Exposure of honey bees and other pollinating species to pesticides

Anne Alix, Mark J. Miles*

Dow AgroSciences, Abingdon, UK

*Corresponding author Email: mjmiles@dow.com

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Abstract

Background: When considering the risk to bees a thorough understanding of the relevant routes of exposure and the magnitude of exposure is necessary.

Results: Bees forage on plants and in particular flowers to obtain food for themselves and for provisioning their young. Foliar applications during flowering will present the most extreme acute exposure situation. Bees can be exposed to direct spray and also to contaminated pollen and nectar taken back to the colony. Spray applications before flowering may lead to exposure in pollen and nectar if the substance has systemic properties and is persistent. For soil/seed treatments exposure may occur in for systemic products due to translocation from the seed or soil to the upper parts of the plant (e.g. nectar and pollen). Other possible routes for soil/seed treatments include dust-off at sowing and guttation water.

Conclusion: Risk assessment requires that relevant routes of exposure for worker bees, hive bees and young should be considered in the risk assessment for both foliar applied and seed/soil treatment pesticides. The availability of exposure models would assist in the development of honey bee and pollinator risk assessment schemes.

Keywords: honey bee, pesticide, risk assessment, exposure

1. Introduction

Plant Protection Products or PPPs (also called pesticides) are part of modern crop management practices. Prior to the placement of PPP on the market and their use an evaluation of the risks posed to the environment is mandatory worldwide requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species.

Guidance already exists and has been available for many years, which aims at characterizing the potential effects of PPPs on honey bees with the corresponding need for an assessment of risk[1,2,3]. General data requirements, risk assessment procedures for spray applied products and special considerations for products which are systemic or are larvicidal or have Insect Growth Regulator (IGR) type properties have been recognized for many years[4,5].

If in practice exposure to honey bees can occur both the hazard (toxicity) of the compound and also the potential exposure to the organism is then considered. The risk assessment usually follows a tiered approach whereby products of low toxicity and low risk are rapidly excluded; whereas products with a potential to harm honey bees are progressed to higher and more realistic tiers of evaluation. Consequently, it is usually not necessary to generate extensive and elaborate measures of toxicity and exposure in tier I risk assessments.

Exposure estimates and/or measurements need to reflect the potential route of exposure and also the level and extent of exposure for the test organism. It is not always necessary or desirable to consider all potential routes of exposure, as long as the needs for the risk assessment can be met. For example, the exposure of birds and other terrestrial vertebrates is considered to be primarily via the consumption of a dose in the animal's diet[6,7] even though other routes (e.g. dermal absorption, inhalation) are also theoretically possible. Even though there are limitations on how exposure is expressed and/or calculated a robust risk assessment is achieved at all tiers.

Bees obtain their requirements by foraging on plants and in particular from flowers to obtain food for them and for provisioning young. Nectar and other sugar sources (e.g. extra floral nectaries and aphid honey dew) are used as an energy source whereas pollen is an important protein source essential for developing young. During these activities they also provide valuable pollination services to plants.

Bees require water, and some species will also forage for nesting material (e.g. leaf cutter bees). In addition to exposure by spray applications bees and the colony can be exposed to collected and processed materials containing pesticide residues stored in the hive. Figures 1 to 4 illustrate the interaction of bees within the crop and off-crop area and the majority of scenarios for which bees can be exposed to applications of PPPs.

Tab. 1 Estimated level of residues in different matrices after the application of Plant Protection Products as spray applications

Time of application	Location of residues	Expected exposure to residues	Expected level of residues	Remarks
Pre-emergence	Soil and water from puddles formed on the soil surface following heavy rainfall close to application	Negligible to soil Dependant on puddle formation	1 kg/ha /year could result in a PEC puddle of 1.38µg/L	The occurrence of puddles depends on heavy rainfall event and soil structure. Covered by the risk assessment based on HQ calculation as an exposure to direct spray
Before flowering	Plant surface	Negligible, as not attractive to bees	Estimated through the application rate (from g/ha to kg /ha)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
	Guttation droplets (for systemic compounds)	Expected to be negligible due to the low attractiveness of growth stages	Peak concentrations observed in the first droplets (100-500 mg/L) down to <0.001 mg/L later on, after sprayed treatments.	The occurrence of guttation droplets depends upon systemic properties, soil and air humidity. Should be covered by the risk assessment performed for sprayed solutions
Flowering	Pollen, nectar	Importance as function of the attractiveness	Concentration may be estimated with the concentration in spray solution as a worst case. Measures range from 1.5 to 2000 µg/kg pollen (or 340 µg/kg).	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
After flowering	Plant surface	Negligible, as not attractive to bees	Estimated through the application rate (from g to kg /ha)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
All	Off crop non flowering vegetation and/or non flowering crop receiving spray drift	Negligible, as not attractive to bees	Estimated drift rate as a % of application rate (2.77% to 29.2% at 1-3 meters, pending upon the spraying technique)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
	Off crop flowering vegetation and/or flowering crop receiving spray drift	Importance as function of the attractiveness	Concentration may be estimated with the concentration in spray solution adjusted by the drift, as a worst case. Measures range from 1.5 to 2000 µg/kg pollen (or 340µg/kg).	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed

2. Exposure scenarios for honey bees and other pollinators

It is first necessary to consider if during the course of the use of a PPP bees will be exposed by considering the details of the product and its pattern of use. In some cases exposure of bees is not possible. For example, winter applications when bees are not flying, pre-emergence use of herbicides, wound treatments, rodenticide baits, indoor uses, use in glasshouses (where pollinators are not used) seed treatments and granules (except where there is systemic activity) and products for dipping bulbs etc. are likely to lead to negligible exposure to bees and in such cases a risk assessment is not required. A second consideration is the attractiveness of the crop plant. If the crop is not attractive to bees then again exposure will be minimal. However, other factors need to be considered such as the presence of other food sources in the treated area (e.g. flowering weeds, aphid honey dew). In general a crop is not attractive to bees when harvested before flowering, however, some crops intrinsically unattractive to bees may also be visited due to extra-floral nectaries (e.g. field beans, cotton).

These initial steps are considered in current risk assessment schemes. Under the recently published EPPO guidance separate pathways on the decision making tree are presented to cover the differences in exposure from sprayed and soil applied products. Likewise, this is covered in the US by the problem formulation stage. However, it is possible to summarize the characteristics of standard scenarios to describe the potential routes of exposure for honey bees and other pollinators. Key factors are the method of application (spray or soil/seed treatment) and whether the product contains an active substance toxic to bees with systemic activity. Tables 1 and 2 list the various scenarios where bees and other pollinators can be exposed and also gives an indication of possible residues levels present in matrices of relevance to bees[8,9,10,11,12]. Further explanations of the exposure scenarios due to spray applications and soil/seed treatment uses are given in the following sections.

2.1 Exposure to spray applications

The timing of spray applications is critical when considering the exposure of bees. Depending on the use pattern of the product applications can be made to bare soil, young seedlings, before flower, at flower or post flowering (Figures 1 to 3). Spray applications at or close to flowering pose the greatest likelihood of acute exposure for bees. This can be via direct sprays or to residues on plants, flowers and possibly in nectar and pollen. The properties of the molecule (chemical stability, presence of residues, breakdown into metabolites and mobility in plants) can also influence the route and duration of exposure.

2.1.1 Exposure to direct spray and residues on plants and flowers

Foliar applications during flowering typically lead to exposure which may be considered for the complete duration of the flowering period as a worse case. For example many tree crops which rely on pollination typically flower for a period of 2 weeks. Foragers may be exposed to direct spray and also to residues in/on plants and flowers (Figure 1). By spraying when bees are not actively foraging it is possible to reduce exposure and limit it to aged plant residues. Exposure is expected to be at its highest level at or shortly after spray and decline thereafter. Exposure will decline over time due to ageing, growth dilution and also due to visits to flowers not present or open on the day of application. Exposure in this context can be simply expressed in terms of the application rate i.e. g a.s./ha (grams of active substance per hectare).

Applications made out of the flowering period of the crop or to a crop which does not flower during the growing season significantly reduces the exposure to bees as they tend to work areas where there are adequate food sources available. However; exposure may occur on flowering weeds within the cropped (or in-field) area and due to drift to off-field areas with flowering plants such as hedgerows (Figures 2 and 3). In the case of the in-field area good weed control by the application of an herbicide or by mechanical means can be a suitable risk management measure unless this conflicts with local biodiversity objectives. For the off-field exposure to flowering plants or adjacent flowering crops, exposure can be expressed as g a.s./ha and adjusted by a suitable validated drift factor as a percentage of the field application rate.

Tab. 2 Estimated level of residues in different matrices after the application of Plant Protection Products seed coating or trunk injection

Application of the PPP	Time of application	Location of residues	Expected exposure to residues	Expected level of residues	Remarks
Seed coating	Drilling	Off crop and/or flowering crop receiving dusts, if pneumatic drillers with air pressure	Importance as function of the attractiveness	From 0.004 to 0.44 g/ha without mitigation measures to 0.002 to 0.12 g/ha when mitigation are implemented.	Emission and dispersion of dusts are very variable and specific drift values cannot be defined. Dusts should be reduced to a minimum in order to limit environmental exposure. Drift values after risk mitigation should be used in the risk assessment.
		Nectar, pollen if systemic at crop flowering	Importance as function of the attractiveness	Measures range from 1 to 6 µg/kg). A default value of 1 mg/kg is used as a tier 1 in the risk assessment scheme, based on residues measures in whole plants (EPPO, 2010).	Risk assessment scheme developed in EPPO, 2010.
		Guttation droplets if systemic	Expected to be negligible due to the low attractiveness of growth stages	Peak concentrations in the first droplets (100-500 mg/L) down to < 0,001 mg/L later on (variable pending upon crop, systemic properties and growth stage).	The occurrence of guttation droplets depends upon systemic properties, soil and air humidity. Should be covered by the risk assessment performed for sprayed solutions as an exposure to direct spray is assumed
Trunk injection	Pre flowering, flowering	Pollen, nectar	Importance as function of the attractiveness	Concentration may reach 300 µg/kg in some trees	Concentrations are based on efficient concentration in trees to control pests. Concentrations depend on the distance to injection point. Could be covered by a risk assessment considering overspray at flowering.

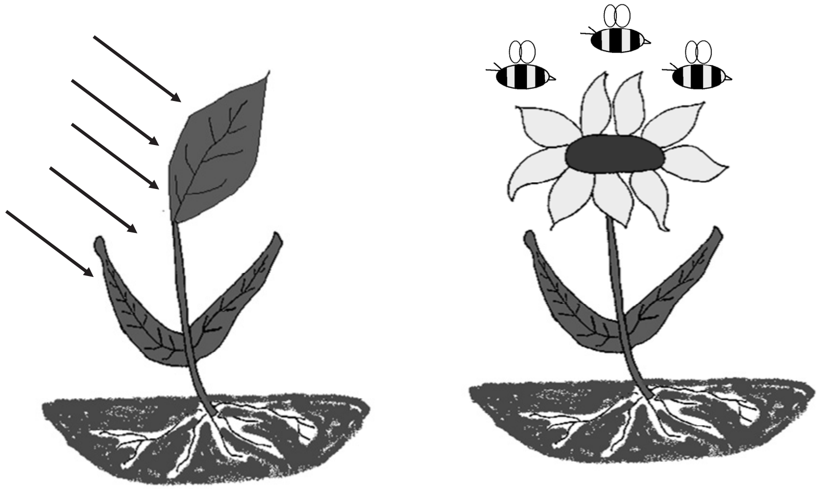

Fig. 1 Foliar spray applications before flowering. Bees are not expected to be exposed at application. However there could be a potential for exposure to residues on foliage and movement into flowers, pollen, and nectar if the plant protection product has systemic properties and is persistent. Diagonal arrows indicate spray application.

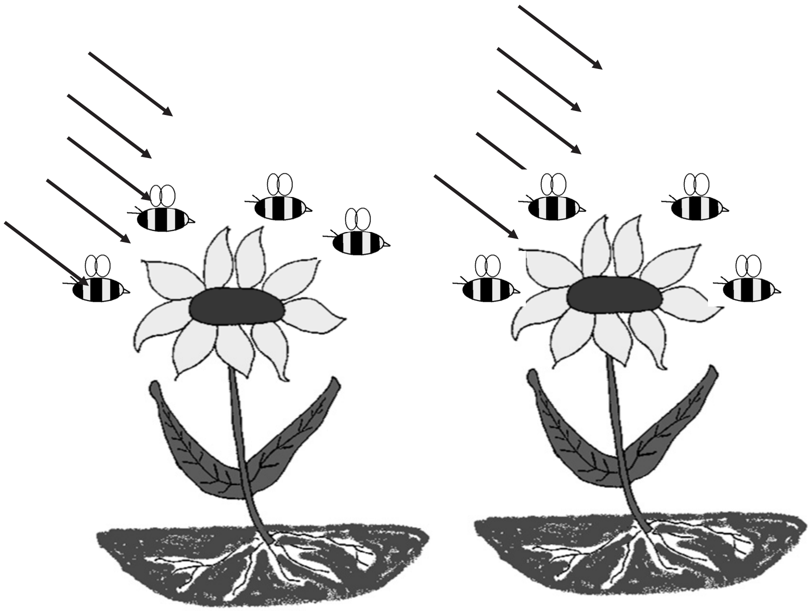

Fig. 2 Foliar spray applications during flowering. Day time applications may lead to direct spraying of bees, exposure to residues and direct contamination of pollen/nectar/flowers. If the plant protection product has systemic properties and is persistent there is the potential for movement into flowers, pollen and nectar. Applications made out of bee flight may reduce exposure. Diagonal arrows indicate spray application.

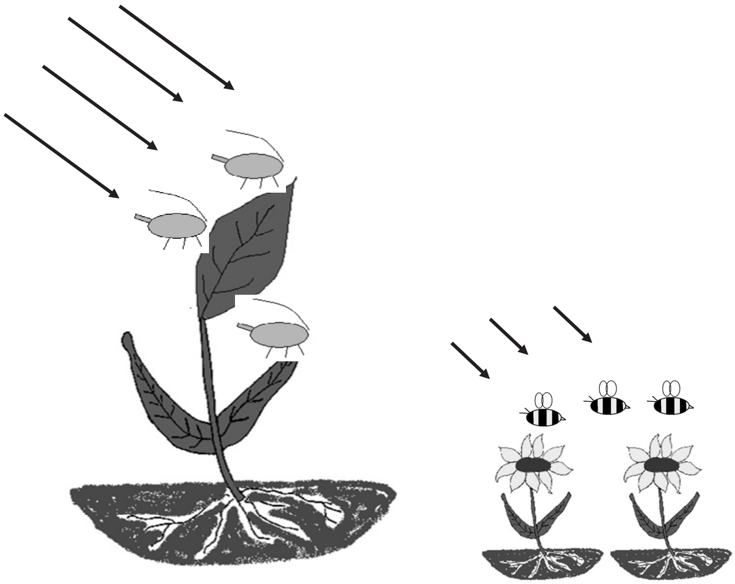

Fig. 3 Foliar spray applications after flowering or to non-flowering crop which are not highly attractive to bees. There is a potential for exposure via aphid honeydew, however if the plant protection product is an insecticide aphids may not be present. Off-crop exposure due to drift on adjacent flowering crops and off-field areas may be possible. Long diagonal arrows indicate spray application, short arrows drift.

2.1.2 Exposure to residues in nectar and pollen due to spray applications

Contamination of nectar and pollen by pesticide spray applications is possible when applications are made during flowering. The degree of exposure can depend on the architecture of the flower as open flowers (such as winter oilseed rape, apple blossom) would expect to receive a higher exposure compared to some other flowers that receive less exposure (e.g. wild blueberry flowers which hang upside down). Contamination of pollen is far more likely than that of nectar by spray applications to open flowers. In relation to exposure to foragers this is covered by exposure to direct spray and residues on plants and flowers in section 2.1.1 above. Due to the location of nectaries in many flowers, residues are likely to be low to negligible arising from spray applications, especially when compared to flowers and pollen which may receive direct spray.

Generally, applications outside of the flowering period would not lead to exposure to bees through nectar and pollen. However, in the case of products with systemic activity, pre-flowering spray applications may lead to substances and/or metabolites being present in pollen and nectar. This is because the systemic properties of molecule may allow for the material to be absorbed by the plant and translocated into flower parts (Figure 1). These residues are also subject to degradation over time, by plant metabolism, diluted by plant growth and movement within the plant, as they are not expected to concentrate in pollen or nectar[13]. Overall, the levels of residue found in pollen and nectar due to spray applications of systemic products pre-flowering will be considerably lower than those arising from direct applications to flowering crops. In the case of compounds persistent in soil, applications may lead to residues in pollen and nectar in following flowering crops. It should be possible to exclude exposure by demonstrating the absence of residues in pollen in nectar following a spray application of a systemic product.

2.2 Exposure to non-sprayed products (soil and seed treatment applications)

The exposure of bees to residues of a product applied as a soil/seed treatment may occur in the case of residue transfer (either the active substance or a degradation product) from the seed or soil to the upper parts of the plant and in particular in matrices of interest to bees (pollen, nectar and honey dew) if the crop is visited by bees. Although the risk to bees due to these types of products has been recognized for many years, it is only recently that a formal risk assessment procedure has been documented and validated[2,14].

2.2.1 Exposure to systemic products: flowering crops

Seed/soil treatment products are used in a wide range of crops. Some of these have systemic properties providing protection from pests and soil borne diseases. Flowering crops such as oilseed rape, sunflowers and many others may be treated with insecticides with systemic activity and provide targeted protection from many sap feeding pests. Residues may also transfer to pollen and nectar and also into aphid honeydew. Bees foraging on these plants can be exposed to nectar and pollen concentrations and if these are not present at lethal levels, these can be taken back to the colony. In the case of honeydew, it is clear than concentrations which do not affect aphids are unlikely also to affect bees. Some plants such as maize do not produce nectar but do produce a plentiful supply of pollen which is utilized and stored by honey bees (Figure 4).

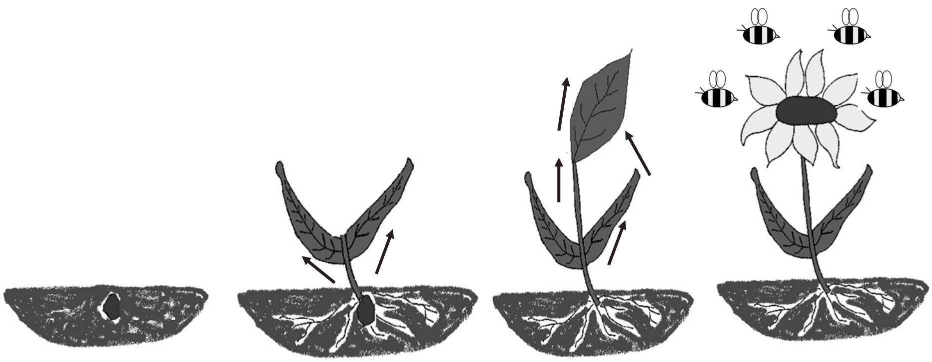

Fig. 4 Exposure to seed and soil treatment. Bees are not expected to be exposed at application (excepted in the case of abraded dust). However there could be a potential for exposure to residues in foliage, pollen, and nectar if the plant protection product has systemic properties and is persistent. At early stages of plant growth guttation fluid may be produced under certain circumstances. Upward arrows indicate movement of plant protection product within the plant.

2.2.2 Exposure to systemic products: non-flowering crops

Seed/soil treatment products are also used to protect crop plants which do not flower before harvest (e.g. many *Brassica* crops) or are unattractive to bees. In these cases the exposure for bees is much less and could be considered negligible. In these cases an assessment of risk may be necessary for a following flowering crop. In the case of systemic compounds which are persistent in soil and these residues remain in the soil to the following season they may transfer into the flowing crop. If this crop is attractive to bees then exposure may occur even if no systemic products are used during the growing season of the following flowering crop.

2.2.3 Exposure due to abraded dust at sowing

Exposure of honey bees may also occur through routes such as contact with or the consumption of dust originating from seed coated with PPPs. An example of this is the case that occurred in Germany in 2008 following the sowing of seeds coated with insecticides[15,16]. It was shown that under certain circumstances the emission of dusts at sowing may be possible resulting in bee kills. The relevance of this exposure route to bees is still subject to further consideration and work. It may be possible to reduce dust emission and dispersion through appropriate risk mitigation measures such as quality coating, equipment of sowing machines with dust drift reducing devices (deflectors) and recommendations with regard to weather (wind) conditions at sowing. The level of dust emitted and dispersed may significantly vary pending on coating and sowing conditions so that many countries have implemented strict risk management measures that will develop into good practices in this area. In France for example, an ordinance has been adopted that defines conditions for coating and drilling corresponding to the good agricultural practice, which will be the basis for further risk assessments [17].

2.2.4 Exposure due to guttation fluid

Exposure of bees may also occur due to the consumption of guttation droplets. The movement of systemic products from the treated seed/soil into the plant may result in the presence of active substance or degradation products in guttation fluid which could be used as a water source by bees. Information on the conditions and relevance of exposure are still needed for a proper risk assessment, in particular with regard to the conditions of occurrence of droplets and the concentrations of PPP in the fluid.

2.3 Routes of exposure for honey bees and wild bees

The characteristics of standard scenarios to be considered when evaluating the potential routes of exposure for honey bees and other pollinators from PPP's, are described in Tables 3 and 4. These tables present the likelihood and anticipated levels of for honey bees relative to wild bees. These differences may be important when considering the exposure of various pollinators. For a more detailed account on this matter see the publication of the OPERA bee working group[18].

Tab. 3 The relative importance of exposure of honey bees and wild bees via various exposure routes of plant protection products as spray applications.

	Honey bees		Wild bees	
Exposure	**Adult**	**Larvae**	**Adults**	**Larvae**
Direct spray	+++	-	+++	-
Spray drift	++	-	++	++
Floral residues	+++ to +	-	+++ to +	-
Nectar	- to ++	+	- to ++	+
Pollen	+ to +++	++	+ to +++	++ to +++
Foliar Residues	+	-	+ to +++	- to +++
Water	+ to ++	+	+	+
Nesting Material	+	+	+ to +++	+ to +++
Exposure to Soil	-	-	- to +++	- to +++

Tab. 4 The relative importance of exposure of honey bees and wild bees via various exposure routes of plant protection products as seed coatings, trunk injections and soil drench applications.

	Honey bees		Wild bees	
Exposure	**Adult**	**Larvae**	**Adult**	**Larvae**
Dust (off-field)	+ to +++	+	+ to +++	++
Nectar	to ++	+	to ++	+
Pollen	+ to +++	++	+ to +++	++ to +++
Foliar residues	+	-	+	+
Guttation water	+	+	+	+
Exposure to soil	to +	-	- to +	- to +

2.4 Conclusions on exposure due to spray applications and non-sprayed products

Exposure for bees and other pollinators is related to flowering as bees forage to collect food resources and due to the attractiveness of the crop. Bees can be exposed to PPPs by both spray and non-sprayed applied products. For spray applications is it typical to express exposure in terms of the application rate (g a.s./ha).

For spray applied products, treatment outside of flowering (or to crops which will not flower before harvest) will limit exposure. Honey dew may attract bees to non-flowering and unattractive crop plants and there is a risk of overspray. Likewise the presence of flowering weeds may make an area attractive. To assist with these issues it would be useful to develop a list of crop plants which are not attractive or are less attractive to bees (e.g. sugar beet, potatoes). Recent work undertaken by a working group of the French Agency on the Safety of Food (AFSSA Draft working document – Guidelines Related to Setting Maximum Residue Limits in Honey – EC Guidance document Part C4) with the aim to provide a guidance document for defining Maximum Residue Limit (MRL) for PPPs in honey has proposed a list of the melliferous plants being attractive to bees based on the presence or absence of nectar and honeydew. However, this list does not include plants such as maize, which may be attractive to bees and hence be considered in the risk assessment even if they do not produce nectar. Some recommendations on the factors to consider in assessing the level of attractiveness of a crop are also proposed, such as the presence in the foraging area of other sources of nectar/honeydew of higher/lower level of attractiveness that may influence the behaviour of bees towards the crop of interest. Similarly, the presence of bee-attractive flowering weeds or of 'secondary' crops in a non attractive crop may favour visits and lead to some exposure. A description of agricultural practices associated to the crop of concern may help in deciding if visits and exposure are expected or not.

Foliar applications of systemic products at pre-flowering crop growth stages may lead to exposure during flowering due to the transfer of residues from the foliage to flower parts in pollen and nectar. However, it are spray applications made during flowering which pose the highest potential for exposure to bees. For crops which are attractive to bees, foragers can be exposed to direct overspray, dry residues on flowers and also to contaminated pollen and nectar. Pollen and nectar can be taken to the hive if the application is not lethal to forager bees and if there is concern a risk assessment for larvae and/or brood may be triggered.

The exposure of bees to residues of a product applied as a soil/seed treatment may occur in the case of residue transfer from the seed or soil to the upper parts of the plant and in particular matrices of interest to bees (pollen, nectar and honey dew) if the crop is visited by bees. These residues in such bee relevant matrices may potentially lead to forager and colony exposure with the exposure express as mg a.s./kg. A review conducted in 2009 of residue data measured in all types of plant parts (leaves, fruit, green part, inflorescence, whole plant, grain) taken close to flowering and where available residues in nectar and pollen, showed that the majority of samples have less than 1 mg a.s. per kg matrix (95th percentile = 0.55 mg/kg, n = 62)[19]. Considering only the residues measured in pollen and nectar residues did not reach more than 0.1 mg a.s./kg. However, this dataset is limited in scope and in the absence of actual measured data it is a recommended to apply a generic worst case value of 1 mg a.s./kg to represent residues due to soil and seed treatments in plants. Under this scenario pollen and nectar can be taken to the hive if the residue is not lethal to forager bees. If there is concern a risk assessment for larvae and/or brood may be triggered.

3. Linking exposure to risk

A risk assessment scheme for sprayed products (both non-systemic and systemic pesticides applied directly to bee-attractive crops), has been in place for nearly 20 years whereas that for systemic pesticides applied as granules, seed treatments and soil drenches or as pre-flowering applications has only recently been developed[2]. An appropriate risk assessment for bees relies on the combination of suitable exposure and toxicity measurements which is beyond the scope of this paper.

Exposure due to spray applications is typically expressed in terms of the application rate in g a.s./ha. An initial screening risk assessment is presented in the EPPO guidance which uses an Hazard Quotient (HQ) validated on field observations. The HQ is calculated as the ratio of the application rate (g a.s./ha) to toxicity values for both contact and oral routes of exposure (as µg a.i./bee). Products with HQ values in excess of 50 are advanced for further evaluation of risk. In the US further evaluation is triggered for substances with contact LD50 values less than 11 µg a.i./bee. Higher tier evaluations typically involve the exposure of worker bees or colonies to spray treated flowering plants or foliage.

In the case of bees exposed to residues of a product applied as a soil/seed treatment the resulting exposure is expressed as a concentration in pollen and nectar (mg a.s./kg). This need to be converted to a dietary exposure (as µg a.s./bee) and compared to the oral toxicity values and the calculation of a toxicity exposure ratio (TER) performed which is then compared to a relevant trigger. This screening step is recommended in the most recent update of the EPPO guidance [2] and has been validated against currently available data[14].

Higher tier evaluations may focus on the refinement of toxicity values and/or measured residues in matrices attractive to bees or be based on semi-field and field tests where the test material is applied according to good agricultural practice and exposure under field conditions is closely reproduced. However there is a strong need for models to predict the exposure of bees to various application scenarios for honey bees both in the field and within the colony.

For both exposure routes (spray and soil/see treatment), evaluation under realistic field conditions in either semi-field or field tests represent the highest level of testing. Further to this, should there be unresolved uncertainty, post-registration monitoring studies are a suitable option and possible condition of registration for a product.

4. Overall conclusions on exposure

More we examine the scenarios for which there is a potential exposure for bees the more there seem to be. This is in part due to the colony structure of honey bees but also because the biology of this single domesticated species is well known. In terms of ecotoxicity, more information is probably known about this single species than any other in a regulatory scheme. It is not practical or necessary in scientifically valid risk tiered assessment schemes to consider all possible routes of exposure however models of exposure would greatly assist in the risk assessment procedure. A robust and useful risk assessment scheme should rapidly exclude products of low concern to allow efforts to be focused on high risk products. Initial risk assessments can be based on empirically derived relationships (HQ for sprays) and also on TERs for systemic exposure through pollen and nectar. In the first instance this could be achieved through the development of simple exposure models for bees. If a compound is indicated as potentially high risk then exposure may be refined via measured values in bee relevant matrices, but these need to be based on sound scientific risk assessment principles. In the case of higher tier testing and risk assessment, this can be based on exposure of bees under realistic use conditions in tunnel, cage and field tests. In these cases the effects of the direct exposure is measured without the need for complex and detailed analysis of residues and TER calculations.

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