I. Regulatory issues (incl. Revision of EPPO risk assessment and guidelines)

Guidance for the assessment of risks to bees from the use of plant protection products applied as seed coating and soil applications – conclusions of the ICPBR dedicated working group

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Abstract

Background: Soil or seed applied plant protection products (PPPs) aim at bringing the amount of active substance involved to the only parts of the plant that have to be protected. Despite a reduced exposure of non target organisms by this way, an exposure of honey bees through residues in pollen and/or nectar may not be excluded for substances that migrate towards the upper plant parts. Directive 91/414/EEC, related guidance documents and literature data were reviewed and discussed by a working group of the ICPBR (International Commission for Plant-Bee Relationships) with the aim to provide adequate guidance to proceed in a risk assessment in such cases.

Results: The review and expert knowledge collected within ecotoxicology, entomology and plant residue area allowed to identify the key parameters that trigger a risk assessment as well as basic hypotheses to consider in deciding for the experimentations required (laboratory, semi-field and field tests). A stepwise, tiered approach is proposed, which has been checked for its ability to discriminate substances that may pose a risk to bees from substances of low concern.

Conclusion: The present scheme is proposed to update the current EPPO risk assessment scheme with a special issue on systemic PPPs.

Keywords: risk assessment, honey bees, soil or seed treatments, systemic.

Introduction

The Plant Protection Products (PPPs) through seed coating or soil applications on bare soils are intended to concentrate the product in/on the plant parts to be protected, and/or to areas where pests are the most abundant. Exposure of non-target organisms is reduced compared to spray applications, as it is intended to be restricted to the area where the organisms are living in the soil and potentially to vertebrate species that feed from these soil organisms.

An exception to this however may occur when products display systemic properties, as in this case growing plants may contain residues. Exposure of bees may then arise if substantial amounts of residues reach flowers, and particularly nectar and pollen which constitute bee food resources.

Directive 91/414/EEC and related guidance documents do not provide detailed technical guidance on how to proceed to assess the risks to bees posed by substances with systemic properties.[1, 2, 3] This issue was debated at the ICPBR (International Commission for Plant-Bee Relationships) meeting in York in October 2005,[4] and a working group was constituted with the aim to identify the key issues for a new risk assessment and to propose some guidance on a harmonized risk assessment scheme at the European level.

This paper presents the approach to develop this risk assessment scheme followed by the ICPBR working group. Based on a detailed analysis of the conditions for exposure of bees to residues, the scheme proposes a stepwise approach starting with simple calculations based on existing data available in the authorisation dossiers, and ending with field studies. Every assumption is discussed in the light of the review of available data in the fields of bee ecology, ecotoxicity and chemistry of PPPs in relation to expected levels of residues

in plants. The resulting risk assessment scheme has been tested with a data package for PPPs of different categories in order to check whether it discriminates between low risk and high risk products.

Current Regulatory Background (Directive 91/414/EEC and related guidance documents)

Directive 91/414/EEC identifies the conditions for use of PPP for which the exposure of bees cannot be excluded, namely systemic seed dressings, systemic preparations for application to soil and systemic dipping treatments for transplanted crops and bulbs.[1] The relationship between systemic properties and the exposure lead to emphasis on the decision making criteria (annex VI of Directive 91/414/EEC) "where relevant, any information on the persistence of residues in the treated plants". However, little recommendation on how to assess the residues content in the treated plants and to deduce exposure levels is given. The guidance document on terrestrial risk assessment recommends to perform an acute oral toxicity test on bees with the active substance in all cases where a product is to be applied as a soil/seed treatment and involves a systemic substance.[3] Then for substances for which a risk is identified at this stage ("e.g. very low LD_{50}"), it is proposed to "take into account realistic exposure conditions, as for example exposure concentrations as expected in nectar and pollen as indicated by residue studies". Nevertheless, no other indication is provided to trigger this step but it is recommended that exposure, to which the oral LD_{50} (lethal dose 50) could be compared, should be "expressed based on the compound (active substance or metabolite) present in the respective plant parts (e.g. nectar, pollen) to which honey bees could be exposed". The next step, triggered by a risk at this stage, would be to envisage "higher tier studies (cage/tent/tunnel or field studies) with realistic exposure scenarios". Current recommendations thus quite quickly refer to higher tier studies, mainly because "estimates of the concentrations of compounds in the relevant plant parts are rarely available" and "exposure calculations in higher tier studies are already considered within the experimental design (e.g. honey bees foraging on treated field crops)".[3]

Decision making criteria are commonly defined for all PPPs whatever the mode of application, by considering that "where there is a possibility of bees being exposed, no authorization shall be granted if the hazard quotients (HQ) for oral or contact exposure of honey bees are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honey bee larvae, honey bee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use".[1] It is acknowledged however that a critical HQ of 50 was validated against field studies with sprayed products which is therefore relevant for sprayed products,[3,5,6,7] while of that kind there is no validated decision making criteria applicable to a first step risk assessment for non-sprayed compounds. Practically, in the absence of clear criteria on the level of concern raised by a LD_{50} value, to assess the exposure of bees and the potential risks, the evaluations that have been proposed in dossiers mainly follow case-by-case approaches and thus miss the normal harmonisation for usual risk assessments in the regulatory context.[1]

Exposure of bees including hive bees to products applied as soil/seed treatments

Conditions for exposure to residues from soil/seed treatments and for risks to the colony

Basically, the exposure of bees to residues of a product applied for crop protection as a soil/seed treatment may occur if the following three conditions are met: (1) the plant is visited by bees; (2) there is a transfer of residues, either the active substance or a degradation product, from the seed or the soil to the upper part of the plant and to plant matrices of interest to the bees (pollen, nectar, honey dew); (3) for an exposure at the scale of the colony or population scale, contaminated matrices are brought back to the hive by foragers and consumed by the colony. For a risk to occur in the colony from the exposure to these residues, the level of exposure needs to be higher than the threshold for effects, as defined from laboratory and/or higher tier (semi-field, tunnel, field derived) data.

In order to provide detailed recommendations when deciding whether bees can be exposed to residues under specific conditions for use of a PPP, a review of the literature was performed on the issues of (1) the attractiveness of plants to bees; (2) typical level of residues to be expected in plants, and matrices such as

nectar and pollen from systemic transfers; (3) the predictability of systemic properties; and (4) the stability of residues in hive matrices, which determines the exposure of the colony on the long term. In addition, available information to determine to what extent the current data generated on adult bees are representative of the sensitivity of the species including larvae were reviewed, as it is determinant in identifying the step at which additional data i.e. toxicity data on different growth stages, should be needed.

The possible relevance of an exposure to contaminated honeydew following a soil or seed treatment has been considered. In fact a concentration of a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should in principle not harm a bee foraging on the produced honeydew, unless the compound is highly selective towards non-aphid insects. Selectivity tests should in principle allow highlighting such a selectivity, which would then trigger a specific, tailor-made risk assessment.

Attractiveness of a crop to honey bees

In the context of potential risk to honey bees, the attractiveness of a plant to bees has to be considered according to the possible presence of pollen, nectar (and honeydew) on the crop i.e. a crop can be considered as not attractive to bees when it is harvested before flowering.

A comprehensive list of attractive cropped plants has not been published in the past. Tasei (2001) proposed a list of the crops being visited by honey bees limited to oil seed crops, orchards or vegetable crops, and identified other crops as being only occasionally visited, such as vine or cereals, in case of food shortages.[8] Recent work undertaken in a working group of the AFSSA (French Agency on the Safety of Food) 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 not of nectar and honeydew.[9] This list does not include plants such as maize, which may be attractive to bees –and thus 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.

Particular attention should be given to the persistence of residues in soil which may result in an exposure in the case of transfer into rotational crops. The question of the attractiveness is then also raised for crops that enter in the rotation with the treated crop. Criteria to identify persistent substances have been defined in the Directive 91/414/EEC, which in general trigger for additional residue studies involving crop rotation.[1] In the case of residue transfer in rotational crops, investigations to address specifically the risks to bees from attractive plants grown during the rotation with the treated crop become necessary.

Typical residues to be expected in plants, nectar and pollen

Very few published data provide information on the level of residues of PPPs that may be expected in plants or parts of plants following a soil or seed treatment. Some authors could quantify residues in nectar and pollen contaminated by systemic substances being sprayed on crops when blossoms were covered during the spray.[10, 11, 12] Investigations focussing on soil or seed treatment and on residues in nectar or pollen are even more rare. Transfer of substances into honey and royal jelly was proven to be measurable for some systemic substances applied by spray.[13] Evidence of translocation of residues into nectar after soil treatment with granules was demonstrated quite early but investigations based on modern analytical methods are more recent and mainly focus on insecticides[14, 15, 16, 17, 18, 19, 20]

Data on residues of PPPs in plants are systematically generated in the context of the dossiers submitted in support of the evaluation process of PPPs, at least in all cases where the plant is intended to be consumed by humans or animals.[1] A compilation of data generated in various plant species treated with systemic insecticides is presented in Figure 1. This compilation gather residue concentrations measured in all types of plant parts (leaves, fruit, green part, inflorescence, whole plant, and grain) at the period being as close as

possible to blossom, as well as residues measured in nectar and pollen. The results display a majority of samples with less than 1 mg active substance (a .s.)/kg matrix (95th percentile = 0.55 mg/kg, n = 62), the same being observed for degradation products. Taking the matrix nectar and pollen separately, residue concentration would not reach more than 0.1 mg a.s./kg. Compared to the dose applied, the fraction that reaches nectar or pollen may in fact correspond to variable fractions of the dose applied, depending on the plant species and environmental conditions.[15,21] Whether the residue levels measured in whole plants may reflect what is expected to be found in nectar or pollen, very little information could be found in the literature, but the statement that the translocation of pesticides is specified to be measurably less effective to fruiting structures than to other plant parts[22]. This could be related to the role played by flowers hampering as a barrier. Therefore the assimilation of the residues in nectar or pollen to levels equal to those found in whole plants or relevant plant parts at the time of flowering constitutes an assumption protective enough to be considered at a first step. This is consistent with a default transfer factor of 1 considered from the whole plant to the honey in the MRL working group.[9]

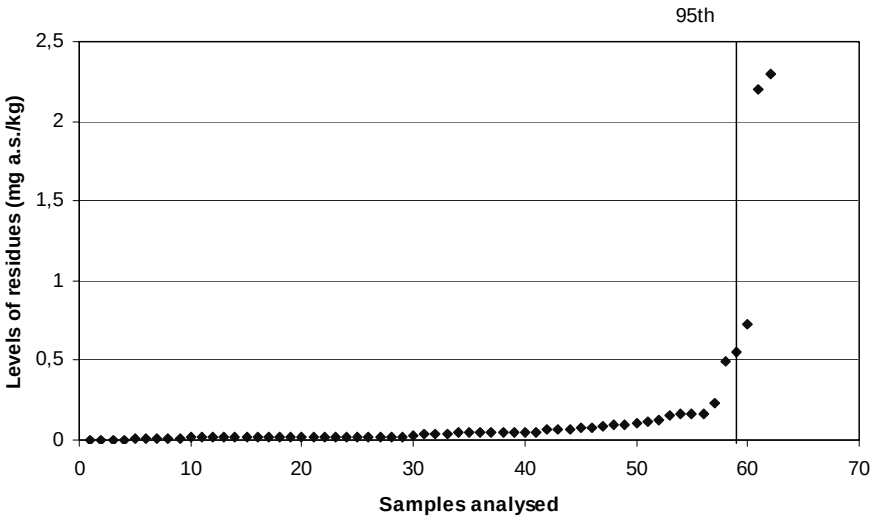

Figure 1 Compilation of residue data in various plant species treated with systemic insecticides. Residue levels, in mg a.s./kg plant, of systemic compounds in diverse types of plant parts (leaves, fruit, green part, inflorescence, whole plant, grain) at the period close to blossoming, as well as residues measured in nectar and pollen (n=62).

Predictability of systemic properties

Attempts to predict systemic properties for PPPs based on chemical and crop factors have been made. From the analysis of phloem translocation of herbicides together with lipophilic properties as the octan-1-ol/water partition coefficient (LogPow) and with dissociation properties as the pKa, the translocation to the plant is expected to be negligible for LogPow values of 4 and above[23] A study on a wider range of substances (ca 400 substances) indicated that the mobility in phloem is satisfyingly predicted by the LogPow, with the pKa modulating the LogPow influence in extreme values[24] Other factors than acidity and polarity may however be implicated into translocation, being related to the substance (such as molecular weight), to the plant (development of the root system, transpiration or nectar production) or to the environment (humidity, light conditions).[15, 22, 23, 24]

In the regulatory context, the information derived from residue studies and plant metabolism studies (residue section of Annex II and Annex III dossiers according to Directive 91/414/EEC), is in general sufficient to identify if the substance will be transferred to the plant during its growth, and if it is further degraded into major degradation products. Similarly, possible uptake of major soil degradation products in plants is identified in these residue studies. This information may then be used to determine whether the substance and/or its residues are to be further considered for a risk assessment to bees. In this respect, the limit of quantification and detection of the analytical methods used in the residue studies must be checked in order to ensure that they were low enough to detect residue levels that exert toxic effects to honey bees. Otherwise additional investigations may have to be considered to demonstrate the absence of translocation at effect levels.

Stability of residues in hive matrices

Again studies that investigate the stability of PPP residues in hive matrices are rather rare. A study on insecticides showed a rather stable behaviour in honey, which may have been related to the absence of Mixed Function Oxydase (MFO) enzymes in the honey sac[25] The residue concentrations in honey, to which larvae are exposed, may also depend on the condensation achievement of honey[26] Therefore it seems premature to consider the variation of residue stability in time in exposure assessments as it may already be done for other organisms exposed to PPPs through the consumption of plant matrices.[3, 27, 28]

Relative sensitivity of larvae compared to adults

Published data that compare the acute toxicity of various pesticides to larvae and adults revealed an important variability.[29] On the 31 substances tested as technical grade or formulated product, three were less toxic to larvae than to adults (the toxicity was considered different when LD_{50} were higher or lower with at least an order of magnitude), 21 were equally toxic and six were more toxic to larvae, no comparison could be made for one substance. No conclusion could be drawn on the predictability of the toxicity to larvae from the chemical family or the mode of action of the tested substance. The highest differences (e.g. ratio between both LD_{50} > 100) were observed for both "simple poisons" (diazinon, profenophos) and Insect Growth Regulators (IGR) (chlorfluazuron). For substances showing a higher toxicity to larvae, the ratio between LD_{50} ranged from 30 (oxamyl) to > 200 000 (chlorfluazuron), the latter being non toxic to adults (LD_{50} > 100 µg/bee, limit test). Differences reached a ratio of 3 to 100. A more recent study that compared the sensitivity of eight substances in adults and larvae, based on the laboratory test of Aupinel *et al.* (2005)[30, 31] indicated that larvae were less sensitive to the assessed compounds than adults with the exception of pirimicarb and metalaxyl[32] In fact, substances acting specifically on growth stages such as Insect Growth Regulators will in many case exert more significant effects when assessed on development parameters than when assessed on adult survival. It may also be true for substances that display, from screening and efficacy studies, and from tests with other non target arthropods, effects specific to juvenile stages. Thus the sensitivity of larvae as well as the related risk assessment should for the time being be considered separately from that of the adults.

Proposed risk assessment scheme

Triggering a risk assessment by establishing exposure

From the review presented above, the relevant parameters that trigger a risk assessment from the exposure to residue of soil/seed treatments are confirmed to be: (1) the attractiveness of plants to bees; (2) a systemic transfer towards pollen and/or nectar and (3) in the case of larvae, a specific risk assessment triggered by the mode of action of the substance of concern, as well as by any observed effect on growth or development as observed on invertebrate species or any other data available in the dossier. The proposed route of entry in a risk assessment scheme takes all these parameters into consideration (Figure 2).

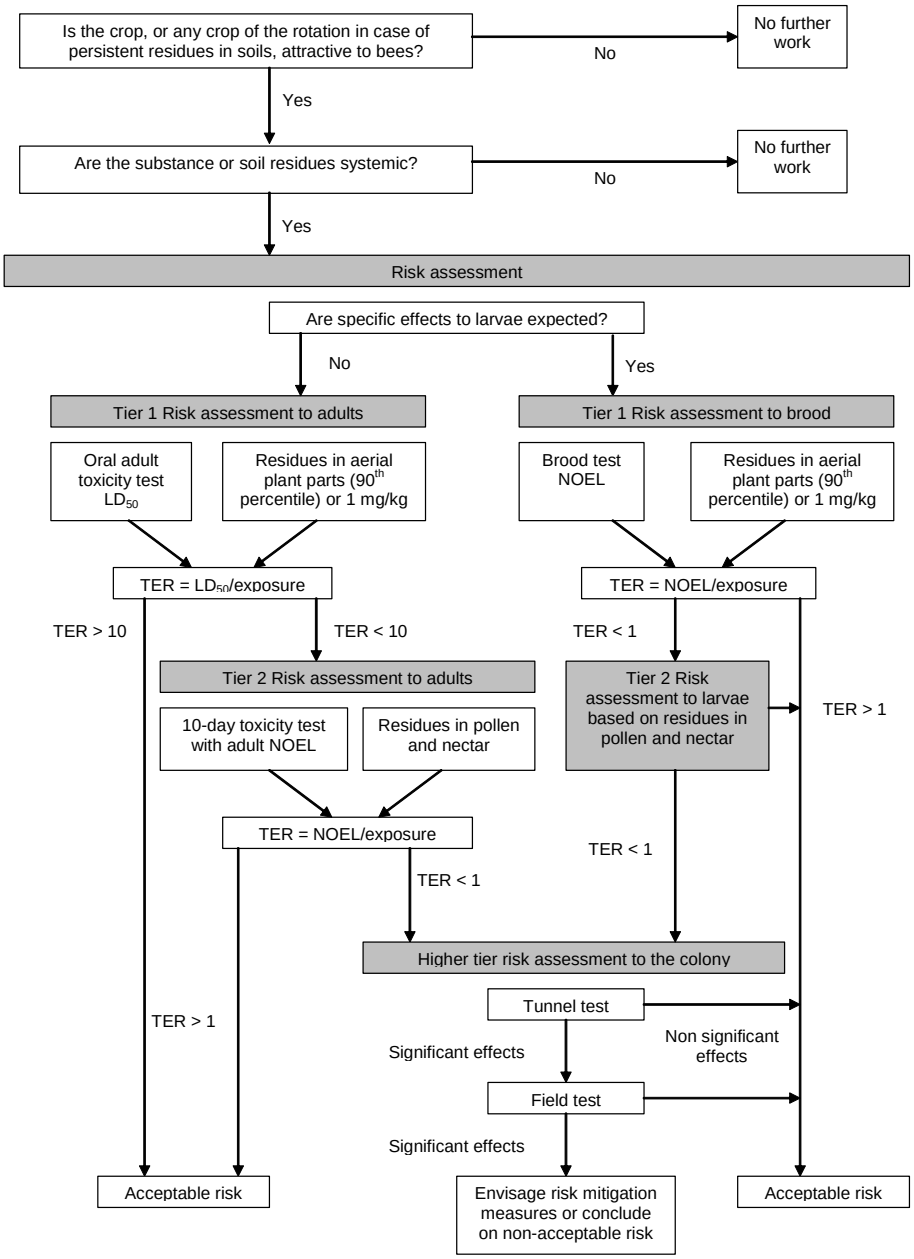

Figure 2 Proposed decision making scheme to evaluate the risks to honey bees in the case of plant protection products applied as a soil/seed treatment. Note that it is possible to skip the Tier 2 and to move directly to a higher-tiered approach.

The attractiveness of the cropped plant to honeybees may be considered as an entry point for this risk assessment. Useful guidance in this respect, as well as recommendations on the criteria to also consider such as the presence in the foraging area of other sources of nectar/honeydew of higher/lower level of attractiveness –which may influence the behaviour of bees towards the crop of interest-, may be found in the document of MRL working group.[9] As stated above, the issue of attractiveness should be considered by also integrating the degradation profile of the substance and its residues in soil, since persistent compounds may also be subject to translocation in plants entering the rotation. In this case, the attractiveness of these plants has also to be taken into account.

At this step, systemic properties trigger the exposure. The prediction of systemic properties may be associated to uncertainties if it is based on chemical properties only. It is therefore proposed to consider all data provided in the residue section of the dossiers submitted in support of authorisation at the national level. A particular attention should be given to the limit of quantification with which they were determined in relation to ecotoxicity thresholds.

It appeared that the sensitivity of larvae cannot be directly extrapolated from that of adults, and exposure of larvae may also be different. The exposure of larvae is triggered by the presence of residues in the hive, which in fact may not be excluded a priori as far as foragers may be in contact with contaminated nectar or pollen at non-lethal levels. The exposure of larvae may however occur through other types of food than for adults. Therefore a separate assessment scheme is proposed for larvae, which should be triggered by the mode of action of the substance of concern as well as by any effect on growth or development observed on invertebrate species, from data available in the dossier.

Risk assessment for adults

In the case that exposure may not be excluded, it becomes essential to assess to what extend this exposure may be of concern. As stated earlier, the level of exposure from the residues actually reaching pollen and/or nectar is rarely available in the current data package. However, the assimilation of the residues in nectar or pollen, to equal levels as found in whole plants or relevant plant parts at the time of flowering (or the generic worst-case value of 1 mg/kg, see 3.3), may provide a protective estimate of exposure levels in a first step.

The assessment of toxic effects may be performed based on current methods that are suitable in this respect.[2,33,34] The main route of exposure of honeybees to soil/seed treatment PPP is probably oral through the consumption of contaminated pollen and nectar, although a contact exposure can not be excluded for bees carrying pollen that contains residues. In this respect the first tier risk assessment could focus on acute oral risks.

The possible risks to bees may as a first tier be quantified through the calculation of a Toxicity Exposure Ratio (TER), as it is currently done for other terrestrial and aquatic organisms.[1] TERs usually correspond to the ratio between a toxicity figure and an exposure levels, expressed in the same units. A TER gives an indication of the margin of safety achieved between the toxicity figure and the exposure level. An acute Toxicity Exposure Ratio (acute TER) may then be calculated based on the acute oral toxicity figure for adult bees and on the assessment of the exposure through estimates of the concentration in the aerial parts of the plant. Because it is an assessment of acute risks, exposure estimates may reflect maximal expected residue levels. The 90th percentile of the data set of residue data for the relevant crop should therefore be selected at this step.

The oral LD_{50} is usually expressed in µg a.s. per bee and residues in plant parts are expressed in mg a.s./kg. Therefore a conversion of residue data is necessary to express exposure as an amount of residue ingested. This conversion may be done by multiplying the 90th percentile of residue concentration (mg a.s./kg plant part) by the daily food ingestion that reflects the dietary need in sugar for a bee. The maximum food ingestion may be estimated from Rortais c. s. at 128 mg /bee/day for nectar foragers.[35] This data set is currently proposed as it is considered to satisfyingly represent food consumption estimates of the different categories of bees. Other figures for food ingestion may become available and could be used if it is demonstrated that they better represent reality.

The calculation of a TER gives an approximation of how close the likely exposure of bees is to a toxicologically significant level. The margin of safety should be sufficient to cover the uncertainty related to longer exposure periods and possible related increased effects. An attempt was made to quantify the range of this uncertainty from existing data. The comparison of toxicity values for adults from acute tests and from chronic (10-day) tests could be done for seven substances.[32] The results showed that the LD_{50} expressed in µg a.s./bee/day as derived from 10-day studies could be derived from 48h LD_{50} by applying an adjustment factor of 10, for acute toxicity data ranging from 0.13 to 90 µg/bee. Despite the need for further work to confirm this correlation with a wider range of compounds, this factor is considered sufficient to cover uncertainties related to the influence of duration of exposure on toxicity levels, considering assumptions regarding exposure levels retained for the tier 1 calculations.

This tier 1 approach is presented in Figure 2. Considering the assumptions that are made to perform this first tier calculation, TER values above 10 are proposed to indicate acceptable risks to bees. In the contrary, TER values below 10 highlight a possible risk to bees and should trigger for a higher tier risk assessment.

Some refinement may be done in a tier 2 approach, by refining the estimate of toxic threshold and/or by refining exposure estimates based on measured level of residues in the relevant material for honeybees.

Additional information with regard to toxic effects may be incorporated by including the duration of exposure of adults in the assessment of effect thresholds. This may be performed by conducting a toxicity test in which worker honeybees are fed with treated sucrose for 10 days in order to calculate a 10-day NOEL (mg a.s./bee/day). The method of Decourtye c.s. allows such an assessment and could be used, despite it is not available as an OECD or EPPO method yet.[36] As stated above, a lower LC_{50} is measured over a 10-day period than after a 4-h ingestion period.[32] Thus uncertainty with regard to chronic exposure to fresh residues is considered to be mostly addressed by the test. If a NOEL derived from 10-day test is used in the TER calculation, the 50th percentile for residue concentration may be used, as it is considered more relevant to reflect a chronic exposure.[3, 27] Revising the toxicity data by generating a 10-day test leads to perform an assessment of the risks for a short-term exposure.

A refinement with regard to exposure may be done by performing measurements of the level of residues in pollen, and for nectariferous plants, in nectar. Measurement should preferably be done in plants grown from coated seeds or sown in a treated soil according to the intended Good Agricultural Practice (GAPs) as the residue levels have to reflect the most probable levels in the crop. Possible build up of residue in soil due to residue persistence, based on Directive 91/414/EEC criteria, and other uses of the substance in the rotation, should be considered if expected. As for plant-based exposure estimates, the mean value of residues levels in pollen or nectar could be used in the TER calculation and compared to 10-day derived toxicity data.

A refinement of both effects and exposure may also be envisaged especially when there is evidence that the refinement of either effect threshold or exposure level will not be sufficient to reach the trigger value. Note however that the trigger value to be used should remain unchanged when a sole exposure refinement is performed since in this case there is still a need to extrapolate from acute to chronic time scale. In the case where a 10-day test is performed, it is proposed to calculate the Tier 2 TER based on the NOEL from the test, as the trigger value to be considered should then be set to 1. Again toxicity and exposure data should be expressed in the same unit. As for the tier 1 calculation, a TER value above the relevant trigger should correspond to acceptable risks, and TER values below the trigger should indicate a possible risk to bees, which should be further investigated through higher tier tests (Figure 2).

Semi-field and field trials

Semi-field and field trials usually correspond to higher tier assessments of the effects a treatment may exert on organisms.[1,2,3] Indeed the aim of higher tier assessment is to address the “unless clause” of the risk assessment which is to “establish through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use”. Thus semi-field and field studies should be designed in order to assess the effects at the scale of the colony, including all bee

categories and long-term effects. Suitable methods to investigate effects of PPPs at this scale are proposed in OEPP/EPPO (2001),[2] which can be adapted to soil/seed treatments. General recommendations for an update of these methods are under development.[37] Nevertheless, specific recommendations to the appreciation of effects of soil/seed treatments are proposed below.

Deciding between a semi-field and a (higher-tiered) field test is a case-by-case decision. Basically, semi-field testing is a suitable option before field testing. The advantage of semi-field tests is that potential mortality is easier to assess and that exposure is ensured and can be easily proven. In semi-field tests, bee colonies are exposed in tunnels to a treated crop. Bees cannot avoid exposure to treated plants, while in field tests, where bee colonies are exposed in plots to the treated crops and are thus free of movements between the crop and surrounding areas.

Semi-field and field trials should be conducted under conditions reasonably representative of the uses to be registered, i.e. using the appropriate crop, application rate and sowing rate. Systemic properties depend on the crop itself, and within a crop the level of exposure is expected to evolve in the same way as the application rate. The duration of flowering should be checked in order to ascertain that it is in the expected range under real cropping conditions. If the substance or its residues are persistent, and if the product may be used on several crops in the rotation, the accumulation in soil should be considered in defining the study protocol.

The effect assessment should consider mortality and foraging behaviour, and effects on bee colonies. In the case pollen or nectar containing residues are brought back to the hive, colonies should be monitored during a sufficient time period to also check long lasting or delayed effects on brood development, queen health, etc.

For both semi-field and field trials, it should be demonstrated that the test bees were exposed under the environmental conditions (especially weather conditions in the case of field trials) of the trial. Parameters such as pollen collection, residue analysis, as well as flight intensity, and observation of the activity on flowers of the treated crop are useful information for that purpose. A quantified assessment of the exposure is particularly important in the case of systemic products, as reference substances for systemic products are difficult to define, being also dependant on crop properties. There should always be a comparable untreated control in order to provide a reference point against which to compare the test treatment. The results, as regards significance of effects should be interpreted with similar rules as for other application modes.[2, 37]

Semi-field and field tests are higher tier data that allow a direct assessment of the effects that may be expected under realistic exposure conditions. Therefore, it is possible to move straight to higher tier investigation instead of refining effect or exposure assessment in a tier 2 approach. This is a case by case decision which should be taken based on the results of the first tier assessment, on the information derived from the properties of the substance and the related expected efforts to propose adequate and easily extrapolable higher tier investigation.

Risk assessment for larvae

A specific risk assessment to bee brood may be necessary when effects on immature stages are unpredictable from the toxicity thresholds observed in adults, e.g. IGRs or other compounds with a specific larvicidal activity (Figure 2). In those cases a stepwise approach similar to that proposed for adults i.e. based on laboratory tests would ideally allow to compare the sensitivity thresholds. Laboratory tests investigating intrinsic properties of the product to immature stages, such as for example the test developed by Aupinel c.s. [30, 31], need further ring testing prior to an implementation as a core data requirement. As regards assessment of effects at the brood scale, a suitable method is described by Oomen *et al.* (1992).[38] Micro colonies are exposed through spiked feeding solution and effects on the brood development are assessed. Ideally, the test should be performed at a level of exposure defined in relation to the mean level of exposure as measured in plant parts, or if available, in nectar or pollen, or other environmentally relevant exposure concentration determined experimentally. The maximum level of exposure supposed to kill foragers should also be considered. Interpretation rules are provided by the authors in the published method.[38] It has to be noted that since exposure level may differ from a crop to another, and considering possible persistence issue in soils,

the results of the test may have to be interpreted in light of the expected level of exposure for each crop of concern. For an adequate risk assessment, the test should allow the determination of a NOEL (No Observed Effect Level) in order to assess the risk for bee brood with e.g. the calculation of a TER that would give an approximation of how closely the likely exposure of bee brood, for a particular crop, is to a toxicologically significant level. Exposure estimates could, as for adults, be deduced either from residues in plant parts (Tier 1) or from residue analysis of nectar or pollen (Tier 2) (Figure 2).

There are too few data available, particularly on exposure of brood, to relate larval toxicity (assessed for example by methods described by several authors, e.g. Wittman & Engels, 1981) with field application rate and brood damage.[39] Therefore, if any effects are detected in a bee brood-feeding test, semi-field or field testing becomes necessary.

It is important to note that as soon as higher tier (semi-field or field tests) tests are triggered, based on the results of lower tier risk assessments for adults or brood, the effects and related risks will have to be addressed at the scale of the colony. This meets the requirements of Directive 91/414/EEC, as stated under annex VI.[1] In this context, potential risks to bees identified from this stepwise approach should be interpreted in light of the uncertainties that remain in the assessment outputs (i.e. variability of exposure levels, ability of the ecotoxicity endpoints) to cover the whole life cycle of the species), and to the measures that may be implemented in the aim to limit or avoid the exposure and thus the risks.

Ability of the scheme to discriminate PPPs AND their use according to risks

In order to verify the ability of the proposed risk assessment scheme to discriminate products that may need a refined assessment for an adequate assessment of the risks to bees from products of low concern, the lower tiers (tier 1 and tier 2) were tested against data available for PPPs.

Acute toxicity studies on adults are performed for all active substances under PPP regulation,[1] so that the risk assessment scheme could be checked for adults. Tier 1 and Tier 2 calculations were performed for all the active substances, existing and new, for which a positive decision with regard to a possible use within Member States has been undertaken at the European level (i.e. included in Annex I of Directive 91/414/EEC). Oral acute LD_{50} were extracted from the French national database *Agritox* as a reference basis for toxicity thresholds.[40] This database is updated with reference data as produced for the re-evaluation of active substances as summarized in the review reports resulting from the European peer review. The database gathered toxicity endpoints expressed as µg a.s./bee, and was built with data generated for technical ingredient exclusively.

Tier 1 TERs were calculated with residue consumption deduced from expected levels in plants. As proposed above, a worst case estimate of 1 mg a.s./kg matrix was considered. This concentration was converted to a daily dose by multiplying this default value (1 mg a.s./kg plant part) by the daily food ingestion reflecting the dietary need in sugar for a nectar foraging bee i.e. 128 mg /bee/day. Resulting Tier 1 TERs were compared to the trigger of 10. Results are presented in table 1.

Table 1 Percentage of active substances that fail the proposed Tier 1 and Tier 2 of the risk assessment (n = 171), which means, from the proposed scheme (see Figure 2): 'Envisage risk mitigation measures or conclude on non-acceptable risk'. Data extracted from the *Agritox* database (http://www.dive.afssa.fr/agritox/index.php)

	Percentage of active substances that do not pass at the first Tiers of the risk assessment (TER < trigger value, n = 171)	**Mode of action**
TER Tier 1 (trigger value: 10)	15.2 %	24 insecticides 1 fungicide 1 nematicide
TER Tier 2 (trigger value: 1)	11.1 %	19 insecticides

The overall discriminating ability of the scheme may be assessed based on an analysis of the Tier 1 calculations. Tier 1 TERs were below the trigger of 10 for 15.2% of the active substances, thus triggering for a Tier 2 risk assessment. The 26 substances in this case consisted mainly of insecticides and acaricides (24 substances), 1 fungicide and 1 nematicide. As insecticides usually display the lowest LD_{50} values, the discriminating ability of the scheme is judged as satisfying. Among the 145 substances for which a Tier 1 TER of 10 or above was calculated, 13 were substances acting as insecticides or acaricides. These substances belong to various chemical families, the most represented being nicotinoids (2) and benzoylureas (2) the latter acting as insect growth regulators. The other substances belong to pyrazolamines, phenoxypyrazoles, triazines, azomethines, tetrazines, benzohydrazides, pyridines, oxazolines or carbamates, and act for example specifically as acaricides. Thus their LD_{50} is found to be higher than 1.8 µg a.s./bee in all cases. In the case of substances that act specifically on developmental stages (proposed as Insect Growth Regulators) or for which an action on juvenile stages has been highlighted from studies on other arthropod species living in terrestrial or aquatic ecosystems, it is in any case recommended to perform a risk assessment focused on larvae (see Figure 2). Note also that within these 13 substances none has been developed to be used as a seed treatment.

Laboratory 10-day toxicity studies are scarcely available for substances or even for PPPs, as the test has not been recommended in the regulatory context. Similarly, residue concentrations in nectar or pollen are available for a very limited number of substances, and related crops. As a consequence, Tier 2 calculations including 10-day test derived NOEC or exposure estimates from nectar or pollen could not be generated. Instead, short-term TERs were calculated considering in a first instance that the results of the study do not indicate stronger effects in the 10-day tests than in acute tests. An arbitrary factor of 3 was considered to extrapolate a NOEL from the LD_{50} in a same test. Of course calculations should in principle rely on a NOEL value as deduced from the study performed. Since a 10-day study is considered to be available, calculations were compared to a trigger value of 1.

A similar approach was followed to check the discriminating ability of the scheme at the Tier 2 level. Tier 2 TERs were below the trigger of 1 for 11.1% of the active substances. The 19 substances in this case consisted mainly of insecticides. This Tier 2 calculation differs from the previous step based on a revised toxicity assessment that supposes no increased toxicity with exposure duration. It is clear that in cases where a 10-fold increased toxicity is observed compared to data from the acute test, this step will become more discriminant than the previous step, and that further refinement of the exposure will become necessary. In this example, a default value was used whereas the scheme recommends the use of the mean residue levels in plant in order to estimate exposure levels. This refinement may also contribute to discriminate substances based on risk assessment criteria.

Conclusions

There was a need for technical guidance addressing the question of the risks to honey bees posed by soil-systemic plant protection product uses under the particular exposure conditions constituted by contaminated pollen or nectar. In developing this guidance, the ICPBR working group considered that the risks posed by plant protection products to the environment have to be dealt with under harmonized conditions within European Member States, and that the risks to non-target organisms should be assessed having in mind a common view about what constitutes an effect at the population level. For these reasons, the proposed scheme meets both EPPO and SANCO guidance documents conception rules, and the approach retained is similar to that for other organisms. A stepwise approach is developed, based on evidence for exposure as an entry route, and that first rely on any existing and relevant data in order to avoid a systematic requirement for field tests. The presented scheme is proposed to update the current EPPO guidance document with a special issue on soil/seed applied PPP, as well as it provides recommendations for conducting higher tier (tunnel and field) studies dealing specifically with soil/seed treatments. Practice is now needed with an emphasis on the higher tier steps, in order to adjust study protocols and conditions for study requirements in the perspective of future amendments of the EPPO scheme.

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Environmental risk assessment scheme for plant protection products - Chapter 10: Honeybees – Proposed scheme

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Specific scope: This standard provides a scheme for assessment of the potential environmental risks presented by systemic plant protection products for honeybees. It is intended as an addition to EPPO standard PP 3/10(2) 'Environmental risk assessment scheme for plant protection products', Chapter 10: Honeybees, revised in 2002-09.

Specific approval and amendment: ICPBR/EPPO working group Honey bees.

Introduction

The sub-scheme in this chapter deals with the potential risks to pollinating insects from the use of soil-systemic plant protection products (PPPs). It specifically addresses the assessment of risks to honeybees (*Apis mellifera*) and their brood and colonies arising from exposure of bees to soil-systemic insecticides and other soil-systemic plant protection products.

As for the assessment of risks arising from sprayed PPPs, it is acknowledged that the most reliable risk assessment is based on data collected under conditions which most resemble normal practice (i.e. by field