Annex E – Water Scenario

Annex to:

EFSA (European Food Safety Authority), Supplementary information to the revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)

[add individual author names in the same order as it is on the first page, followed by a comma, in the format: Surname Initial(s), Surname Initial(s) and Surname Initial(s)], 20YY. [Full title, including output category]. EFSA Journal 20YY;volume(issue):NNNN, 24 pp. doi:10.2903/j.efsa.20YY.NNNN

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Annex E – Review of the relevance of the exposure to honey bees via the consumption of contaminated water (water scenario)
Table of contents

1. Introduction
2. Aims and document outline
3. Sources of water for bees
4. Overview of water use by honey bees
5. Overview of the water scenario in EFSA (2013)
6. The use of guttation water by honey bees
7. Practical issues in assessing water use by honey bees
8. Summary and conclusion
9. References

Appendix A – Update to the proposed protocol
Appendix B – Analysis of exposure by guttation water in the 25 reports of field experiments re-
evaluated by Zumkier et al. (2019)
1. **Introduction**

In this Annex, the relevance of the assessment of risk from exposure to contaminated water as described in the previous guidance document (EFSA, 2013), known as "the water scenario", is reviewed. The detailed methodology of the process was described in the protocol for the revision (Annex A of the supplementary document). The draft of this protocol was shared on the 20th of March 2020 with the ad hoc stakeholder group and with experts from Member states. All comments received during this stakeholder consultation were carefully checked, and many were considered in the final version of the protocol for the revision (Annex A), section 4.1.6. However, during the implementation of the process some major deviations were made from the original protocol, namely an *a posteriori* recognition that it was impossible to complete the planned methodology due to a lack of evidence. Details of the major issues in completing the method described in the protocol for the revision (Annex A) are addressed in Appendix A of this document.

2. **Aims and document outline**

Like all animals, bees require water to survive, so the presence of detectable levels of plant protection products (PPP) in water in the environment led to the development of a water scenario in the previous guidance document (EFSA, 2013). The scenario attempts to quantify the risk of dietary exposure to plant protection products when present in permanent (e.g., rivers, lakes, ponds) or temporary water sources (e.g., puddles, guttation fluid). Whilst the presence of PPPs in water has been widely reported (Guttation fluid: (Girolami et al., 2009, Reetz et al., 2011); Puddles: (Samson-Robert et al., 2014)), there was a lack of evidence that exposure via water was an ecologically relevant exposure route (See references in EFSA PPR Panel (2012)), but a water scenario was included as a precautionary measure. Here, we review the relevance and practicalities of implementing the water scenario.

This Annex is broken down into the following parts:

1. The sources of water for bees
2. Water use by honey bees
3. Overview of the water scenario in the EFSA (2013) guidance document
4. The use of guttation water by honey bees
5. Practical issues in assessing water use by honey bees
6. Summary and conclusion

These parts cover a general overview of water use in bees (Sections 3-4), issues with the current scenario (Sections 5-6) and the WG conclusions on the inclusion of the water scenario in the updated risk assessment (Section 7).

3. **Sources of water for bees**

Bees can potentially obtain water from three sources. First, water can be produced within the body of the bees as a by-product of metabolising food (some examples: *Bombus lapidarius* (Bertsch, 1984); Carpenter bee, (Nicolson and Louw, 1982); Honey bee, (Louw and Hadley, 1985)). All bees generate metabolic water to some degree, but the amount generated depends on multiple factors including metabolic rate, body size and the bee's level of activity. At rest metabolic water may only provide a small proportion of the water a bee needs to remain alive but at times of intense activity, e.g., whilst transporting food back to the hive, the amount of metabolic water generated can be equal to the amount of water lost by evaporative water loss (Louw and Hadley, 1985).

Second, bees also extract water from food. Nectar can contain between 6.3-85% sugar by weight (see Annex C of supplementary document and Pamminger et al. (2019) and the average sugar content of nectar for plants pollinated by bees is approximately 40% ((Pamminger et al., 2019, Pyke and Waser, 1981)) with the majority of the remaining mass consisting of water. Whilst nectar foraging is often considered as a means of obtaining carbohydrates, it is also a significant source of water. For adult

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solitary bees, foraging on nectar is thought to be the primary way to obtain water (Nicolson, 2009), for example, one species of Mason bee, *Chalicodoma sicula*, adjusts their foraging to fill their water need before searching for the sugars required to sustain themselves or for nest provisioning. Similarly, at least one study indicates that honey bees alter their foraging preferences by foraging on more dilute sugar water when the colony need for water is high (Ohguchi and Aoki, 1983).

Third, honey bees exhibit a unique behaviour amongst bees where specialised workers visit water sources and retrieve water to the hive. Within eusocial bee colonies, the internal conditions are regulated to maintain a relatively constant internal environment (Seeley, 1995). However, the size and level of social organisation in a honey bee colony is atypical for a eusocial bee and, as a consequence of having many more individuals and larger hives, they have evolved specific behaviours that allow the internal environment of the hive to be maintained. One adaptation is the ability to regulate both the humidity and temperature within the hive by the use of water (Human et al., 2006). Whilst there have been sporadic reports of bumblebees visiting water (Ferry and Corbet, 1996), honey bees are the only bees to regularly collect and transport water into the hive.

Of these three sources of water, the first and second apply to all bees, whilst the third point only applies to honey bees. As honey bees are the only type of bee known to actively collect water in large quantities, we provide more details of water use by honey bees below.

### 4. Overview of water use by honey bees

Honey bees generally maintain a continuous flow of water to the hive (e.g. (Lindauer, 1955)). A colony of honey bees need water for several functions: (i) to quench thirst of the individual adult bees (i.e. maintain body fluid homeostasis), (ii) to produce glandular secretions and dilute honey for feeding the brood, (iii) to cool the nest on hot days and (iv) to maintain the relative humidity to prevent desiccation of the brood (Reviewed in Ostwald et al. (2016)). As nectar is mostly water, the colony’s water needs are often covered by its collection of nectar (Ostwald et al., 2016).

However, honey bee colonies sometimes require supplemental water. This may happen when the nectar flow to the hive is low, e.g., after a number of days with cold or rainy weather preventing the bees from flying out, or in times of nectar shortages, or to cool the beehive (Lindauer, 1955). As honey bee colonies do not maintain large water stores in the hive, the colony must quickly boost its water collection when the water demand is high (Ostwald et al., 2016) meaning that, even in periods of high water demands, the colonies needs may be rapidly met by the activities of water foragers, e.g. within half an hour after a cold spell in spring with brood in need of food (Lindauer, 1955). Here, we focus on collection of water and not water contained in nectar. This includes water that originates from ephemeral or transient water sources, such as guttation water, dew or puddles, or permanent water sources, such as rivers, streams, ditches or ponds.

There is a lot of uncertainty regarding water foraging as a behaviour. Water foraging is highly dependent on local environmental conditions, and the number of water foragers appears to be limited to a small number of bees relative to the number of bees that forage for food (Lindauer, 1955). Whilst the number of studies into water collection at the colony level is low relative to those related to foraging for food, the general consensus is that only a small proportion of adult bees in a hive are specialist water collectors (((Kühnholz and Seeley, 1997, Lindauer, 1955, Ostwald et al., 2016, Page and Fondrk, 1995, Reetz et al., 2012, Visscher et al., 1996)). For example, in greenhouse studies with two honey bee colonies experiencing brood nest hyperthermia, Ostwald et al. (2016) only identified 7 and 19 water foraging bees in colonies of approximately 3000 workers, indicating that only 0.23 and 0.63% of foraging bees were water collectors. Kühnholz and Seeley (1997) observed only 12 and 20 water collectors from hives with 4100 and 4150 bees, i.e., 0.49 and 0.29% of the bees in the hive. Similarly, in a study using 10 commercial bee colonies only 15 and 3 water collecting foragers were identified out of the 723 and 601 sampled foragers, i.e., only 0.5 – 2.1% of returning foraging bees (numbers estimated from Figure 3, (Page and Fondrk, 1995)).
Although the absolute number of individual water foragers appears to be low, in some cases, water foragers can constitute a large proportion of bees entering and leaving the hive. In studies of heat stress, where hives were heated, the proportion of water collecting bees varied between close to 0% of returning workers under no heat stress to 10-18% of the returning foragers when the colonies were heated (Kühnholz and Seeley, 1997). However, it is important to note that there are likely to be big differences in the behaviour of individuals in the hive responding to a short term and rapid increase in temperature and water collected to meet the physiological needs of the individuals in the colony. So, whilst water collecting bees may be easy to observe during heat stress, the low numbers of dedicated water foragers and the use of water present in nectar means it may be more difficult to monitor when water is only needed for consumption.

5. Overview of the water scenario in EFSA (2013)

In the previous guidance document (EFSA, 2013), the method for calculating a risk assessment for water is based on the following equation for calculating the exposure toxicity ratio (ETR):

\[
ETR = \frac{W \times PEC}{LD_{50}}
\]

Where: (i) W is the amount of water an individual bee consumes (µL/bee), (ii) PEC is the Predicted Environmental Concentration of the PPP in the water (µg/µL), and (iii) LD\textsubscript{50} is the median lethal dose of the PPP, as determined through laboratory testing (µg/bee).

It is important to note that the calculation of the ETR uses an LD\textsubscript{50} determined from laboratory studies of dietary exposure to plant protection products. This means that the ETR calculated is for a dietary scenario – there is no incorporation of other exposure routes such as contact, e.g., by transporting water in the crop, or after water has been deposited within the hive, or inhalation as the water and the PPPs evaporate. Therefore, water that is not consumed and metabolised by bees, e.g., for cooling the hive, is considered irrelevant for the purposes of risk assessment under the water scenario in EFSA (2013). Acknowledging that the water scenario is a dietary scenario has important implications for the implementation of the water scenario; even if we can determine how much foraged water enters the hive, we still need to know what proportion is consumed by the bees and what is used for other purposes to calculate an ETR.

The water scenario in EFSA (2013) covers acute and chronic exposure in adult worker bees and exposure in larval bees, over five days. Whilst the water scenario in EFSA (2013) was theoretically sound, there are issues with the values of water consumed by individual bees used to calculate the ETR. This value is critical as the calculation of the ETR is reliant on knowing how much foraged water (W) individual bees consume. We currently lack this information. For example, when calculating the ETR for pollen and nectar, the consumption values were represented by the shortcut values presented in Tables J4-8 in EFSA (2013) which are directly equivalent to W. The shortcut values for sucrose and pollen in the previous guidance document (EFSA, 2013) were based on multiple studies of bee feeding to determine the sugar and pollen requirements of adults engaging in either foraging or in-hive tasks and developing larvae.

For adult honey bees, the value for W used in EFSA (2013) was based on one study (Free and Spencer-Booth, 1958) which was designed to investigate clustering behaviour in bees under different temperatures and not water consumption. The experimental conditions are highly artificial and are not representative of either in hive or foraging conditions. In addition to the issues with the experimental design, the “worst case assumption” chosen in EFSA (2013) appears to represent a scenario where the bees are being placed under thermal stress. Given these issues, the working group has decided it is not possible to use this study to estimate water consumption in honey bees and we were unable to identify other sources of data (See Appendix B of this document for a detailed discussion of the suitability of Free and Spencer-Booth (1958) to estimate water consumption in adult bees).
For larval honey bees, the water scenario in EFSA (2013) tries to account for the origins of all the water in the diet during larval development. The accounting system used relies on some implausible assumptions. The main being the assumption that water is uncontaminated if it comes from honey and is contaminated and therefore comes from water foraged outside of the colony if it enters the diet in any other way. Similarly, the larval water scenario uses the water content of larval food minus the amount of water in the volume of honey required to provide the required carbohydrates to set W at 111 µl contaminated water over five days.

For worker larvae, the composition of larval food in the first three days is highly consistent and consists primarily of glandular secretions (Wang et al., 2016). On days four and five the composition changes (Wang et al., 2016) as a mixture of honey/nectar/beebread, which may or may not include the addition of an unknown amount of foraged water, is incorporated into the larval diet. It is unclear why there is an inconsistency about how processed larval food is treated in the larval water scenario in EFSA (2013). In particular, why honey is seen as uncontaminated but water in worker/royal jelly, a glandular secretion, is treated as water, potentially containing 72% saturation value of the PPP (EFSA, 2013). By assuming that all non-honey derived water is contaminated, the water scenario overestimates the level of larval exposure during development. This assertion is supported by multiple studies which report actual residue levels in royal jelly as being far lower than what would be expected given the Tier 1 assumptions ((Davis and Shuel, 1988, DeGrandi-Hoffman et al., 2013, Johnson and Percel, 2013, Böhme et al., 2018, Böhme et al., 2019)).

Even if we continue to accept the implausible assumptions mentioned in the previous paragraph we still do not know how much, or even if, foraged water enters the larval diet, therefore a Tier 2 refinement is impossible. However, an assessment for the effects of water in larvae is unnecessary as the issue of PPPs in larval food is already directly addressed within the risk assessment framework. The guidance document (EFSA, 2013) already requires direct measurements of the levels of PPP in larval food at Tier 2, which includes any water derived residues. There are already multiple studies that have measured pesticide residues in larval food ((Davis and Shuel, 1988, DeGrandi-Hoffman et al., 2013, Johnson and Percel, 2013, Böhme et al., 2018, Böhme et al., 2019)) which provide both methods for deriving actual measurements of the levels of PPP in larval food through development.

### 6. The use of guttation water by honey bees

The water scenario relates to exposure via contaminated water from all sources. However, one source, guttation fluid, was the primary focus of both the previous guidance document (EFSA, 2013) and the majority of experimental studies for exposure via water. Therefore, for the rest of the document we will use foraging from guttation fluid as a worst-case scenario for water foraging. Guttation refers to the process of exudation of liquid droplets from the tip, edges and axial surfaces of leaves. It occurs in a wide range of plants when conditions favour rapid absorption of water and low transpiration (Sanjay, 2016), such as early morning or late in the evening. Guttation fluid originates in the sap of the plant and consists primarily of water, but also contains any other compounds present in the sap, such as carbohydrates to set W at 111 µl.

In EFSA (2013), foraging on guttation fluid was proposed as a realistic worst-case scenario because guttation fluid can contain high concentrations of PPP, it is associated with plants that are in close proximity to the colonies (i.e., at the edge of the treated field), and guttation was assumed to meet the entire colonies water demands. This was despite a recognition in the previous guidance document (EFSA, 2013) that there was little strong evidence for bees using large volumes of guttation fluid. Here, we review and discuss the relevance of guttation as an exposure route using recent regulatory field studies conducted between 2010-2017, designed to detect the influence of guttation on honey bee colonies that were recently reviewed in Zumkier (2019). These experiments aimed to evaluate the effect of PPPs on honey bee colony through exposure via collected guttation water of the treated crop by:
1. quantifying the concentration of the PPP in the guttation fluid,
2. quantifying the occurrence of guttation fluid,
3. demonstrating a relevant exposure route by direct observation of foraging on guttation fluid and
4. identifying the ecotoxicological effects on the colony in the hive.

We assess the relevance of the guttation water scenarios for the risk assessment for bees, based upon the results of these 25 field studies (Appendix C), but focus explicitly on the relevance of guttation water as exposure route.

The reports used in Christl et al. (2019) were designed to observe foraging from guttation water in the field and incorporated at least some of the best practice that were suggested in EFSA (2013) for designing these studies (See Appendix C). Each report concluded that guttation fluid of seed-treated crops do not have adverse or unacceptable effects on honey bee colonies under typical commercial conditions by recording colony size, a direct assessment of the SPG, and other secondary characteristics, such as foraging behaviour. However, most of the reports (21 of the 25) recorded fewer than ten observations of bees collecting guttation water and we concluded that they were not valid assessments of water foraging behaviour in honey bees (See Appendix C). The low number of observations mean that these studies did not demonstrate that the colonies were exposed via guttation fluid. Given the lack of standardization in methods we cannot determine if the studies that failed to observe water foraging (i) used methods that were unsuitable for monitoring water foraging behaviour or if (ii) there was actually little or no foraging on guttation fluid, thus we are disqualifying them as valid assessments of water foraging for our purposes. The inability of the majority of these regulatory studies to observe bees foraging on guttation fluid illustrates the difficulty in quantitatively assessing this behaviour in the field using current techniques.

Additionally, we were unable to find experimental field studies in the open literature that could demonstrate a causal link between guttation and an effect on honey bee colony size and two recent reviews reached similar conclusions ((Pistorius et al., 2011, Schmolke et al., 2018)).

The main conclusion from Zumkier (2019) was that neither the detection of residues in guttation droplets nor the occurrence of guttation are sufficient to conclude an exposure, and hence a risk, for honey bees. The WG agree that none of the studies listed in Appendix C provide any evidence for a negative effect of PPPs present in guttation fluid on honey bee colonies, which includes not reducing the size of the colonies by 10% as specified by the current SPG. However, we cannot determine if this is because PPPs present in guttation fluid are harmless or if the experimental designs used in these experiments were unsuitable for detecting these effects. In order to make this distinction we would require standardized and validated methods for assessing water foraging in bees.

**7. Practical issues in assessing water use by honey bees**

There are a number of practical issues associated with the assessment of water use by bees.

**Tier 1**: The Tier 1 assessment in EFSA (2013) is based on calculating an exposure toxicity ratio using the “uptake of adult bees” using guttation as the worst-case exposure scenario uses the maximum solubility of the compound in water as the predicted environmental concentration, and the LD$_{50}$ calculated from laboratory feeding tests. However, there are issues with the value of W (detailed in section 3). Without extensive experience in measuring water consumption in honey bees or a thorough method development the working group are not able to identify a protocol to measure water consumption in bees.

**Tier 2**: Tier 2 allows for refinement of the calculation based on crop specific data. For example, the concentration of the PPP can be reduced from the maximum solubility to a 90th percentile value of the concentration measured in crop guttation water. In theory, it should be possible to modify the water consumed by an adult bee here, however, the working group are not able to identify a protocol to measure water consumption in bees.
Tier 3: The main issues with incorporating water collection into the higher tier risk assessment is that we lack the ability to: (i) accurately assess water use in the colony, (ii) reliably observe water collecting behaviour in the field, (iii) relate the presence of PPPs in water to a reduction of 10% of the colony size, and (iv) evaluate whether the study has been performed in a realistic worst-case environment.

Assessing water use in the colony: Honey bee colonies gather water to lower the brood nest temperature through evaporative cooling and preparing food with proper water content for the larval brood (Seeley 1955). This means that a colony’s water need rises both on hot days, when the colony starts to heat, and on cool days, when nectar foraging is hampered, there are likely to be complex interaction between the amount of water present in food (nectar and honey) and the amount of foraged water. Our lack of understanding about how much water enters a hive is compounded by the fact that the fate of water within the hive is also unknown. For example, we cannot ascertain how much of the water is being used for thermoregulation, and therefore irrelevant to the current water scenario, and how much is being consumed by the bees which is a requirement of the current water scenario.

Observing water collection in the field: Whilst observing bee behaviour in specific locations or on individual colonies is not particularly difficult or technically challenging (e.g. (Lindauer, 1955)), carrying it out on a scale that allows us to make a robust assessment of the effects of PPPs on behaviour is more challenging. The WG currently consider there to be a lack of methods to effectively monitor the behaviour of a small number of bees that engaged in a behaviour that is both temporally variable and dependent on local environmental conditions in a standardised manner. The lack of observations in the majority of the studies reviewed in Zumkier (2019) illustrate this point. Even when designing experiments to explicitly monitor water foraging from guttation, which we believe represents a worst-case scenario that combines high concentrations of PPP in water and a lack of alternate water sources to encourage the bees to meet all of their water requirements from guttation fluid in the immediate area of the hive, observing the bees foraging on guttation fluid still proved difficult (See Appendix C).

Detecting the effects of PPP in water on honey bee colonies: Following on from the point in the previous paragraph, to our knowledge, no study has produced strong evidence that PPPs in guttation fluid affect honey bee colony size to an extent that exceeds the SPG. This implies that if any effects of exposure to PPP in guttation water exist, the effect sizes appear to be too small to be detected at the colony level using our current experimental techniques and designs.

Whilst the absence of evidence is not evidence of absence the lack of standardised protocols to monitor the amount of water entering, and being consumed in the hive, hampers the process of quantifying the influence of PPPs in water. The methods described in EFSA (2013) to assess the effects of pesticides in guttation on honey bees attempt to link the occurrence of guttation, pesticide residue analysis, dead bees and colony size. However, no ring tested protocol exists, and there is a lack of practical experience, relative to assessments for the influence of PPPs in pollen and nectar, in how to design these experiments to. If exposure via foraging on water is to be incorporated in future guidance documents, there needs to be a concerted effort to develop appropriate methods that can detect harmful effects of PPPs present in water sources with a given level of accuracy.

Evaluate whether the studies have been performed for a sufficiently worst-case exposure. Even if it were possible to reliably demonstrate that (i) the water that entered the hive was used for consumption by the bees in the hive, (ii) the water was collected from the guttating treated crop and (iii) there were no effects of PPPs on the honey bee colonies, this would not be sufficient to demonstrate that the desired degree of protection of honey bee colonies would be attained. The reason is that it should be ascertained that the guttation water to which the hive bees are exposed represents a ‘realistic worst case’ exposure, i.e. that the concentration in the guttation water entering the hive represents the desired spatio-temporal percentile probability of occurrence in time and place for the exposure via guttation water. For instance, the concentration in guttation water collected in spring from a winter seed-treated crop is significantly lower than the concentration shortly after emergence in autumn and thus, a risk assessment for guttation water in spring does not represent the desired ‘realistic worst-case’ exposure. The desired percentile probability may be operationalised by selection of the yearly maximum daily PEC in guttation water entering the hive over a series of multiple years, and for a number of hives, but no
methodology has been developed yet. For example, how to establish that the measured concentration is the yearly maximum concentration in the guttation water, how many years and hives would be needed, and/or which agro-environmental conditions would be required for the study areas (similar to what Appendix G of EFSA, 2013 has done for field studies for higher tier residue value for nectar or pollen). So, it is not yet possible to evaluate whether the sufficiently worst-case exposure conditions regarding guttation water would have been reached.

8. Summary and conclusion:

The water scenario in the previous guidance document (EFSA, 2013) is a honey bee specific dietary scenario that describes a potentially relevant exposure route which the WG concludes cannot currently be incorporated into an effective risk assessment scheme. Therefore, the WG has decided to remove the water scenario from the updated risk assessment. This conclusion is based on the three major points outlined in sections 3-7 of this document and includes: a lack of fundamental information on how much water individual bees consume which impeding our ability to design a realistic lower tier assessment (Sections 3-5), a lack of evidence that exposure to PPPs via water is a realistic and important exposure route (Section 6), and the practical difficulties of assessing effects of PPPs in foraged guttation water on honey bee colonies in a field scenario, invalidating the highest and reference tier of the risk assessment (Section 7).

There are a number of outstanding challenges to overcome before water can be incorporated into risk assessment: we require a better understand the dynamics of water use in honey bee hives, a reliable fit-for-purpose protocol to accurately assess the water requirements of a hive, and the individuals within it and ideally an understanding of how the water needs are met by various sources of water in the environment. In addition, there should be guidance how to evaluate the degree of worst-case environmental scenarios of the field studies performed.

Finally, even if the amount of water entering the hive can be accurately determined we require an understanding of how much of that water is consumed by the bees (relevant for risk assessment as described in EFSA (2013)) and the amount used for hive maintenance (irrelevant for risk assessment as described in EFSA (2013)) also needs to be determined.

Given the issues mentioned in this document we simply reemphasise that whilst water foraging is potentially a relevant exposure route, we currently lack the relevant information and technical ability to effectively formulate an effective risk assessment, therefore we have elected to remove the water scenario from the updated risk assessment.

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10.1080/00218839.2019.1614727


10.3390/insects4010071
Annex E – Water Scenario


https://doi.org/10.1002/jez.140235


Appendix A — Update to the proposed protocol

Here we address each question raised in the draft protocol for the revision (Annex A) and briefly discuss if and how it was addressed. In order to investigate the relevance of the exposure via consumption of contaminated water it is necessary to understand how much of the collected water the bees consume, from which sources this water comes and when. The two general questions and related sub-questions to answer are reported in Table A1. However, we were unable to answer the questions proposed in the published protocol. Here we address each question in turn and explain why we were unable to complete each task.

Table A1: Each of the questions proposed in the protocol for the revision (Annex A)

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer or Reason</th>
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<tbody>
<tr>
<td>Q1 What is the water consumption of adult honey bees and larvae?</td>
<td></td>
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<tr>
<td>Q1.1. How does the total water demand of bee colonies relate to water consumption only?</td>
<td></td>
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<tr>
<td>Q1.2. What different sources cover the water needs of adult bees? Can this be quantified? Does the distribution between the different sources vary during the day and during the season, and can this be quantified?</td>
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<tr>
<td>Q1.3. Same question as 1.2 for larvae.</td>
<td></td>
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<tr>
<td>Q1.4. How many days of the year adult honey bees or larvae consume water collected by the water foraging bees (i.e. not only from the water contained in nectar or honey)? Which conditions (both environmental and bee behavioural) determine whether water is collected?</td>
<td></td>
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<tr>
<td>Q2 Is the exposure to pesticides by collected water (esp. guttation water) a relevant scenario?</td>
<td></td>
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<tr>
<td>Q2.1 Adult honey bees: what is the proportion of the daily pesticide intake via consumption of collected water compared to the daily pesticide intake via nectar and pollen?</td>
<td></td>
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<tr>
<td>Q2.2 Larvae: what is the proportion of the daily pesticide intake via consumption of collected water compared to the daily pesticide intake via (royal or worker) jelly, nectar and pollen?</td>
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<tr>
<td>Q2.3 Can the occurrence of guttation be characterised over the day?</td>
<td>This is expected to be a function of (i) crop type, (ii) crop and BBCH crop development stage, and (iii) environmental conditions</td>
</tr>
<tr>
<td>Q2.4 Can the concentration of pesticides in the guttation water be quantified as a function of time after application or sowing?</td>
<td>This is expected to be a function of (i) compound, (ii) BBCH crop development stage, (iii) application technique (seed treatment or spray-downward or up- and sideward) and (iv) application timing</td>
</tr>
<tr>
<td>Q2.5 How do the pesticide concentrations in guttation water, puddle water and surface water compare in general?</td>
<td></td>
</tr>
</tbody>
</table>
Q1. What is the water consumption of adult honey bees and larvae?

Q1.1. How does the total water demand of bee colonies relate to water consumption only?

We carried out a non-systematic review of the open literature and the data does not appear to be available, answering this question would require specific studies. We currently have some estimates of how much water a honey bee colony will collect per year, or in times of thermal stress. However, we cannot link the total amount of foraged water entering the hive to the amount consumed by adult and larval bees which is an essential component of the risk assessment.

Q1.2. What different sources cover the water needs of adult bees? Can this be quantified? Does the distribution between the different sources vary during the day and during the season, and can this be quantified?

Honey bees can and do collect water from various sources which includes “pure” water sources such as dew, puddles or ponds in addition to water contained within nectar. However, there is no information about how much of it is for consumption by individual water foragers, how much is for hive maintenance, and how much is consumed by other adult bees in the hive.

Can this be quantified?

At a colony level yes. Water consumption at the level of the colony has been successfully studied but as the traditional methods for monitoring water foraging and consumption are time and labour intensive, it is not an active area of research. To our knowledge there is little information for the amount of water individual adults consume within the colony.

Does the distribution between the different sources vary during the day and during the season, and can this be quantified?

Honey bees can use a variety of water sources, for example, some of the studies in Zumkier (2019) provide just one example of honey bees collecting water from guttation fluid and dew at the same time. This means that they almost certainly can change the source of water at different times of the day/year depending on water availability.

Q1.3. What different sources cover the water needs of larvae? Can this be quantified? Does the distribution between the different sources vary during the day and during the season, and can this be quantified?

The issues with the various sources of water are identical to those for adults, water can come from multiple sources. However, for larvae water is provided as a component of their food, which is processed by nurse bees. Can this be quantified?

The water content of larval food can be quantified. For example, the composition of Worker and Royal Jelly is >60% water and the constancy of moisture content is assured, by the continuous provision of fresh supplies of this substance by nurses (Sabatini et al., 2009). However, for at least the first three days the larvae are fed solely glandular secretions which are a form of processed food produced by the nurse bees containing protein, lipids, sugars, vitamins, and water and not foraged water. There appear to be only trace levels of contamination in royal jelly (see section 4.2.3 of the protocol for the revision (Annex A)), and how much, or even if, foraged water directly enters the larval food is unknown.

Q1.4. How many days of the year adult honey bees or larvae consume water collected by the water foraging bees (i.e. not only from the water contained in nectar or honey)? Which conditions (both environmental and bee behavioural) determine whether water is collected?

We were unable to estimate the amount of water that is collected for consumption, as opposed to hive maintenance.

Whilst we know the broad conditions that that drive water foraging in bees, temperature and nectar flow, we lack the information on the requirements and utilisation of water to estimate the number of days bees collect water for consumption.
Q2 Is the exposure to pesticides by collected water (esp. guttation water) a relevant scenario?

Most Q2 questions have become irrelevant, because the water scenarios have been taken out the risk assessment. In addition, we have shown (section 6) that any non-negligible exposure to guttation water is hard to demonstrate, and cannot be expressed in a sound probability of occurrence in time and space, required for the risk assessment.

Q2.1 Adult honey bees: what is the proportion of the daily pesticide intake via consumption of collected water compared to the daily pesticide intake via nectar and pollen

We lack data relating the use of collected water within the hive or the volume of water that individual bees need to consume to survive. Without this basic information we cannot assess what proportion of pesticide exposure in a bees diet originates from water and thus the scenario is not evaluated.

Q2.2 Larvae: what is the proportion of the daily pesticide intake via consumption of collected water compared to the daily pesticide intake via (royal or worker) jelly, nectar and pollen?

Refer to Q1.3 above. Larvae are fed on jelly during the first 3 days, containing only traces of pesticides and as the use of water within the hive is not known, it is unknown whether collected water would end up in larval food after their initial 3-d development.

Q2.3. Can the occurrence of guttation be characterised over the day? This is expected to be a function of (i) crop type, (ii) BBCH crop development stage, and (iii) environmental conditions.

As the water scenarios have been taken out, these issues were not studied into further detail.

Q2.4. Can the concentration of pesticides in the guttation water be quantified as a function of time after application?

This is expected to be a function of (i) compound, (ii) crop and BBCH crop development stage, (iii) application technique (seed treatment or spray-downward or up- and sideward) and (iv) application timing.

As the water scenarios have been taken out, these issues were not studied into further detail.

Q2.5 How do the pesticide concentrations in guttation water, puddle water and surface water compare in general?

As the water scenarios have been taken out, these issues were not studied into further detail.
A.1. Analysis of data from Free and Spencer-Booth (1958)

The rationale for reviewing the water consumption estimates is detailed below and based on the data from Free and Spencer-Booth (1958).

The WG first tried to exclude any temperature treatments or groups that would be unsuitable for determining water consumption. The original paper suggested that the death rates were at their lowest and most stable between 25-35°C (Supplementary Figure 2A) but that the two smallest groups showed anomalous spikes in mortality at 30°C (data not shown). If we take that as correct, then we should assume that the temperatures in this range were the least likely to put the caged bees under thermal stress; so, we should exclude groups <25° and >35°C for estimating water consumption. Given the unexplained variation in the death rates in the two smallest groups these have also been excluded.

At lower temperatures the bees require carbohydrates to generate heat which likely explains the dramatic reduction in sugar intake in the warmer cages. The dashed line in Supplementary Figure 2B indicates the lower boundary for the daily sugar intake for a forager bee (lower boundary for a nurse bee is very similar at 34 μg/day) recorded in EFSA (2013) which shows just how different this experiment is from bees in a hive. However, we might still be able to gain some insight into the water requirements for bees. The rate of decline in sugar intake at higher temperatures appears to drop off between 25-30°C, i.e. sugar consumption drops by 20 μg between 15 and 25°C but only drops by 5 μg between 25-35°C, indicating that these values (somewhere between 5-15 μg per day) likely reflects the amount of sugar required to keep a bee alive, when we don’t impose any energetic costs that require supplementary feeding e.g. in hive tasks or having to generate metabolic heat. This agrees with the mortality data.

Figure 2C shows the average total water uptake per bee, which is the sum of the water in the 66% sugar syrup, and the pure water provided to the caged bees. Supplementary Figure 2D shows the uptake of pure water relative to the water from the sugar syrup. At low temperatures (<25°C), the water intake is relatively high because the bees need to increase their sugar intake by consuming large volumes of syrup and ignoring the foraged water. When bees take on more water than they need, they simply excrete the excess. The steady decline in sucrose and water uptake at temperatures up to 30°C show that the bees reducing their food intake when they don’t require the nutrients from the food. The water intake was minimised at 30°C indicating that this is likely as good an estimation of a bee’s water requirements as we can get from this experiment.

There is an argument that we should use the values from 35°C treatment, as these temperatures are commonly found within the hive, however, this would be unwise. The experimental conditions in this study were highly unrepresentative of the conditions in the hive or whilst foraging; the bees were held in a temperature-controlled cage with no in-hive or foraging tasks and the cages had no humidity control – both of these factors are likely to affect a bee’s energy requirements and transpiration rates which in turn impact on a bee’s water requirements. We know that the hive is maintained in a state of homeostasis, where both temperature and humidity are regulated. If we assume that honey bees have evolved so they are not constantly in a state of stress (such as might be indicated by a tripling in the amount of water uptake between the 30°C and 35°C treatments) then we need to use measurements where there is minimal evidence of either cold stress (causing increases in syrup consumption) or heat stress (causing rapid increases in water consumption).

Given the issues raised in this document and after comments made during the consultation process the WG decided that we could not justify using a single study which was not designed to monitor water use by honey bees in a realistic manner to set the water requirement for adult bees.
Figure A 1: Supplementary information. Mortality rates for each temperature treatment (Plot A), the average daily amount of sucrose consumed per bee with the dashed lines indicating sugar requirements of a forager bee in a hive from EFSA (2013) (Plot B), average daily water uptake per bee with an estimate for the amount of water a bee requires to survive (Plot C), the proportion of pure water as a percentage of total water uptake and my estimated value for the proportion of water that comes from pure water (Plot D).
Appendix B — Analysis of exposure by guttation water in the 25 reports of field experiments re-evaluated by Zumkier et al. (2019)

B.1. Introduction

Here, we review how water foraging has been assessed at the highest tier by using 25 reports of field experiments that were submitted by applicants between 2010-2017, and that were re-evaluated in the report by Zumkier (2019). The aim of these experiments was to evaluate the effect of PPPs on honey bee colonies that were exposed via guttation water from the treated crop. The fact that PPPs are found in guttation water of some crops has been reviewed extensively in other studies (e.g. (Girolami et al., 2009, Tapparo et al., 2011, Nikolakis et al., 2015)). Therefore, the aim of the current assessment is to determine whether guttation fluid is collected frequently enough and at high enough volumes for PPPs to reduce the colony size, and thus represents a relevant exposure scenario. To do so, it was investigated whether the study design of the 25 available studies met the criteria described in the previous guidance document for maximising exposure, and whether it could be considered suitable to demonstrate that the bees in the studies were actually exposed to PPPs through guttation fluid.


The previous guidance document (EFSA, 2013) proposes field studies for the assessment of PPPs in water for Tiers 2 and 3. In Tier 2, field studies establish the 90th percentile concentration of the PPP in guttation water (box 4 and 7, Tier 2 of EFSA (2013)) to refine the Tier 1 ETRs based on the default values and require between 2-5 studies to establish realistic worst case environmental concentrations that the bees may encounter. In Tier 3, field studies are used to assess both the concentration of PPP in guttation water and the effects on the colony (box 8, highest tier, Tier 3). We will concentrate on the Tier 3 assessment, representing the surrogate reference tier, that intends to represent reality as well as possible in a field test. Tier 3 incorporates measurements of PPPs in water and also tries to establish (i) that bees are collecting guttation fluid and (ii) if there are reductions in colony size that exceed the 7% reduction included in EFSA (2013). In a Tier 3 risk assessment, the likelihood that the honey bees will use guttation fluid should be maximised, therefore, the hives should be located away from alternative sources of water and the guttation fluid must contain higher than median values of the PPP.

The design of the higher tier studies focused on generating an assessment that was as close to a realistic worst-case exposure scenario as possible by focusing on the distance to permanent water sources, which were identified as being the main driver for exposure (EFSA, 2013). This was operationalized by requiring an experimental design that simulated a 90th percentile probability of occurrence in time and space for the exposure through guttation water by placing the experimental hives used for risk assessment in an environment with no alternative sources of water to guttation. The water scenario therefore required that there were no other sources of water within a 90th percentile distance in the area of use to permanent water sources and that the concentration of the PPP was greater than the median concentration of the PPP in the guttation water (box 8 of Figure 1 in Chapter 3 of EFSA, 2013), thus avoiding stacking two 90th percentiles and ending up in a more extreme worst-case scenario.

None of the studies we reviewed, even those carried out after the publication of EFSA (2013), implemented these criteria. However, we still feel these studies can inform our assessment of the water scenario, given that some of the specifications in the water scenario were difficult to implement. First, the previous guidance document (EFSA, 2013) suggested assessing the distances of permanent water bodies in the area of use, be this at the scale of the whole EU, regulatory zone, climactic zones or member state level. This should be defined as the 90th percentile distance from the hive to the nearest permanent water sources using geographic information system (GIS) procedures. However, what a human perceives to be a “permanent water sources” that is large enough to be recorded on a map and available for GIS analysis, e.g., drainage ditches, rivers, ponds, lakes, may not be what a bee perceives as a permanent water source, e.g., water troughs, leaking pipes or taps, or boggy ground, which are unlikely to be recorded without extreme effort on the local scale and will be impossible to determine at the national or regional level. As none of the studies carried out a GIS analysis of the area of use, it is therefore impossible to tell if the studies met the distance requirements from EFSA (2013), however, even if these assessments had been carried out, they would be unlikely to accurately represent the local water foraging landscape from a bee’s perspective. Despite this issue at least 16 of the 25 field studies
attempted to isolate the bees from other sources of water and recorded the distance to the nearest large alternative water source. Thirteen of the 25 attempted reports noted that there were no large water sources within at least 100m from the hives, adjacent to the treated plots.

Concerning the criterion of concentrations in guttation water above the median, EFSA (2013) did not specify precisely how to establish these or include specific median RUD values like those supplied for nectar and pollen (Appendix F, EFSA (2013)). So, there was no clear guidance on how to ascertain that the exposure was sufficient. However, most studies reviewed in Zumkier (2019), measured concentrations in guttation water, at emergence and at several successive assessments during the study (as was indicated in Appendix U of EFSA (2013)) and thus did demonstrate that PPPs were present in the guttation water.

B.3. Aim of the field studies

Each of the 25 reports could be considered as the highest tier of the risk assessment as described in Figure 1 in Chapter 3.5 of the previous guidance document (EFSA, 2013). This type of field studies aimed to create a situation in which the water collecting bees are likely to take up contaminated guttation water. So, the aim of these studies was to determine if

1. guttation water had been collected
2. there were any detectable ecotoxicological effects on the colony
3. demonstrate that foraging from guttation water does not occur to a significant extent, even when guttation water was present close to the hives and alternative water sources were far away.

The third point implies that the field study aims to show that the guttation water exposure route is unlikely, even when the conditions for this route are optimal. In these cases it is crucial-

- to create a 90th percentile worst case situation for water sources, i.e. ensuring that permanent water sources are so far that they are unattractive (and avoiding the presence of transient water sources as much as possible, to ensure the choice of guttation water as water source by the bees), and
- to create optimal conditions for observing the number of bees collecting guttation water from the treated crop.

In order to provide an insight in the type of experiments performed for a Tier 3 assessment, we describe below a study which successfully monitored the uptake of guttation water by water foragers and thus demonstrated exposure to the PPP via guttation water.

B.4. A study into the effects of Clothianidin & Beta-Cyfluthrin in guttation fluid

The study was conducted on seed treated winter oil seed rape (Hofmann et al, 2010, N4), which was conducted at six fields (3 treated, 3 controls), where five hives were placed at the edge of each field within 0.5 m of the crop. The sites were chosen so that there were no permanent water bodies within 250 m of the hives and monitored daily to record the presence of guttation and dew, and to assess the behaviour of the bees. The observations occurred autumn (7 weeks after emergence, Sept-Oct) and spring (4 weeks up to the beginning of flowering of the crop, April). Monitoring was done in the morning (from sunrise onwards) and evening, at three assessment areas on each plot: two in-crop and one off-crop zones of several 10s m² each, less than 10 m to the hives. The behavioural assessments during the guttation period consisted of 35-minute observations.

Guttation was frequent, occurring on 80% and 76% of the observation days in autumn and spring respectively, and generally lasted for a couple of hours, usually stopping by 11 am. Residues in guttation water were measured with a maximum of 0.41 mg/L in autumn 2009 and 0.02 mg/L in spring 2010.
Bees were only observed taking up dew or guttation water in the morning and not the evening. Of the 7906 honey bees observed within the assessment areas, 568 and 278 honey bees were seen taking up dew and guttation fluid respectively. The majority of these observations occurred in the area directly in front of the hive.

In this study the following points probably contributed to the effective observation of bees taking up guttation water:

- Permanent water sources were located at least 250 m from the hive, presumably promoting the use of the nearby guttation water;
- The observation areas were located very close to the hive. As water collection is an energy-consuming activity, water collectors are assumed to collect water as close to the hive as possible.
- Moreover, the observation areas were sufficiently large to increase the possibility to observe bees, i.e. 68 m² in-crop within 10 m from the hive and 26 m² off-crop within 5 m from the hive,
- The observations were well timed to co-occur with guttation, were carried out on a daily basis for a relatively long period (35 minutes, repeated up to 4 times).

**B.5. Evaluation criteria to determine whether colonies were exposed to PPPs via guttation water**

We evaluated all 25 reports to establish whether studies had effectively determined if the bees were collecting guttation water. The criteria we used for assessing this were:

1. The hives should be at least at 100 m distance (an arbitrary value chosen by the working group to represent a sufficient distance) from alternative water sources;
2. Concentrations in the guttation water should be measured and reported. We here exclude studies that did not measure the residue levels in the guttation water.
3. In the observation areas the number of active bees, including their activity, such as taking up guttation water should be recorded, as well as the observation time. The size of the observation areas should be sufficiently large (at least a few 10s of m²) and they should be located close to the hive (0-20 m)
4. In this way the guttation water foraging behaviour can be scaled per unit area and time and be compared to bee activity in general.
5. There should preferably be co-occurrence of expected high pesticide concentrations in guttation water (for seed treated crops shortly after emergence) and water need for the brood (the most sensitive endpoint, highest activity in spring), e.g, an autumn sown seed-treated crop often does not represent a realistic worst-case situation.

**B.6. Evaluation results**

1. **Hives should at least be at 100 m distance from alternative water sources**

   Eight of the reports did not indicate the distances to permanent water sources to the hives, while 17 did. In seven of the 17 reports permanent water sources were close to the hives (<100 m), while the remaining 10 mentioned distances of at least 100 m. Few reports mentioned distances to transient water sources; good examples of detailed definition and mapping of transient water sources are Striffler et al. (2015 a and b; study code N23 and N24). None of the studies expressed the distances in terms of percentile of occurrence in the area of use, as suggested by EFSA (2013).

2. **Concentrations in the guttation water should be measured.**

   Eight of the 25 studies did not report residue concentrations in guttation water, of the 17 remaining studies 11 had maximum residue concentrations above 1 mg/L.
3. Number and activities of bees in the observation areas, their size and location and the observation time should be recorded

All reports recorded the number of bees observed collecting water. In 19 of the 25 reports observation areas of 1-4 m² each were used at distances ranging from 5 to 25 m from the hive (up to 150 m in one case, Knabe et al, 2010; study code N15). In the six remaining reports areas ranged from approximately 20 to 44 m² at distances ranging from 2 to 200 m, but only 2 to 6 m for the four reports monitoring significant numbers of water collecting bees. These four reports included off-crop areas in the monitoring.

In most studies (23), observations were made on a daily basis. However, two studies only carried out observations once a week. In 20 of the 25 reports observations were repeated during the morning and later in the day. Most reports failed to record any bees collecting guttation water, and only four recorded observing of more than seven bees collecting guttation water (Table B1).

4. Co-occurrence of expected high pesticide concentrations in guttation water and water need for the brood

In the 7 studies with seed-treated winter cereals (studies N4, N5, N6, N9, N19, N20 and N21) the crop emergence is in autumn. In autumn, the measured concentrations in guttation water are distinctly higher than in spring (Table B2), but brood production stops and the amount of water that the colony needs to forage for consumption should be low relative to the spring period. So, seed-treated winter cereals may not reflect a realistic worst-case conditions with respect to risks by exposure to guttation water.
### Table B1: Number of foraging bees observed (total and collecting guttation water or dew) and total observation time for the reports which reported bees collecting guttation water.

<table>
<thead>
<tr>
<th>Study code</th>
<th>Number of observed bees:</th>
<th>Observation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Guttation</td>
</tr>
<tr>
<td>N4</td>
<td>7906</td>
<td>278</td>
</tr>
<tr>
<td>N5</td>
<td>3148</td>
<td>334</td>
</tr>
<tr>
<td>N6</td>
<td>3276</td>
<td>343</td>
</tr>
<tr>
<td>N9</td>
<td>6973</td>
<td>505</td>
</tr>
<tr>
<td>N12</td>
<td>Not reported</td>
<td>7</td>
</tr>
<tr>
<td>N13</td>
<td>Not reported</td>
<td>1</td>
</tr>
<tr>
<td>N14</td>
<td>299</td>
<td>1</td>
</tr>
<tr>
<td>N22</td>
<td>18</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table B2: The maximum PPP concentrations measured in the seven reports that recorded Autumn and Spring guttation

<table>
<thead>
<tr>
<th>Study code</th>
<th>Crop</th>
<th>Compound</th>
<th>Maximum residue (mg/L):</th>
<th>Autumn</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>winter oilseed rape</td>
<td>clothianidin</td>
<td>0.41</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>winter barley</td>
<td>imidacloprid</td>
<td>15</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>N6</td>
<td>winter wheat</td>
<td>imidacloprid</td>
<td>6.9</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>N9</td>
<td>winter barley</td>
<td>clothianidin*</td>
<td>8.51</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>N19</td>
<td>winter oilseed rape</td>
<td>thiamethoxam</td>
<td>11.137</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td>N20</td>
<td>winter oilseed rape</td>
<td>thiamethoxam</td>
<td>7.520</td>
<td>0.0040</td>
<td></td>
</tr>
<tr>
<td>N21</td>
<td>winter oilseed rape</td>
<td>thiamethoxam</td>
<td>0.274</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*Clothianidin and Imidacloprid applied as a single combined treatment
B.7. Conclusion

Each individual report concluded that guttation water from seed-treated crops does not reduce colony size by more than 10% or induce any detectable adverse effects on honey bee colonies. However, none of the studies met the criteria for a valid assessment based on EFSA (2013) and most (21/25) of the studies failed to meet our criteria for a valid assessment, largely based on their failure to demonstrate that the colony had been exposed to PPP via foraging from guttation fluid (Table B1).

It is also important to note that the lack of a ring tested or standardised method for assessing honey bee water foraging means we have no way of assessing how suitable the methods used are for assessing water foraging. Therefore, it is impossible to know if the reason for not observing water foraging was because honey bees rarely engage in foraging from guttation fluid or if the methods used were unsuitable to detect the behaviour. This means that the results of these studies are inconclusive.

It is interesting that the studies with the highest numbers of observations of water collection from guttation fluid all involved regular, relatively long observation periods, made close to the hive and over relatively large areas. However, even in the studies where foraging from guttation fluid was observed, when expressed per unit time, the number of individuals was small, approximately 1-3 bees per hour, in experiments across multiple plots and hives (Table B1). There are certain intrinsic characteristics of water foraging behaviour in honey bees, e.g., low number of water collecting bees, specific environmental conditions required to increase water demand and the short time period needed to alleviate the water shortage, that make it easy to miss water collection events and thus it will be difficult to demonstrate that guttation water is (or is not) a relevant exposure route.

This means that the highest tier of the risk assessment scheme, proposed in the previous guidance document (EFSA, 2013) seems very difficult to operationalize, which presents a serious problem for assessing the risks for the colony by the guttation water exposure route.

References in Zumkier et al. (2019)

Below, the full reference of each of the 25 studies that were re-evaluated in the report by Zumkier (2019) are given, together with the study code (N1 to N25) used in the report by Zumkier (2019) and in the text above:

N1: Liepold, K. Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Alsace (France), 2010. Eurofins. Study code S09-01402. (Sponsor Bayer CropScience)

N2: Liepold, K. Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Champagne (France), 2010. Eurofins. Study code S09-01403. (Sponsor Bayer CropScience)

N3: Liepold, K. Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Languedoc-Roussillon (France), 2010. Eurofins. Study code S09-01404. (Sponsor Bayer CropScience)

N4: Hofmann, S., C. Garrido, J. Lueckmann. Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter oil-seed rape (W-OSR), seed-treated with clothianidin & beta-cyfluthrin FS 400 + 80, 2010. Rifcon. Report no. R09107 (Sponsor Bayer CropScience)

N5: Hofmann, S., C. Garrido, J. Lueckmann. Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product, 2012. Rifcon. Report no. R09247-3 (Sponsor Bayer CropScience)

N6: Hofmann, S., J. Lueckmann. Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated with an imidacloprid or a clothianidin combi-product, 2014. Rifcon. Report no. R09247-4 (Sponsor Bayer CropScience)
N7: Rexer, H.U. A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in southern Germany in 2013 and 2014, 2014. Eurofins. Study code S13-00171. (Sponsor Bayer CropScience).

N8: Rexer, H.U. A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in southern Germany in 2013 and 2014, 2014. Eurofins. Study code S13-00170. (Sponsor Bayer CropScience).

N9: Staffel, J., P. Aumeier, S. Hofmann. Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treated product clothionidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012, 2014. Rifcon. Report no. R111130 (Sponsor Bayer CropScience)

N10: Rexer, H.U. A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in southern Germany in 2014 and 2015, 2014. Eurofins. Study code S14-01392. (Sponsor Bayer CropScience).

N11: Rexer, H.U. A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in southern Germany in 2014 and 2015, 2014. Eurofins. Study code S14-01385. (Sponsor Bayer CropScience).


N16: Gonsior, G. Thiamethoxam. Thiamethoxam FS (600) (A9765R) – A field study to investigate the effects of residues in guttation fluid on honeybees (Apis mellifera L.) in sugar beet grown from treated seed in Germany, Celle. Interim report, 2015. Eurofins. Study code S15-01050. (Sponsor Syngenta, UK)

N17: Dittbrenner, N. Thiamethoxam. Thiamethoxam FS (600) (A9765R) – A field study to investigate the effects of residues in guttation fluid on honeybees (Apis mellifera L.) in sugar beet grown from treated seed in southern Germany. First interim report, 2015. Eurofins. Study code S15-01048. (Sponsor Syngenta, UK)


N19: Gonsior, G. Thiamethoxam. Thiamethoxam FS (A9807F) – A field study to investigate the effects of residues in guttation fluid on honeybees (Apis mellifera L.) in winter oil seed rape in Germany. Second interim report, 2015. Eurofins. Study code S14-04675. (Sponsor Syngenta, UK)


N23: Striffler, B., M. Weiss. Field monitoring to determine the occurrence of bees as well as occurrence and attractiveness of guttation water and honeydew to bees in onion fields in the Netherlands, sown with fipronil-treated seeds (formulation BAS 350 76 I= Mundial, fipronil 500 g/L). Final report, 2015a. Tier3solutions. GLP study no. P13089. (Sponsor BASF-SE, Germany)

N24: Striffler, B., M. Weiss. Field monitoring to determine the occurrence of bees as well as occurrence and attractiveness of guttation water and honeydew to bees in brassica fields in the Netherlands, sown with fipronil-treated seeds (formulation BAS 350 76 I= Mundial, fipronil 500 g/L). Final report, 2015b. Tier3solutions. GLP study no. P14003. (Sponsor BASF-SE, Germany)

N25: Dittbrenner, N. GF-2372 (sulfoxaflor): a field study to investigate the effects of residues in guttation fluid on honeybees (*Apis mellifera* L.) in treated summer oil seed rape in southern Germany. Interim report, 2017. Eurofins. EAS Study code S16-07085. (Sponsor Dow Agrosciences, UK)