Perspective

Received: 20 April 2010 Revised: 13 May 2010 Accepted: 19 May 2010 Published online in Wiley Online Library: 13 July 2010

(wileyonlinelibrary.com) DOI 10.1002/ps.1994

Risk assessment for honey bees and pesticides – recent developments and 'new issues'

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Abstract

In 2008, major areas of discussion at the ICPBR Bee Protection Group meeting were the development of a honey bee risk assessment scheme for systemic pesticides and revision of the test guidelines for semi-field and field studies. The risk assessment scheme for systemic pesticides is based on analysis of conditions for exposure of bees to residues. These are based on a stepwise approach, starting with simple calculations based on existing data in dossiers and progressing to higher-tier semi-field and field studies (the guidelines for these have been modified in line with this). The proposed scheme has been tested with data packages of high- and low-risk PPPs. A future area of interest for the group may be the risks posed by guttation fluid containing systemic pesticides. A recent paper on 'Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees' has focused significant interest on the possible risks posed by the presence of residues of systemic pesticides in guttation fluid to water-collecting honey bees. The occurrence of guttation and the presence of pesticide residues in the fluid are discussed, together with remaining questions that will need to be addressed in answering whether such a route of exposure may pose a risk to honey bees.

Keywords: risk assessment; honey bees; pesticides; guttation; systemic

1 INTRODUCTION

The International Commission for Plant Bee Relationships (ICPBR) Bee Protection Group has been the recognised European expert forum addressing risks of pesticides to bees for 30 years. The group meets formally every 2–3 years (the 10th meeting was held in Bucharest in 2008) and is open to representatives of academia, regulators and industry (there were over 80 delegates from 15 countries at the 2008 meeting). Apart from publication of the scientific papers presented at the meetings,[1–4] the primary outputs are reviews and revision, as appropriate, of EPPO honey bee testing guidelines and risk assessment schemes. This paper reviews the work of the Bee Protection Group in the last 3 years and highlights a recent topic that is likely to be considered at the next meeting of the Group.

In 2008, major areas of discussion at the meeting[4] were the development of a risk assessment scheme for systemic pesticides and revision of the test guidelines for semi-field and field studies. Both have recently been submitted to EPPO for consideration.[5,6]

2 RISK ASSESSMENT FOR SYSTEMIC PESTICIDES

Systemic pesticides are those applied as sprays, soil drenches, seed treatments or granules, which may result in residues in the growing plant. The application of such directed treatments has obvious environmental advantages over widespread spray applications. However, there is the potential for residues to be present in substrates attractive to honey bees, e.g. nectar, pollen or aphid honeydew. These types of pesticide have received increased publicity over the last 10 years, with linkage, without robust scientific evidence to date, to pollinator declines. However, over this period there has been no EU/EPPO guidance available on how to assess risks to bees that are posed by substances with systemic properties.

The ICPBR Bee Protection Group has developed a set of proposals[5] based on analysis of conditions for exposure of bees to residues. These are based on a stepwise approach, starting with simple calculations based on existing data in dossiers and progressing to higher-tier semi-field and field studies (the guidelines for these have been modified in line with this).[6] The proposed scheme has been tested with data packages of high- and low-risk PPPs, and full details are available in Alix *et al*.[5] and summarised here.

2.1 Potential exposure to systemic pesticides

The first stage of the scheme (Fig. 1) is identification of potential exposure. Plant protection products (pesticides) applied as seed coatings and soil applications (bare soil) are intended to concentrate the product in/on plant parts to be protected or where pests are most abundant. This approach potentially reduces exposure of most non-targets when compared with spray applications, but, if the product has systemic properties, then

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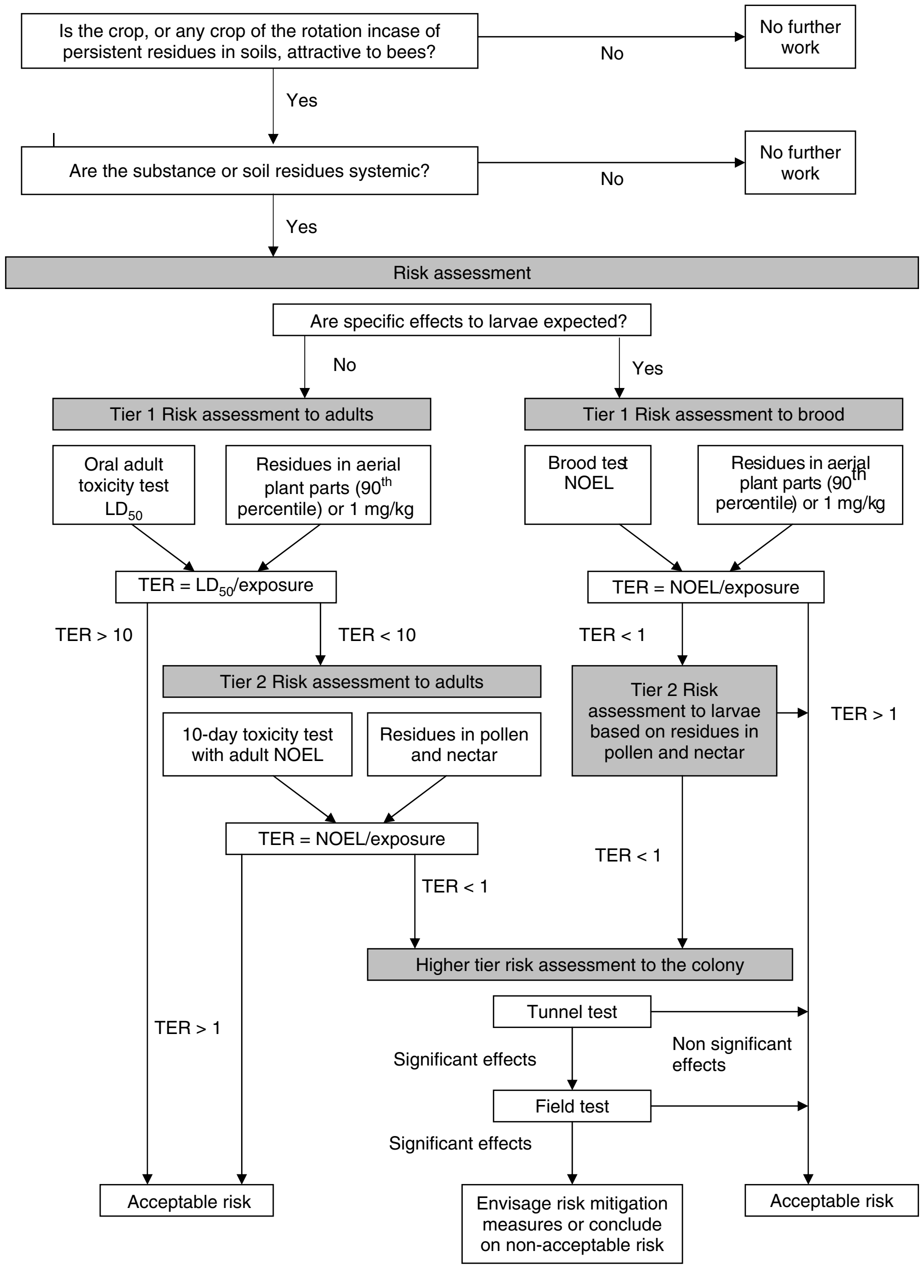

Figure 1. Stepwise approach to evaluate the risks to honey bees in the case of Plant Protection Products applied as a soil/seed/treatment. Note that it is possible to skip the Tier 2 and to move directly to a higher-tiered approach.

growing plants may contain residues, and exposure of bees may arise if significant amounts of residues reach nectar and/or pollen. Thus, if there is the potential for transfer to nectar or pollen and the plant is attractive to bees, or if the product is persistent (Dir 91/414/EEC identifies criteria to identify persistent substances for residue studies involving crop rotation) and plants used in the rotation are attractive, then exposure is likely to occur. Obviously, systemic uses where the crop is harvested before flowering are not of significant concern unless there are flowering weeds present.

In determining whether residues are likely to be present in nectar and pollen, information from residue studies and plant metabolism studies can be used to identify if the substance will be transferred to the plant during growth and major degradation products. However, there are few published data for systemic pesticides in pollen and nectar, and, in considering residue data from the registration dossier (generated for parts of plants intended for consumption), methods should be checked to ensure the LOQ is low enough to be relevant for risk assessment for honey bees.

Dossier data were compiled by AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (leaves, fruit, green part, inflorescence, whole plant, grain) from as close to flowering as possible. The majority of the 62 residue samples contained less than 1 mg AI kg^{-1} active substance (95th percentile 0.55 mg AI kg^{-1}). Similar levels of residues were observed for metabolites. In all cases where data were available, the residues in pollen and nectar were less than 0.1 mg AI kg^{-1}, suggesting, as expected, that translocation of pesticides to fruiting structures is measurably less effective than to other plant parts.

  Pest Manag Sci 2010; **66**: 1157–1162

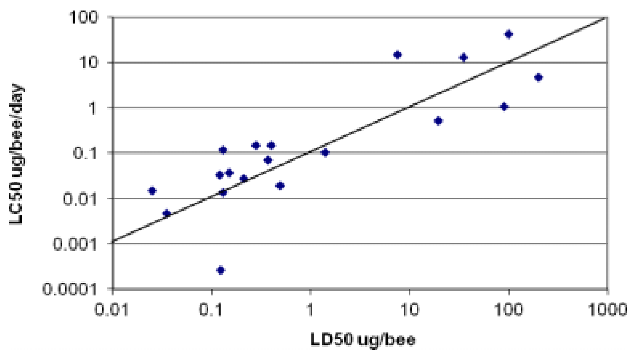

Figure 2. Correlation between 48 hr LD50 and 10-day LC50 data for a range of pesticides in honeybees (Thompson unpublished data).

2.2 Toxicity data

Toxicity to adult workers and brood is considered by the scheme (Fig. 1). Adult worker oral toxicity data for active ingredients are readily available in dossiers. Active ingredients are relevant, as it is these, rather than the formulations, that are likely to be present in pollen or nectar. In terms of toxicity, there are limited data available on the relative sensitivity of adult honey bees and larvae (brood), and therefore direct extrapolation from adult data is not possible. Insect growth regulators are the obvious case where active ingredients are more toxic to larvae owing to their mode of action. Although data for honey bee larvae are not routinely generated for registration, data are available from screening and efficacy studies for other larval stages of insects and should be used to identify if there are concerns about effects on larval stages. When there are such concerns, data for larvae should be generated, e.g. using Oomen *et al.*[7] or the OECD Guidance Document 75.[8] Until more data are generated to allow prediction of the relative sensitivity of different life stages, risk assessments for adults and brood should be considered separately.

2.3 Risk assessment

The proposed risk assessment scheme is discussed in detail in Alix *et al.*[5] and is shown in Fig. 1. In principle it follows the TER approach of comparing toxicity and exposure. The TER trigger value of 10 takes into account that exposure is chronic rather than acute and there is a tenfold difference between the acute oral LD_{50} for a pesticide and the chronic oral 10 day LC_{50} (Fig. 2). At later stages, increasing realism is introduced with semi-field and field studies which address both mortality and sublethal effects on adults and brood.

2.4 Assessment of the scheme

The final stage of the development of the proposed scheme was to assess the ability of the proposed risk assessment scheme to discriminate at an early stage between products that may need a refined assessment and those of low concern. Tier-1 calculations were performed for adults for all Annex 1 listed active ingredients with estimates of worst-case exposure of 1 mg AI kg^{-1} matrix (converted to ingestion rate for foraging bee 128 mg nectar bee^{-1} day^{-1}). This assessment showed that 15% of the 171 Annex 1 listed active ingredients failed at tier 1, and, of these, 24 were insecticides, one was a fungicide and one was a nematicide. The identities of the compounds that failed the trigger provide confidence of the ability of the scheme to highlight products of concern.

3 DUSTS – NOT A RISK ASSESSMENT ISSUE

The Group discussed concerns over dusts that had resulted in honey bee mortality in 2008 in Germany and France.[9–11] These mortalities had occurred following the drift of dust during drilling from poorly treated seed onto nearby flowering crops. The issues were less around risk assessment and more in ensuring that the dusts generated from seed treatments are minimised. Both Germany and France have instigated requirements for reduction of dust in bags of treated seed and ensuring vents from drilling equipment are directed towards the soil to minimise drift; these two modifications are estimated to reduce dust generated during drilling by 99%. These have recently been taken by DG SANCO, together with a requirement for post-registration monitoring of exposure of honey bees to neonicotinoid pesticides following use as seed treatments to ensure that the risks associated with their use are acceptable.

4 GUTTATION – A NEW ISSUE?

A recent paper on 'Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees' was published by Girolami *et al.*,[12] and this has focused significant interest on the possible risks posed by the presence of residues of systemic pesticides in guttation fluid to water-collecting honey bees. In response to concern from beekeepers in France, AFSSA recently published their opinion on the risks to bees by guttation[13] (extrafloral secretions), and a Swiss study on the residues of clothianidin present in guttation fluid from treated maize and the risks to bees has recently been published by the Federal Office of Agriculture.[14]

In considering the risk posed by guttation in plants containing systemic pesticides to bees, there are a number of questions to be addressed.

4.1 What is guttation?

Guttation is a well-described phenomenon (it was first reported by Abraham Munting in 1672), not to be confused with dew or condensation, in which liquid water is released from leaves through water pores (hydathodes) situated at the edges of leaves or from vein endings in the leaf. Guttation occurs in response to increased root pressure and decreased transpiration[15] with diurnal periodicity; it occurs to a greater extent at night and in the early hours after sunrise. Many reports of guttation in the literature are related to its role in the development of disease through the invasion of the hydathodes of leaves by spores and bacteria[16] and the ability of pesticides, particularly fungicides, to act systemically to control these diseases.

4.2 Which crops exhibit guttation?

Although a number of authors have compiled lists of plants observed to guttate, it is now considered a general phenomenon. In fact it has been suggested as an indicator of irrigation requirements in strawberries.[17] In discussing the production of recombinant proteins through collection of guttation fluid, Komarnytsky *et al.*[18] cited species known for their high levels of guttation, such as tomato and monocotyledons, particularly grasses. On waxy leaves such as mustard, cucumber, cabbage, sugarbeet and most cereals and grasses, droplets remain beaded. However, care must be taken in deciding whether guttation has occurred in some crops, such as tobacco, potato, lettuce and beans, as the entire surface of the leaf exudes the fluid. In the absence of a waxy cuticle, this may result in the spreading of the fluid over the surface of the leaf, and in some cases in the pooling of water at the base of leaves and within the convolutions of the leaf.[19,20] There may also be differences between cultivars of the same species. Singh *et al.*[21] demonstrated that rice cultivars with greater panicle weights demonstrated greater guttation rates and suggested that this was an evolutionary strategy for improving water balance and delivery of inorganic solutes to the panicles. The leaves exhibiting guttation also vary with plant species. In strawberries it has been reported on expanding young leaves but not consistently on the older leaves, and this difference was thought to be due to changes in the water pores or loss of vascular function. In wheat, plugging of water pores occurred in older leaves owing to the condensation of the exudate. However, Komarnytsky *et al.*[18] suggest that the change in the leaf from sink to source tissue may also be responsible for this observation. The AFSSA review of the risks to honey bees of guttation fluid generated in maize suggests that, under glasshouse conditions, the peak production is from the first unfolded leaf stage (BBCH 11) to the 6–7-leaf stage (BBCH 18).[13]

4.3 What environmental conditions favour guttation?

Dutrochet (1867, cited by Eaton[22]) was the first to ascribe osmosis as the cause of exudation (guttation) and Wiler (1892, cited by Eaton[22]) showed the positive effect of warming roots. Guttation is particularly prevalent in conditions of high soil moisture and low transpiration, although there may be other factors involved, such as previous levels of water stress, growth stage, root depth and soil water potential.[17] Cool mornings followed by warm days provide excellent conditions for guttation because in warm soils absorption is very active and at the same time, with relatively high humidity, transpiration is reduced almost to zero at night. In regions where, particularly in early spring and late autumn, the daytime temperatures are high and nights rather cool, such as mountain valleys and irrigated desert areas, plants have been observed to guttate frequently and copiously. In the greenhouse situation, guttation is readily induced in many plant species by keeping the soil temperature high (25–32 °C), the relative humidity of the air close to 100% and the soil moisture abundant. Light intensity also plays a role; the guttation of maize coleoptiles increased when exposed to light, guttation reaching a maximum about 2 h after the start of illumination.[20] Other factors reported as affecting the scale of guttation were previous levels of water stress, root depth and soil water potential distribution within the root zone.

4.4 What volume may be exuded and for how long?

Hughes and Brimblecombe[19] assessed the average total volume exuded per grass blade at sunrise as 1.73 µL, with larger droplets at sunrise and smaller droplets towards sunset. They concluded that the same conditions required for dew formation (the dew point of air) also encourage guttation, and the latter is significantly correlated with soil temperature and moisture. From this they developed an equation to calculate the guttation droplet diameter at sunrise for grasses. However, interactions between temperature and soil moisture are likely to vary with plant species and soil type and therefore are of limited value.

Hughes and Brimblecombe[19] also reported that more than one droplet may be exuded per night, although only one would be present on the leaf tip, with others falling onto the grass beneath or rolling down the grass blade. Collection of all the exuded droplets accounted for a mean total of 9.9 µL $leaf^{-1}$ $night^{-1}$ (including the drop present at the tip of the leaf). From this they calculated that, for a grass blade density of 10 464 blades m^{-2}, the average total guttation volume was equivalent to 0.1 mm of precipitation. As the same conditions of high air humidity resulting in dew were reported as being suitable for guttation (a typical night comprised a precipitation equivalent of 0.14 mm dewfall and 0.1 mm guttation), it is interesting to note that the authors considered that the dewfall in this study in the UK was comparable with those reported elsewhere in Europe. In a similar manner, Williams *et al.*[23] also showed that guttation accounted for 33% of total dew (0.195 mm at 08.00 h) on creeping bentgrass maintained as a golf fairway. In southern England the authors calculated that 7–10% of the mean daily net radiation would be required to evaporate the combined dew- and guttation-derived wetness between June and August, with 50% required by October.

4.5 Is guttation fluid likely to be attractive to bees?

Although the liquid exuded during guttation originates from the xylem sap, the concentration of ions, sugars and other molecules may decrease owing to their removal by the upper parts of the plant, in particular the leaves.[15] Goatley and Lewis[16] determined the composition of guttation fluid in rye, wheat and barley seedlings. They showed that the sugar contents in rye and barley were similar, but that the sugar content in wheat was lower than in the other two species. Most of the sugar in barley was glucose (38.7 mg L^{-1}), whereas in rye it comprised glucose (18.7 mg L^{-1}), fructose (10.3 mg L^{-1}) and galactose (10.3 mg L^{-1}). These levels of sugars are unlikely to be attractive to honey bees as a nectar source; Seeley[24] identified 15% sugar as the threshold of interest to nectar-foraging bees.

4.6 What levels of pesticide residues may be present in guttation fluid?

There are a number of factors affecting the residues in guttation fluid, e.g. formulation, metabolism within the plant, application methods, adjuvant, solubility/lipophilicity of the active ingredient and plant species. Examples also include the chemistry of the active ingredient, including a negative correlation between lipophilicity and residues in guttation fluid[25] and selective expression of solutes such as ions.[26,27] Metabolism within the plant also affects residues levels; the systemic herbicides dichlobenil and vernolate are extensively metabolised in plants, and dichlobenil has a high affinity for plant tissue.[27]

Formulation effects on levels of residues for sprayed systemic pesticides are presumed to be due to enhanced penetration of the cuticle in wheat, barley, tomato and grapevine.[25,28] However, differing formulations of soil-applied fungicides can also affect residues. Bickers *et al.*[29] showed that, for tebuconazole and cymoxanil in barley (eight-day-old seedlings) and tomato (14-day-old potted seedlings), there was a significant difference in uptake from slow release formulations compared with wettable powder formulations, with further differences between the active ingredients ascribed to lipophilicity.

Stoller[30] observed differences between plant species in the translocation of pesticides to leaves and then to guttation fluid, depending on the metabolism in the plant. Using Amiben (3-amino-2,5-dichlorobenzoic acid, a herbicide widely used at that time in soybean), which is more effective as a soil-applied than as a foliar herbicide, he showed that 13 species (monocotyledons and dicotyledons) exhibited varying levels of parent compound

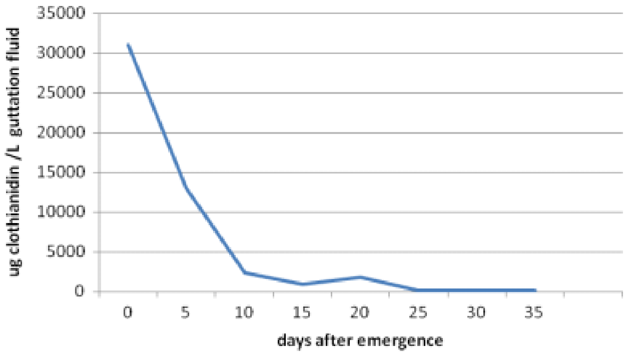

Figure 3. Concentration of clothianidin reported in guttation fluid collected from newly emerged maize plants from seed treated with 0.5 mg ai/seed (mean of 2 studies). Data from Swiss Federal Government for Agriculture14.

in guttation fluid from 1.4% (wheat) to 74% [barnyard grass, *Echinochloa crus-galli* (L.) Beauv.].

Girolami *et al.*[12] undertook laboratory studies with honey bees, in which they fed guttation fluid from treated maize to honey bees in the laboratory. The maize seeds were treated with imidacloprid (0.5 mg Gaucho 350 per seed), clothianidin (1.25 mg Poncho per seed), thiamethoxam (1 mg Cruiser FS per seed) or fipronil (1 mg Regent FS per seed) and grown in open field conditions and in the greenhouse. In the field, guttation droplets were collected at 08.00–09.00 h each morning for the first 3 weeks after emergence (after which guttation decreased), and here 1–3 mL of fluid could be collected from 100 plants (in the greenhouse, 30–150 µL plant^{-1} day^{-1} was collected). The residues in the guttation fluid from plants grown from treated seed were 47 ± 9.96 mg imidacloprid L^{-1}, 23.3 ± 4.2 mg clothianidin L^{-1} and 11.9 ± 3.32 mg thiamethoxam L^{-1}; no fipronil was detected. Therefore, the levels in guttation fluid were 254 times the LD_{50} for imidacloprid, 280 times the LD_{50} for clothianidin and 48 times the LD_{50} for thiamethoxam.

The Swiss Federal Government for Agriculture[14] commissioned a study in 2009 to assess the risks to honey bee colonies during sowing of maize seed treated with clothianidin (Poncho; 25 g AI per 50 000 seeds, i.e. 0.5 mg AI seed^{-1}) through drift of dust and guttation. No effects due to dust drift were observed. Guttation fluid collected from maize after emergence (7–10 days after sowing) was reported to contain 25–37 mg clothianidin L^{-1}, decreasing to around 0.1 mg L^{-1} by 40 days after sowing (as above, the LD_{50} for clothianidin is around 0.084 mg L^{-1}) (Fig. 3).

4.7 What affects the risks to bees?

Owing to the recent interest in guttation fluid as a hazard for honey bees, there have only been limited data published on risks posed to non-target arthropods. Only one study[12] showed a significant effect in honey bees, but this should be treated with caution as the data were generated by feeding collected droplets directly to bees, and in many cases sucrose was added to ensure the honey bees consumed the dose. What is less clear is whether honey bees use guttation fluid on crops as a significant source of water. There have been no published reports to date of honey bees collecting guttation droplets from crops.

Guttation fluid is unlikely to be identified by honey bees as a source of sugar in view of the low levels present. Bees are less subject to dessication than most terrestrial insects because of their nectar diet and high metabolic water production. Water is collected by bees to dilute thickened honey, to produce brood food from stored pollen, to maintain humidity within the hive and to maintain temperature within the brood area. Water is not stored in combs by temperate bee colonies. The amount of water required depends on the outside air temperature and humidity, the strength of the colony and the amount of brood present. Water-carrier bees carry water in the honey stomach and can carry 40–50 µL in this way.[31] Vissher *et al*. (1996), cited by Nicolson,[31] reported mean water loads of 44 mg in honey bees collecting water under desert conditions. When groups of honey bees were confined to a cage at 35–40 °C and provided with a 67% sugar solution, they consumed about 10 µL of water per bee per day. Seeley[24] estimated average annual requirements of 25 L for a single wild colony in cold temperate conditions. Shawki *et al.*[32] reported that bees generally collect water up to 50 m from the hive, suggesting that local sources tend to be used when available. Usual sources of water identified for honey bees are those that are moderately mineralised and include rainwater and puddles, and it has been suggested that the presence of decomposing plant material generates olfactory stimuli attractive to foragers. In the Swiss study[14] discussed above, no increased mortality was identified in honey bee colonies placed at the edges of the treated fields; the colonies developed normally, and no clothianidin residues were detected in the honey bees or in honey sampled from the colonies, suggesting guttation fluid was not collected from the treated crop.

There are a number of gaps in current knowledge, which limit the ability to undertake an assessment of the risks posed by guttation fluid containing pesticide residues to honey bees, and these are likely to be considered at the next ICPBR meeting in determining whether pesticides present in guttation fluid should be considered as a potential risk to bees:

1. Do honey bees use guttation fluid as a source of water in relevant crop species?
2. Can residues be extrapolated between crops for representative formulations with active ingredients of varying physicochemical characteristics?
3. What are the actual residues in a range of representative crops, and what effects do field conditions (e.g. varying soil types) have on the profile of residues present in guttation fluid?
4. Does the drying of guttation fluid on the surface of leaves result in significant residues of systemic pesticides and thus exposure of honey bees resting on leaf surfaces?

ACKNOWLEDGEMENTS

The author would like to thank all members of the ICPBR Bee Protection Group for their input into the proposed risk assessment scheme and test guidelines, particularly Gavin Lewis, Anne Alix, Mike Coulson and Pieter Oomen. This work was supported by Defra and the UK Chemicals Regulation Directorate under projects PS2335 and PS2361.

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