their growing season, others showed guttation only for a short period (Fig. 1, left). Finally, while some crops showed guttation only in younger growth stages, some may show guttation up to inflorescence (Fig. 1, right) (Joachimsmeier et al. 2011).

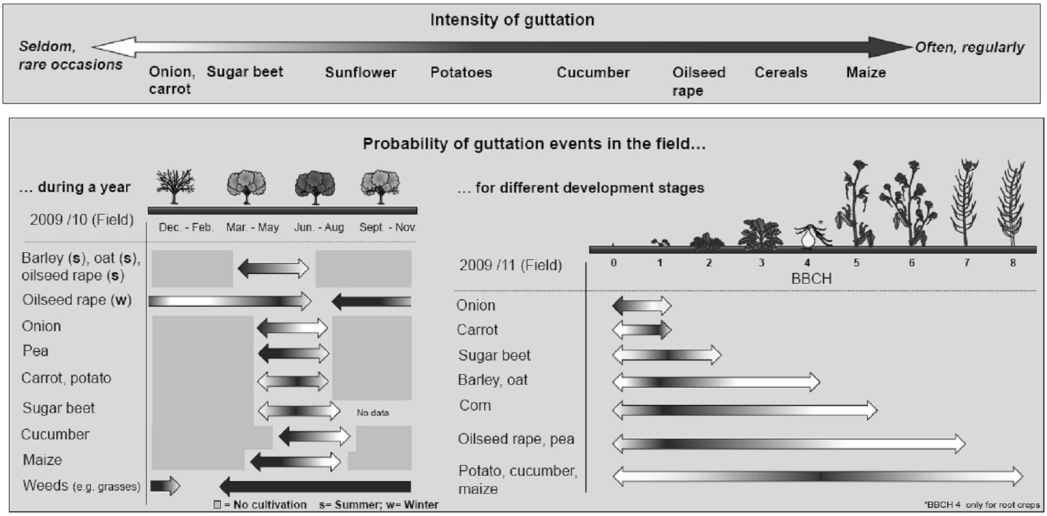

Fig. 1 Intensity and probability of guttation in differenct crops in field conditions (Joachimsmeier et al., 2011)

2.1.3 Residues in guttation droplets and potential risk

Residues of systemic fungicides, herbicides and insecticides may be found in guttation droplets. For all tested crops, peak residue levels occurred at the onset of guttation activity after emergence and declined with time (Fig. 2). Depending on the residue levels, the period of concern may vary from crop to crop. Depending on the toxicity of the active substance, concern for honey bees may be triggered, e.g. in maize high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets were found.

The concentrations in guttation droplets tend to be slightly lower for granules than for seed treatments in young growth stages, nevertheless the potential risk is likely to be comparable. (Fig. 2, left).

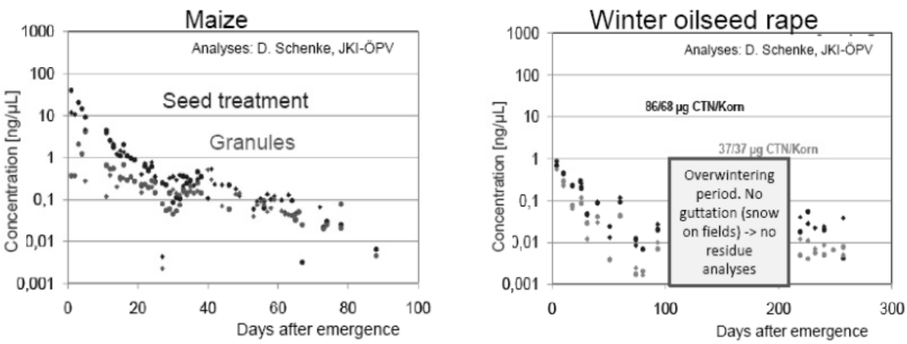

Fig. 2 Residues of a systemic pesticide (clothianidin as an example) in guttation droplets after seed treatment and granular treatment for maize (left) and seed treatment for winter oilseed rape (right)[14]

Highest residues were found in all crops at younger growth stages, showing decline with increasing plant age and growth stage[13,14]. The amount of residues in guttation droplets depends on the crop and its growth stage, the properties of the active substance, the amount of active substance per seed and other factors[15]. (In some trials during sampling at two different times (morning and midday), increased residue concentrations were measured in the midday samples due to the evaporation of the water content with increasing solar radiation[1].

In comparison to other crops studied here, potential risk via guttation is in general higher for maize, which can be assumed to be the 'worst-case' crop, as residues of soil-systemic treatments at emergence and young growth stages are much higher compared to other crops and guttation occurs frequently at time of high water needs of colonies.

To assess the potential risk, in a first step oral toxicity data e.g. LD_{50} values can be used for a calculation of the amount of liquid that would lead to an uptake of a lethal dose e.g. the acute LD_{50}. Other values e.g. NOEC or LC50 values could also be used for a refined calculation both for acute or chronic toxicity. In this case, the LD_{50} is only used to demonstrate a potential risk. In Table 1 such an example of a calculation is given. For a substance with a LD_{50} of 100 ng/bee 100 µl water would need to be consumed at a concentration of 1 ng a.s./µl in guttation droplets. The data e.g. for clothianidin show that at a residue in guttation droplets of 1 ng/µl, a value found in seed treated maize or granular applications for approximately 4 weeks after emergence, only 3.7 µl of water would need to be consumed to achieve the LD_{50} of 3.7 ng/bee. Thus, concern was particularly raised for systemic insecticides with high toxicity for adult bees and/or bee larvae, especially for highly toxic systemic neonicotinoids, e.g. imidacloprid, thiamethoxam and clothianidin.

Tab. 1 Calculation of the amount of guttation water that, if consumed would lead to an uptake of a lethal dose for different active substances

Thiamet-hoxam		**Clothia-nidin**		**Substance A**		**Substance B**	
LD_{50} in ng/bee	**5**		**3,7**		**50**		**100**
Guttation droplets residues ng/µl	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**
0,01	500	0,01	370	0,01	5000	0,01	10000
0,05	100	0,05	74	0,05	1000	0,05	2000
0,1	50	0,1	37	0,1	500	0,1	1000
0,5	10	0,5	7,4	0,5	100	0,5	200
1	5	1	3,7	1	50	1	100
1,5	3,33	1,5	2,47	1,5	33,33	1,5	66,67
2	2,5	2	1,85	2	25	2	50
3	1,67	3	1,23	3	16,67	3	33,33

2.2 Risk evaluation

2.2.1 Methodology for risk evaluation studies

In laboratory studies it is not possible to stimulate the uptake of guttation liquid or pure water by honeybees without adding sugar. Such guttation liquid artificially spiked with sucrose is then used by bees as a carbohydrate source. Thus such laboratory feeding studies constitute a very unrealistic exposure scenario and provide only limited information for risk assessment to assess the risk for honeybees. Such laboratory studies have been used as a fast screening of guttation with feeding tests in cages. The outcome of such tests have shown to be of comparable outcome with OECD 213/214 laboratory toxicity data, resulting in high mortality after feeding sugar-enriched guttation droplets of maize treated with a systemic insecticide[4].

In semi-field studies, controlled conditions in tents or tunnels offer the possibility to simulate water collection from guttation droplets and other water sources, and to study honeybees' reaction to known residue levels in water. Alternative water sources can be excluded to ensure a maximum exposure. Effects on foragers, hive bees, and different brood stages can be measured in worst-case exposure scenarios. Nevertheless, semi-field studies have a limited potential for extrapolation of the findings to field conditions.

In field and monitoring studies honey bees can freely choose water sources. Field and monitoring studies can be designed to cover different scenarios from realistic field conditions to artificially aggravated exposure. In both, it is difficult to conclude on the activity of water foraging bees in the surroundings and to estimate the portion of water foragers using guttation droplets or other sources, and there is no control about the intensity of use of focused water sources. Likewise, the assessments are very labor intensive. Behaviour of foragers, effects on foragers, hive bees and different brood stages, brood development and colony development under realistic worst-case exposure conditions can be investigated. Residue analysis of dead honeybees and guttation fluid can be done for verification of a cause-and-effect chain.

Monitoring studies offer a wide range of possible designs under which presence or absence of the effects on honeybee colonies are determined in different environmental conditions. The significance of the results depends on the design of the study and environmental conditions. As the colonies show individual water foraging behavior and the environmental conditions of the study sites may be variable, the intrinsic variability of the systems can be compensated by appropriate replicate (e.g. colony and field) numbers.

2.2.2 Findings from semi-field, field trials and monitoring

Not surprisingly, when bees were fed with sugar-enriched guttation droplets of maize high mortality or total mortality was observed. In semi-field trials it was clearly demonstrated that increased mortality of worker bees may occur when bees are thirsty and no other water source is available (Fig. 3, left). On the other hand, when an alternative water source was available, no clear increase of mortality was observed (Fig. 3, right). In field conditions at the same site, no increase of mortality was observed for free flying colonies set up at the field border [5].

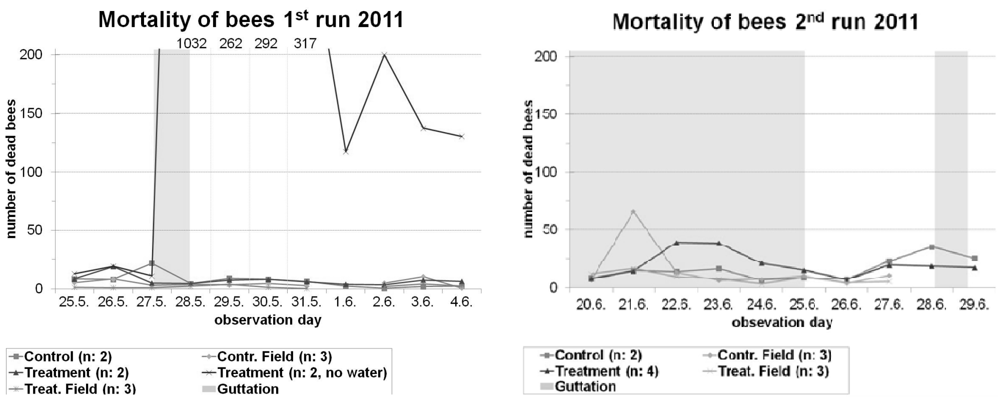

Fig. 3 Mortality of bees following exposure to maize guttation droplets in semi-field and field conditions, semi-field: 2 colonies with, and 2 without alternative water (left) or 4 with additional water (right) [5]

A number of studies with realistic worst case exposure were done by public research and industry. For granular application in maize with the active substance clothianidin, honey bee monitorings were conducted in 2010 and 2011 in different regions of Germany by public research institutes, the Apicultural State Institute LWG Veitshöchheim (Bavaria), the Bee Institute LAVES, Celle (Lower Saxony) and the DLR (Rhineland Palatinate). Colonies were set up at the field border before emergence of the maize crops. At the location in Veitshöchsheim, in both years 2010 and 2011[6] and also in Rhineland Palatinate[16] no noticeable mortality peaks were seen, and it was concluded that mortality, brood and colony development were on a normal level during the whole study and no treatment related effects were seen. However, in dead bee samples from days with no increased mortality, residues of clothianidin were found, indicating that single bees came in contact with the active substance which, however, was not leading to an overall increase of mortality[6].

In the trials conducted in 2010 by LAVES events of clearly increased worker bee mortality were observed, and residues of clothianidin were found in the dead bees (Fig. 4). It was concluded that the mortality was caused by uptake of guttation fluid. Although guttation occurred frequently during this trial, use of guttation fluids leading to increased mortality did not occur regularly but only on single events. Adverse effects on brood and colony development were not observed[21]. In the monitoring done by LAVES in 2011 no noticeable mortality peaks were seen and it was concluded that mortality, brood and colony development were on a normal level and no treatment related effects were observed during the whole study in 2011 (Von der Ohe, pers.com.). As no mortality peaks were seen in the other maize monitoring trials although guttation frequently occurred, it can be concluded that a use of larger amounts of guttation fluids by a larger number of bees only occurs in very specific circumstances. The high variability of effects observed under practical conditions is due to the individual location, climate conditions, water availability and water need. Also in a monitoring trial with seed treated maize in 2011 and also in winter oilseed rape 2010 and 2011 by the JKI (Pistorius, unpublished) no treatment related mortality peaks were observed.

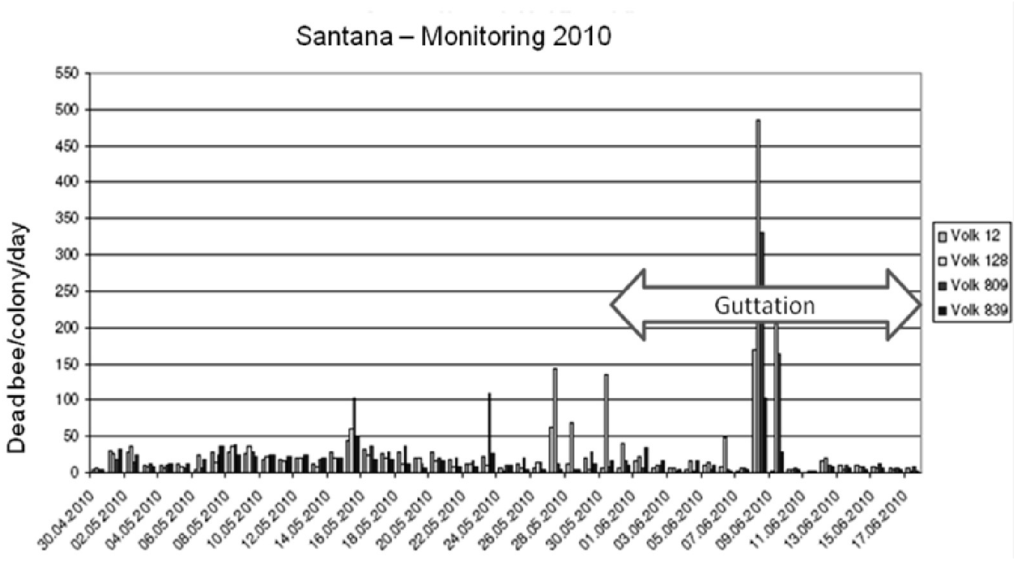

Fig. 4 Daily bee mortality, monitoring trial with clothianidin soil granular application in 2010 [21]

During guttation period in maize and wheat fields, no honeybees were observed collecting guttation drops[18,13]. Nevertheless, as noted from available data and practical experience it seems very difficult to observe bees taking up guttation fluid, even if guttation-related mortality occurred (as shown by residue analysis); mortality assessments seem to provide the more reliable information; however, if not conducted along with residue analysis of dead bees, they cannot differentiate between mortality related to guttation and other causes of mortality.

In the years 2009 and 2010, manufacturers of systemic insecticides conducted a series of field studies on guttation with soil-systemic applications of insecticides in maize, the crop that has been identified as worst case crop with regard to guttation.

Study setups varied with regard to methodological approaches and exposure conditions (e.g. availability of alternative water and food sources) but were nevertheless basically consistent in their experimental approaches. In all studies, exposed colonies were followed up for several weeks or even months in order to account for potential chronic or delayed effects.

A majority of studies employed realistic exposure conditions prevailing during normal agricultural practice. In others, worst-case exposure conditions were tested in terms of availability of alternative water and food sources. A few studies investigated the influence of additional provision of water sources.

Overall, data for more than 170 bee hives exposed to guttating maize have been considered. About two thirds of the hives have been exposed to treated fields, and one third to control fields (no systemic insecticidal seed or soil treatment)[2]. Due to the long exposure periods (most studies from emergence until flowering), the number of 'assessment days' (number of observation days per study x number of observed hives) sums up to more than 10.000 assessment days. On the vast majority of the assessment days and sites in the described studies, no increased worker bee mortality was recorded. Nevertheless, an increased number of dead bees could occasionally be observed for some hives. These events were limited to one or very few days which coincided with the guttation period. Results of analytical investigations suggest that honeybees occasionally use guttation droplets as water source. Causality between individual mortality peaks and colony strength, health or survival could not be concluded for any of these studies. Some details of studies conducted in maize by the manufacturers of systemic insecticides are summarized in table 2.

In their key findings, studies of the manufacturers of systemic insecticides are consistent with the results of comparable studies that were conducted by independent research institutes as described above. Each of the company-owned studies was or will be evaluated and assessed individually by the competent authorities; at least most if not all of these data were available to the JKI before the elaboration of this publication.

2.2.3 Potential risk under field conditions

Shawki et al. [19] assumed honey bees might collect water at distances up to 50 m. Thus, it is likely that at a certain distance between crops and colonies a potential risk is usually reduced to a very low level. Therefore the potential risk of guttation is in the first instance depending on the distance of the colonies to treated crops, because uptake of guttation droplets is mainly determined by the distance between colony and crop and the availability of other water sources. The risk of uptake of contaminated water is higher if the colonies are located in closer proximity to the crop, and lower with increasing distance. If the crop is showing regular guttation activity and seeds are treated with a systemic active substance with high intrinsic bee toxicity and findings of residue levels of high concern in guttation droplets occur, then guttation is a potential risk for individual bees if hives are located near such fields.

For a number of other crops in some countries, e.g. winter oilseed rape or sugar beet crops in Germany and the UK, insecticides for seed treatment containing neonicotinoids have been registered for more than 10 years with no link to honey bee poisoning incidents based on the national investigation schemes (Germany: Pistorius J, 2011, pers. comm., United Kingdom: Thompson H, 2011, pers. comm.).

[2] Experimental colonies with initial strengths that do not reflect realistic apicultural conditions (i.e. < 5,000 bees) were not considered for this evaluation.

Tab. 2 Field studies on guttation in maize with insecticidal seed or soil treatment

		Field Studies on Guttation in Maize with Insecticidal Seed or Soil Treatment											
Country	**Year/Season**	**Treatment Sites**	**Control Sites**	**No of treatment group hives**	**No of conrol treatment hives**	**Setup of hives (time, location)**	**Bee mortality assessment**	**Assessment of colony development**	**Assessment of guttation occurence**	**Assessment of bee exposure**	**Occurence of guttation**	**Treatment effects on mortality**	**Treatment related effects on colony levels**
Austria	2009 (Spring)	30	0	60 (small colonies)	0	Directly at or in treated fields; before crop emer-gence	yes (every second day)	yes (three-week inter-vals)	yes (every second day)	yes (every second day)	Fre-quent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No[1]
France (North / South)	2009 (Spring to Sum-mer)	4	4	24 (full size colonies)	24	Directly at treated fields; before drilling	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Mortality peaks but no difference in the number of peaks between control and treatment.	No
France	2010	19	3	4 per site (full size colonies)	4 per site (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period, bee residues still to be confirmed	No
France	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	fre-quent more so in treat-ment plots	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No
France	2009	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling, directly in field	yes (daily to once every 4 days)	yes (weekly)	yes (daily)	yes (daily)	Fre-quent	No treatment related mortality effects during guttation period	No
France	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No
France (South)	2010 (early Sum-mer)	1	1	6 (full size colonies)	6	Directly at treated fields; before crop emer-gence	yes (daily)	yes (weekly)	yes (daily)	yes (every 2-3 days)	frequ ent	Low mortality throughout the study. Mortality peaks in treatment and control coincided in most cases.	No
Germa ny	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No

As many different systemic active substances of low to moderate toxicity to bees have also been used for seed treatments and soil applications in the past, it can be assumed that in many cases honey bee colonies would have been exposed to guttation water. Due to the fact that no effects on bees had been observed, it can be concluded that in these cases unacceptable effects, e.g. increased mortality, might not occur, e.g. for fungicidal seed treatments (Pistorius J, 2011, pers. comm.).

2.2.4 Implications for the registration of pesticides

Data from experiments with intrinsically highly toxic, systemic insecticides indicate that further studies beyond standard laboratory toxicity data might be needed for a limited number of highly toxic active substances in a worst case crop. Criteria for active substances that may trigger further consideration may be

- systemic properties of active substance (xylem mobility),
- persistence,
- intrinsic toxicity for bees and
- mode of action
- crop

Regulatory decisions need to be made on a case by case basis. While exposure of honey bees to contaminated guttation water will be regularly addressed if the above mentioned criteria are met, specific testing does not need to be a standard regulatory requirement for all substances.

In order to assess the potential risk from guttation, commonly used study designs can principally be used. Nevertheless, some adaptations for semi-field trials and field trials are needed depending on study aim and these should be carefully considered for the study set up (e.g. the location directly at field edge, the set up of colonies at the field to cover crop stages with high residues, absence or availability of alternative water sources). For guttation studies prolonged assessment periods, e.g. on mortality and colony development, are necessary.

3. Conclusions

A large number of studies were conducted by both public research labs and industry to address the potential risk of guttation to bees. From available studies it can be concluded that different crops vary in the intensity and frequency of guttation events, residue levels in guttation liquid depend on the properties of the active substance, the amount of active substance per seed and other factors. Peak residue levels of systemic insecticides in guttation droplets have been measured soon after emergence and in young growth stages. Guttation droplets are one of several possible water sources in the surroundings of a colony and usually are only available at a limited time. The collection of guttation liquid is not an exposure scenario comparable to exposure to nectar and pollen, and the risk is likely to decrease rapidly with distance of the colonies to treated crops and the availability of alternative water sources nearby. In both field trials and monitoring from research institutes and industry, occasional increased mortalities of worker bees were reported from single events in such trials, where colonies were placed directly next to the sown maize crop. However, data indicate that even when such mortalities occurred no long-term effects on colony strength and brood development were seen. The potential risk from guttation seems to depend in the first instance of the distance of the colonies to treated crops. As guttation issues with particular focus on honeybees have been investigated for a few years only, the conclusions represent the current state of knowledge. Further basic research on mechanisms of water collection of the bees and use of water in the hive are recommended.

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References

1. Apenet report, 2010. http://www.reterurale.it/downloads/APENET_2010_Report_EN%206_11.pdf [accessed 04 January 2012].
2. Engelhard B, Illies I, Pistorius J, Gottschalch V, Wallner K.: Überprüfung einer Bodenanwendung von Actara (Wirkstoff Thiamethoxam) im Hopfen auf das Verhalten der Bienen und eventueller schädlicher Auswirkungen. *Schriftenreihe der Bayrischen Landesanstalt für Landwirtschaft*, Nr 8, 2011.
3. European Commission – Health & Consumer Protection Directorate-General, 2002. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final, 17 October 2002.
4. Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A, Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *Journal of Economical Entomology*, **102**, 1808-1815, (2009).
5. Frommberger M., Pistorius J., Schier A., Joachimsmeier I., Schenke D., Guttation and the risk for honey bee colonies (*Apis mellifera* L.): a worst case semi-field scenario in maize with special consideration of impact on bee brood and brood development. ICPBR Wageningen, 11th Symposium Hazards of Pesticides to Bees, 2-4.11.2011.
6. Illies I, Berg S, Pistorius J, Bischoff G, Effects on honey bee colonies following a granular application of Santana containing the active ingredient clothianidin in maize in 2010 and 2011. ICPBR Wageningen, 11th Symposium Hazards of Pesticides to Bees, 2-4.11.2011.
7. Joachimsmeier I, Pistorius J, Heimbach U, Schenke D, Zwerger P, Kirchner W, Details on occurrence and frequency of guttation in different crops in Germany. ICPBR Wageningen, 11th Symposium Hazards of Pesticides to Bees, 2-4.11.2011.
8. Johansson, T. S. K. and Johansson, M. P. (1978). Providing honeybees with water, *Bee World* **59**, 11-17.
9. Kuehnholz S, Seeley TD, The control of water collection in honey bee colonies. *Behavioural Exology and Sociobiology* **41**: 407-422, (1997).
10. Lindauer M, Temperaturregulation und Wasserhaushalt im Bienenstaat. *Zeitschrift für vergleichende Physiologie* **36**: 391-432(1954).
11. Pistorius J. ,Brobyn T, Lorsch J A, Suzuki H, Maus C, Lückmann J, Wallner K, Campbell P, Forster R. Marolleau F, Becker R.: Presentation of the ICPBR Working group on assessment of risks posed by guttation. ICPBR Wageningen, 11th Symposium Hazards of Pesticides to Bees, 3.11.2011.
12. Pistorius J, Wallner K, Joachimsmeier I, Reetz J, Schenke D, von der Ohe W, Illies I.: Review on activities in Germany to assess the occurrence, residues and possible risk of guttation for honey bees. ICPBR Wageningen, 11th Symposium Hazards of Pesticides to Bees, 2-4.11.2011.
13. Reetz J E, Zühlke S, Spiteller M, Wallner K. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? *Apidologie*, in press DOI: 10.1007/s13592-011-0049-1. (2011).
14. Schenke D, Joachimsmeier I, Pistorius J, Heimbach U. (2010). Pesticides in guttation droplets following seed treatment – Preliminary results from greenhouse experiments. Poster *SETAC Europe 20th Annual Meeting* 23-24 May 2010, Seville, Spain.
15. Schenke D, Joachimsmeier I, Pistorius J, Heimbach U. (2011). Pesticides in guttation droplets following seed treatment- field studies. *SETAC* North America 32nd *Annual Meeting,* 13-17 November 2011, Boston, US.
16. Schulz A, (2011) Beurteilung der Bienenschädlichkeit von Santana in einer Feldstudie, Dienstleistungszentrum Ländlicher Raum, Westerwald-Osteifel, Fachzentrum Bienen und Imkerei (FBI), Mayen 2011.
17. Seeley TD, (1997) Honigbienen: Im Mikrokosmos des Bienenstocks, *Birkäuser Verlag*, Basel.
18. Sgolastra F, Lanzoni A, Lilli A, Serra G, Medrzycki P, Porrini C. Valutazione del rischio per le api all'esposizione di gocce di guttazione derivante da mais conciato. *Atti Giornate Fitopatologiche,* 2010, **1**: 537-540. (2010).
19. Shawki M AA, Titera D, Kazda J, Kohoutkova J, Taborsky V, Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®. Plant *Protect. Sci*, **42**: 9–14. (2006).
20. Thompson H, Risk assessment for honey bees and pesticides – recent developments and 'new issues'. *Pest Manag Sci*; **66**: 1157–1162. (2010).
21. Von der Ohe W., 2010. LAVES. Santana: Maisaussaat mit insektizidem Granulat (a.i. clothianidin) 58.1 – 6022 Santana / 2010, Bericht 09.11.2010
22. Von der Ohe W., 2011. LAVES Santana: Maisaussaat mit insektizidem Granulat (a.i. clothianidin) (pers. comm, not yet published).