

Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group

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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

tests or by monitoring the product in use). However, beside financial and time costs, these tests pose the question of extrapolating results from one crop to others, since exposure of pollinators is not directly related to the application rate, but also results from systemic properties of the active compound and attractiveness of the crop, which itself is related to agricultural practice. The tiered approach thereafter proposed is thus aiming at triggering higher tier (tunnel and field) testing to the sole cases where an exposure to level of residues of concern can not be excluded. As for other sub-schemes, it is always possible on principle to go straight to higher-tier tests if there is evidence that these tests will be triggered, or for convenience. It should be kept in mind that a multiplication of field tests might be quite heavy since extrapolating exposure conditions between crops can not easily be made.

Risk assessment scheme

Details of the product and its pattern of use

1. Take from Chapter 2 the basic information on the product and its pattern of use.
If this is an insecticide for soil treatment (granules ...) or a seed treatment: **go to 2**

Possibility of exposure

2. Is the crop (see note 1) or a rotational crop (see note 2) attractive to bees?

If yes: **go to 3**

If no: **go to 10**

3. Is the active substance or its residues systemic in plants (see Note 3)?

If yes: **go to 4 & 5**

If no: **go to 10**

Preliminary screening based on toxicity and exposure level (Tier 1)

4. Assess the acute toxicity of the active substance to worker honeybees by conducting acute contact and oral laboratory tests. Determine acute LD₅₀ for both exposure routes.

Calculate the ratio (TER) between the LD₅₀ (oral) and exposure. Exposure is assessed through the amount of residues that may be ingested by a bee in one day (see Note 4).

If ratio > 10: **go to 11**

If ratio < 10: **go to 7**

5. Does the compound exert sublethal effects on growth or development (risk assessment for bee brood triggered)? (see Note 5).

If yes: **go to 6**

If no: **go to 11**

6. Conduct a bee brood-feeding test with definition of NOEL and TER calculation (see Note 6)

If ratio > 1: **go to 11**

If ratio < 1: **go to 8**

Second tier risk assessment for adults

7. Refine the risk assessment on effects and/or exposure side.

Lethal effects can for example be assessed over a prolonged period that represents the duration of exposure of foragers during flowering (determination of a 10-day NOEL).

Exposure assessment may also be refined by measuring residues in pollen and nectar of the treated crop.

Calculate the new ratio between the NOEL (oral) and exposure. Exposure is assessed through the amount of residues that may be ingested by a bee in one day (see Note 7).

If ratio > 1: **go to 11**

If ratio < 1: **go to 8**

Semi-field trials

8. Conduct a semi-field field trial in conditions representative of use (application rate, crops ...) (see Note 8). Are effects on colony survival and development significant (see Note 9)?

If no: **go to 11**

If yes: **go to 9**

Field trials

9. Conduct a field trial in conditions representative of use (application rate, crops ...) (see Note 8). Are effects on colony survival and development significant (see Note 9)?

If no: **go to 11**

If yes: **go to 12**

Categories of risk

The preceding stages of assessment allow uses of plant protection products to be allocated to three categories of potential risk to honeybees.

10. Categorize as negligible risk to bees:

11. Categorize as low risk to bees:

12. Categorize as high risk to bees: **go to 13**

13. Review the data which led to the high-risk category and check whether the conclusions are correct (see note 10).

If yes, confirm assessment: **go to 14**

If no, obtain more information as needed: **go to 8**

Risk management

14. The following points give guidance on the steps that might be appropriate in order to mitigate effects on honeybees, for products in each of the categories of risk.

If risk is low (i.e. level of exposure leads to acceptable risks) or negligible (i.e. no exposure): set no restrictions on use.

If there is a high risk consider conditions that would limit or exclude exposure of bees. For example, allow use only in crops which are not visited by bees. Consider the persistence of residues in soil and possible exposure through rotational crop and consider related recommendation with regard to rotational crops in contaminated soils. Mitigation measures should be proposed as moving beehives away from the treated crops.

According to the Directive 2003/82/EC, these indications or restrictions should be mentioned in standard phrases for safety precautions for the environment as SPe8: "Dangerous to bees/To protect bees and pollinating insects ...". Specific phrases may be proposed based on the conditions that would lead to a limited or excluded exposure of bees.

Explanatory notes

Note 1 Establish if the crop is attractive to bees

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, i.e. weeds, which may influence the behaviour of bees towards the crop of interest, may be found in the document of MRL working group (EC, 2009). In general, a crop can be considered as not attractive to bees when it is harvested before flowering. Some plants being not intrinsically attractive to bees may be visited due to extra floral nectarines, e.g. in field beans or due to honeydew produced by aphids on

crops otherwise not attractive to bees. 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 to be expected or not.

Note 2 Establish if rotational crops have to be considered in the risk assessment

The persistence of the product in soil may result in an exposure of bees, in the case of the growth of an attractive plant in the rotation. Criteria to identify persistent substances have been defined in Directive 91/414/EC, which in general require additional residue studies involving crop rotation. In the case of residue transfer into rotational crops, investigations to address specifically the risks to bees from attractive plants grown during the rotation with the treated crop become necessary.

Note 3 Establish if the substance or its residues present systemic properties

The exposure of honeybees to plant protection products used for soil or seed treatments may occur in the case of transfer of the active substance itself or its degradation products to the parts of the plant that may be consumed by bees, i.e. nectar or pollen, or honeydew. Exposure to contaminated honeydew is, however, not considered a relevant route in the case of soil and seed treatments, as (a concentration of) a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should in principle not be capable of harming a bee foraging on the produced honeydew, unless the compound is highly selective towards non-aphid insects. Selectivity information (as apparent from the registration dossier) should in principle allow highlighting such a selectivity, which would then trigger for a dedicated risk assessment according to the present sub-scheme.

Information derived from residue studies and plant metabolism studies (residue section of Annex II and Annex III dossiers according to Directive 91/414/EC), is in general sufficient to identify if the substance is transferred into the plant during its growth, and if it is further degraded into major degradation products. Similarly, possible uptake in plants of major soil degradation products is identified in these residue studies. In case of uptake and transfer into the plant, the PPP is systemic, and the answer to question 3 is ‘yes’.

The sensitivity (i.e. limit of quantification and detection) of the analytical methods that were 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. If uncertain that detection methods were sensitive enough, additional investigations have to be considered to demonstrate the absence of residue translocation at toxic levels. Beside this verification, studies that specifically investigate the presence of residues in flowers, nectar or pollen are not necessary at this stage.

Note 4 First tier risk assessment

Suitable methods for acute oral and contact toxicity tests are described by OEPP/EPPO 170 (2001), OECD (1998a, b).

The main route of exposure of honeybees to soil/seed treatment is oral through the consumption of contaminated pollen and nectar, although a contact exposure can not be excluded for bees carrying pollen that contain residues. It has to be noted, however, that topical exposure through contaminated nectar may also occur for sprayed, non-systemic compounds.

In this respect the first tier risk assessment focuses on acute oral risks. A first tier toxicity exposure ratio (TER) is calculated based on the acute oral toxicity figure for adult bees and on an assessment of the exposure through, ideally, pollen and nectar. Residues in pollen and nectar are rarely quantified in residue studies that are available in the residue section of dossiers as these studies are performed for other (risk to consumers) purposes. The transfer and fate of products and their residues in plants is not homogeneous, and transfers to the blossom depend on their ability to cross the flower barrier. Thus estimates of the concentration in the aerial parts of the plant may be considered as an overestimation of residual concentration in nectar and pollen, and thus provide a useful margin of safety as a first assessment step. In the case such data on residues in plant material are not considered reliable or available, a generic worst case

value of 1 mg (a.s.)/kg plant matrix is proposed. This value is deduced from a compilation of the data generated in various plant species treated with systemic insecticides and the consequent 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 displayed 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 matrices nectar and pollen separately, residue concentrations would not reach more than 0.1 mg a.s./kg.

Because it is a worst case assessment, exposure estimates should reflect the maximal expected residue levels. When based on measured residue in plant matrices, the 90th percentile of the data set of residue data for the relevant crop should be selected at this step.

The oral LD₅₀ is measured in µg active substance per bee and residues in plant parts are expressed in mg/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 et al., 2005 at 128 mg /bee/day for nectar foragers. The data set provided by Rortais et al. (2005) is 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 closely the likely exposure of bees is to a toxicologically significant level. The margin of safety achieved should be sufficient to cover the uncertainty related to longer exposure periods and possible related increased effects. To quantify the range of this uncertainty, the comparison of toxicity values for adults from acute tests and from chronic (10-day) tests was done for 7 substances (Defra, 2007). The results show that the LD₅₀ expressed in µg a.s./bee/day as derived from 10-day studies can be derived from 48h LD₅₀ by applying an adjustment factor of 10, for acute toxicity data ranging from 0.13 to 90µg/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 exposure duration on toxicity levels.

Note that for low toxicity figures (e.g. LD₅₀ of 10 µg a.s./bee and above, TER calculations will always result in values above the trigger (= low risk) even with exposure levels estimated from concentrations in aerial parts. However, a definite cut-off value for entering in the risk assessment scheme through a Tier 1 TER is difficult to establish. As the Tier 1 calculation does not involve additional experiments but the acute oral toxicity test in adults, some toxicity-based trigger is not deemed necessary.

Note 5 IGR

Insect growth regulators (IGRs) and substances that display effects specifically to juvenile stages, apparent from screening and efficacy studies and from tests with other non target arthropods (including terrestrial and aquatic), have to be assessed more precisely with a bee brood-feeding test (Note 6).

Note 6 Bee brood-feeding tests

A suitable method is described by Oomen et al. (1992). The test should be performed at the highest expected level of exposure (the maximum level of exposure is supposed to kill foragers) as measured in plant parts, or if available, in nectar or pollen, or other environmentally relevant exposure concentration determined experimentally.

As the level of exposure will vary from a crop to another and probably also between samples of a same crop, it is not necessary to duplicate the study to take the variability of exposure levels into account. Rather, 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. Note that since exposure level may differ from a crop to another, and considering possible persistence issues in soils, TER calculations should be done for each crop separately, to ensure that the trigger is reached in any case.

There are too little 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 rates and brood damage. Therefore, if any effects are detected in a bee brood-feeding test, semi-field or field testing becomes necessary.

Note 7 Second tier risk assessment

Additional information with regard to toxic effects may be incorporated by including the duration of exposure of foragers in the assessment of effect. This should be performed by conducting a toxicity test in which worker honeybees are fed treated sucrose for 10 days to calculate a 10-day NOEL (mg a.s./bee/day). The method of Decourtye et al. (2005) could be used, although it is not available as an OECD or EPPO method yet. Usually a lower LC₅₀ is measured over a 10-day period than after a several hours ingestion period (Defra 2007). Thus uncertainty with regard to chronic exposure to fresh residues is considered to be addressed by the test.

A refinement of the exposure may be made by measurements of the residues in pollen, and if relevant, nectar, in plants grown from coated seeds or sown in a treated soil according to the intended Good Agricultural Practices (GAPs). Residue levels have to reflect the levels expected from the crop. Possible build up in soil due to residue persistence, based on Directive 91/414/EC criteria, and use of the substance in the rotation should be considered if expected. Since exposure has to reflect a period of several days, the mean value of the concentrations measured in samples could be used in the TER calculation.

The tier 2 TER should be calculated, with the NOEL from the 10-day chronic toxicity test in bees and/or the measured level of residues in the relevant material for honeybees (mean residue data). A further refinement of both effects and exposure is not necessary but it is rather to be considered as a possibility, especially when there is evidence that the refinement of either effect threshold or exposure level will be sufficient to reach the trigger value. If a 10-day test derived NOEL 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. Note however that the trigger value remains unchanged in the case of a single exposure refinement since the uncertainty with regard to chronic effects remains. Again toxicity and exposure data should be expressed in the same unit.

Note 8 Semi-field and field trials

Suitable methods for semi-field and field trials are discussed in OEPP/EPPO (2001) and can be adapted to soil/seed treatments.

Semi-field and field trials should be conducted under conditions reasonably representative of the uses to be prescribed (appropriate application and sowing rate and crop). This allows also for testing under specific conditions of exposure (e.g. in relation to duration of flowering) to be expected. If the substance or its residues are persistent and the product may be used on several crops in the rotation, the accumulation in soil should be considered the study protocol.

Possible effects on adult survival and foraging behaviour and on bee colonies should be checked. In 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.

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 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 are useful information for that purpose. A quantified assessment of the exposure is particularly important for 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(s).

Semi-field testing is a suitable option before field testing. The advantage is that potential mortality is easier to assess and that exposure is ensured and can be easily proven.

Semi-field testing is readily feasible by exposing bees to a treated crop in tunnels. This reflects a truly realistic scenario in so far as that there can not be a certain target exposure level accurately pre-determined, since a certain dressing rate of seeds will not necessarily lead to an exactly predictable residue level in nectar and pollen. Of course this is also a characteristic of natural conditions that a certain level variation can occur. An alternative for special design tunnel studies, where exposure of the bees to a certain pre-determined residue level is aimed at, could be the exposure to spiked nectar and pollen in a tunnel, as far as technically feasible. The choice between these options should then be made on a case-by-case basis according to the particular circumstances of the situation.

Note 9 Significance of semi field/field results

Effects as a result of the experimental treatment in semi-field or field trials may be difficult to assess and to distinguish from other sources of mortality. Statistical analysis of the results normally solves this problem and studies should be designed to allow statistical treatment to be performed.

Current procedures, including pollen collection, possible residue analysis of collected pollen and direct observations of foraging behaviour should provide sufficient information concerning exposure to the test compound to enable reliable interpretation of results. Although exposure a priori is ensured in cage or tunnel trials, analysis of nectar, pollen or blossoms is recommended in order to verify the residue level in bee-relevant matrices.

Note 10 Additional investigation

Special effects (larval toxicity, long residual effect, disorienting effects on bees, etc.) identified by the field test may in some cases require further investigation using specific methods, particularly in the case these effects are observed under realistic exposure conditions, since this means they may be expected also under the intended conditions for use of the PPP. Such investigations should then be dedicated to appreciate the importance and significance of effects and to help in setting risk mitigation measures.

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