Proposed revision of the higher tier testing requirements for EPPO Standard PP1/170: Test methods for evaluating the side-effects of plant protection products on honeybees

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Abstract

Background: Regulatory evaluations for the effects of pesticides on honeybees in the EU are based on the honeybee test guidelines and risk assessment scheme of the European Plant Protection Organisation (EPPO). While this is considered to be robust and effective, it is also recognised that a continuous process of review and appropriate development is necessary. A working group of the International Commission for Plant-Bee Relationships (ICPBR) had been set up to review the current guidance set out in the EPPO PP1/170 standard for higher tier testing i.e. semi-field (cage) test and full field studies. The aim of this group was to utilise the considerable experience obtained with honey bee testing. This paper presents the working group's proposed revision to the EPPO standard PP1/170, taking into account feedback received from the 10th ICPBR Symposium in Bucharest.

Results: The primary aim of the group has been to produce guidance that is sufficiently detailed yet suitably flexible so that it enables tests to be conducted and evaluated without being too prescriptive. In particular, it recognises that higher tier testing may arise as a result of various initial concerns e.g. adult toxicity, brood effects and systemic toxicity. The guidance is designed to provide the different emphasis that is required to meet the specific requirements of individual studies.

Conclusion: The revision of higher tier testing for honeybees presented in this paper is proposed as an update to the current EPPO PP1/170 standard.

Keywords: honey bees, test guidelines, higher tier, semi-field (cage) tests, field tests.

Introduction

Currently in the EU, regulatory evaluations for the effects of pesticides on honey bees are based on the honey bee test guidelines and risk assessment scheme of the European Plant Protection Organisation (EPPO)[1]. The International Commission for Plant-Bee Relationships (ICPBR) Bee Protection Group provides the technical input to the EPPO standard PP1/170[2] and associated risk assessment scheme[3]. While the current EU risk assessment scheme is considered to be robust and effective it is also recognised that a continuous process of review and appropriate development is necessary. This needs to be done in a considered way with the development of a consensus view amongst the expert representatives within the group. This allows any new information to be evaluated and its significance in relation to the risk for honey bees assessed. Accordingly, a review was carried out in 1999 at the 7th ICPBR symposium in Avignon, France[4] and this resulted in the current versions of the EPPO standard PP1/170[2] and the associated risk assessment scheme[3]. More recently, EPPO had asked the ICPBR Bee Protection Group to undertake a similar exercise at the 10th Symposium in Bucharest.

At the previous meeting of the Bee Protection Group (in 2005 at the Central Science Laboratory, York, UK)[5], a working group was set up to review the current guidance for higher tier testing i.e. semi-field (cage) test and full field studies. The aim of the EPPO standard PP1/170 is to provide sufficient guidance to allow the studies to be conducted without being too prescriptive. It was considered that this should be looked at again in the light of experience obtained with the working of this guideline over many years. In particular, it was recognised that developments in other areas highlighted the fact that higher tier testing might be triggered via a number of different routes e.g. adult toxicity, brood effects, systemic activity etc.

Accordingly, it is important that the guidance is sufficiently detailed and flexible to address the different emphasis that each requires.

This paper presents the working group's proposed revision to the higher tier testing requirements of the EPPO standard PP1/170, revised in response to comments received at the 10th ICPBR Symposium in Bucharest and after circulation to all delegates following the meeting.

Semi-field tests

Semi-field testing (cage, tunnel or tent tests) are higher tier studies that may be triggered as a result of the standard Tier 1 risk assessment i.e. contact or oral hazard quotients >50. In addition, it may be triggered as a result of possible concerns about systemic activity identified during the Tier 1 assessment or by information about insect growth regulator (IGR) properties. Semi-field testing can also be modified for specific assessments with honeybees e.g. repellency and other behavioural effects, effects of aged residues, the evaluation of the hazard of the application of plant protection products to honeybees foraging the honeydew secreted by aphids or for specific testing of brood effects. It is therefore important that this guideline is interpreted with appropriate flexibility to ensure that all these requirements can be accommodated. Similarly, it is important when designing a semi-field study that the aims and objectives are clearly specified.

Experimental conditions

Principle of the trial

Honey bees from small colonies are forced to forage on a flowering crop in field cages (to provide realistic worst-case exposure). Typically, the test products and a toxic standard known to present a high hazard to bees (e.g. dimethoate) are applied in separate cages during bee flight, while other cages are left as untreated or water-sprayed controls. The toxic standard is used to confirm that the bees are exposed to the treatment and to calibrate the magnitude of the possible effects under trial conditions. Its selection should be based on the specific concerns being addressed. In those cases where the trial conditions do not allow the use of a toxic standard (e.g. in the case of assessment of systemic activity), this needs to be justified and it should be demonstrated otherwise that bees have been exposed. The effects of the treatment on bees are assessed just before and several times after application.

Trial conditions

As a guide, cages should contain a minimal crop area of 40 m^2. However, cages of a smaller or significantly larger size may be appropriate depending on the objectives of the study. A number of factors need to be considered when selecting the appropriate cage size e.g. nature and attractiveness of the test crop, objectives of the study (short versus longer term effects) and the size of the test colonies. For screening purposes and the study of specific questions such as short term mortality assessments on aged residues, smaller cages (of at least 12 m^2) may be appropriate. For increased realism or where increased foraging area is required, larger cages may be appropriate. The cage should have a mesh size that the bees cannot escape through e.g. ≤3 mm.

In the first instance, rape, mustard, *Phacelia* or another crop highly attractive to bees should be used as test plants e.g. in the case of a standard semi-field trial based on acute toxicity. In other cases, identification of a surrogate (worst-case) test crop may be more difficult e.g. for systemic compounds, where the test crop should be one for intended use. Other factors may then need to be considered when extrapolating between crops (e.g. plant metabolism data). Less attractive crops (on which use of the product is proposed) may be appropriate e.g. if significant effects are seen or expected with the standard attractive crops. This will have implications for the design and conduct of the study, e.g. a toxic standard may not be appropriate and the levels of foraging expected will be lower. Normally, treatments should be applied when the test crop is in full flower except where justified e.g. when recommended product use is pre-flowering.

On cereals, where aphid honeydew is being simulated, sucrose solution is sprayed onto a suitable crop e.g. wheat, in such a manner as to maintain sufficient attraction. Such testing may require larger areas of crop to provide sufficient forage for the test colonies and thus may require the use of a larger cage. For such a test, trial conditions and methods described by Shires *et al.* (1984)[6] are suitable.

Preparation of the bees

Use one small healthy queen-right colony per cage containing approximately 3,000 to 5,000 bees and at least three full frames containing all brood stages and stores of nectar/pollen (but not excessive in order to ensure exposure to the treatments), or a nucleus. The size of the colony may need to be adjusted according to the aims and conditions of the study. Thus, normal field colonies may be used in larger cages while in small cages only one brood frame and one frame with nectar/pollen may be sufficient. For the assessment of brood effects, smaller colonies may also be appropriate e.g. 'Mini-Plus-Beuten' hives, according to the method of OECD Guidance Document 75[7]. Feeding of the colonies during the trial may be necessary depending on the available forage and water should be offered.

Design of the trial

Treatments: test product(s), toxic standard known to present a high hazard to bees (e.g. dimethoate for a standard assessment based on acute toxicity) and a control without plant protection product. The choice of toxic standard will depend on the objectives of the study (e.g. fenoxycarb for an IGR compound) and may not be appropriate in some cases (e.g. for systemic compounds). The control should normally receive a water spray unless there is a justified reason for not doing this.

Test units: cages with one colony each.

Replicates: sufficient to allow appropriate risk assessment. Normally, the minimum number of replicates should be three in order to enable statistical analysis but a lower number may be appropriate in some cases, for example with crops that need a large area (e.g. orchard trees) or where a high number of treatment groups are required. Where this is the case, smaller cages may allow replicate numbers to be maintained although this needs to be considered in the context of the study objectives and the nature of the information required.

Application of treatments

Test Product(s): use formulated products only.

Timing of application

Normally the products should be applied during the daytime when bees are foraging most actively. However, this may be modified if appropriate for the objectives of the study e.g. when testing systemic compounds applied pre-flowering (seed dressings and soil applied products) or for assessing mitigation measures (application before bees are active). To assess aged residues, application is carried out at intervals before exposure, which can take place in the same way as for directly sprayed treatments. Untreated pot-grown plants in the cages are then replaced with the treated ones after appropriate ageing intervals. There should not be any rainfall before directly sprayed applications have dried e.g. for about 2 hours after application.

Shortly before application the number of foraging bees per m^2, and how the assessments are carried out, should be recorded. Where a toxic standard has not been used, a foraging density of at least 5 bees/m^2 is required on bee attractive crops (e.g. *Phacelia*) in order to verify exposure. However, in other cases foraging levels need to be related to the specific conditions of the trial e.g. for less attractive crops and pre-flowering application of systemic compounds (where exposure is related to a more sustained period during flowering).

Application rates

The product should normally be applied at the highest rate specified for the intended use in flowering crops. Lower application rates may be applied e.g. if the off-crop risk needs to be assessed (using drift rates of application), when exposure on weeds in orchards are tested (ground deposition rates), or in cases where

products which are intended for use in three-dimensional crops and where the use rate is dependent on the canopy height (but the test is being conducted in a 'two-dimensional' surrogate crop). Normally a single application will be sufficient but multiple applications (according to the GAP) may be appropriate in specific cases e.g. for sprayed compounds that have the potential to move to the flowers via foliar uptake.

Mode of assessment

Pre-treatment assessments should be sufficient to demonstrate stable background mortality and to show that the bees have acclimatised to the test conditions and are actively foraging on the crop. Typically, for a standard study with a sprayed product this means that the colonies need to be introduced into the cages approximately 2-3 days prior to treatment. This will not be possible where a pre-flowering treatment is being tested. In this case, the hives are introduced at flowering and exposure starts straight away. In the case of aged residues, exposure can take place by replacing untreated pot-grown plants used to acclimatise the bees with plants previously treated at appropriate intervals.

Conduct mortality and behavioural assessments at least 2 days prior to treatment (to demonstrate the bees are acclimatised) and then just before and at several intervals after treatment (preferably daily but at least on days 0, 1, 2, 3, 5 and 7). Additional assessments can be carried out if appropriate e.g. on treatment day. Longer post-treatment periods may be required in some cases but will be limited by the confinement of the colonies (subject to specific test conditions). Normally 7 days is the appropriate post-treatment exposure period, which will be limited by the flowering period of the crop or the confinement of the bees to a limited foraging area. Record flight and/or foraging activity in the cages as given by the number of bees/m^2 (y monitoring a fixed area e.g. 1 m^2, or using transects along the length of sub-plots (if present), in both cases for a defined period. The details of these assessments will depend on a number of factors e.g. cage size and attractiveness of the crop, but they should be sufficiently reliable to quantify the activity level. The behaviour of the bees on the crop and around the hive should be recorded using a standardised approach. Count the dead bees in dead-bee traps and those dying in the rest of the cage (e.g. from water permeable sheets placed along paths or around the edge of the crop).

The condition of the test colonies (including brood status) should be assessed once just before exposure (e.g. when moving the colonies into the cages) and once at the end of exposure. However, due to their confinement post-treatment assessments are of limited use unless the trial has been specifically designed to address this (e.g. OECD guidance document 75 [7]). Other assessments should be made as appropriate to the type of test product and the test design. As the colonies are confined and their foraging activity is greatly restricted, additional endpoints that are sometimes included in longer-term, full field trials e.g. pollen and nectar storage and hive weight development, are generally not appropriate for cage tests. If such restrictions represent a significant limitation in the context of the study objectives it may be necessary to go straight to a field trial (an option always available within the context of the risk assessment scheme). Residue analysis may be appropriate in specific cases to verify exposure e.g. systemic compounds. Record temperature, humidity, rainfall and cloud cover at appropriate intervals throughout the assessment period (in the cages where appropriate). Alternatively, use data from the nearest official weather station.

If it is appropriate to follow the colonies for longer periods (e.g. to assess colony development or to consider the possibility of delayed effects or delayed exposure from stored pollen/nectar) they will need to be moved into the open at another site. The hives of all treatment groups should be set up together at the same post-treatment location where no further pesticide exposure is expected (i.e. no flowering crops present), in order that they are not exposed to different location-specific factors. The collection of untreated pollen and nectar from non-crop plants by the test colonies at this stage cannot be avoided and reflects normal field conditions.

Results

Repeat tests where control mortality is excessively high and also where effects in the toxic standard treatment are low*****(see Footnote p. 41). While there should be a statistically significant increase in effects with the toxic standard compared to the untreated control (as appropriate to the mode of action of the compound) the actual level will depend on the trial conditions (e.g. the attractiveness of the test crop) and so it is not always appropriate to set a required level.

Mortality, behavioural and colony assessment data must always be provided and any other data which is relevant to the properties of the product being tested. Adjustments may be needed for differences between colonies in pre-treatment levels of some parameters e.g. mortality and foraging levels.

Statistical analysis should normally be performed using appropriate methods, which should be indicated. If statistical analysis is not used, this should be justified. When interpreting the results, it needs to be recognised that there are endpoints which are intrinsically suitable for statistical evaluation (e.g. mortality data) whereas others may be not (e.g. behavioural endpoints). In addition, the evaluation needs to consider the range of parameters assessed and their relative importance, which will depend on the specific objectives and design of each study and must be considered on a case-by-case basis. The evaluation of the results also needs to take into account the biological significance of any effects seen in the context of each colony and the test conditions and this will involve some degree of expert judgement.

Field tests

As for semi-field studies, field testing may be required as a result of a number of possible reasons e.g. the Tier 1 risk assessment based on hazard quotients, systemic activity, concerns about potential brood effects or based on the results of cage studies. Again, it is important that this guideline is interpreted with appropriate flexibility to ensure that the specific requirements are addressed and that the aims and objectives of each field study are clearly specified

Experimental conditions

Principle of the trial

Honey bee colonies should be placed in or on the edge of large test fields of flowering crops. The fields should be chosen so that bees are mainly exposed to the flowering field in which the hives are placed. Test fields should be well separated to minimise bees foraging on neighbouring treatments. The treatments are applied to separate test fields, normally during the daytime when bees are foraging most actively. However, this may be modified if appropriate for the objectives of the study e.g. when testing systemic compounds applied pre-flowering or for assessing mitigation measures.

A toxic standard is usually not suitable for field trials. In specific cases a toxic standard known to present a high hazard to bees may be used. In those cases where a toxic standard is not included, it should be demonstrated otherwise that bees have been exposed. Reference products that present known hazards to bees may also be included for comparison with the test product. Assessments are made to assess possible effects on the bees shortly before and several times after application.

As with the semi-field tests, it is intended that this guideline should be interpreted with appropriate flexibility to accommodate differing requirements arising from initial (lower tier) assessments. The aims and objectives should be clearly identified to reflect this.

Selection of the crop

In the first instance, rape, mustard, *Phacelia* or another crop highly attractive to bees should be used as test plants in the case of a standard field trial based on acute toxicity. In other cases, identification of a surrogate (worst-case) test crop may be more difficult e.g. for systemic compounds, where the test crop should be one

for intended use. Other factors may then need to be considered when extrapolating between crops (e.g. plant metabolism data). Less attractive crops (on which use of the product is proposed) may be appropriate e.g. if significant effects are seen or expected with the standard attractive crops. This will have implications for the design and conduct of the study e.g. a toxic standard may not be appropriate and the levels of foraging expected will be lower. Normally, treatments should be applied when the test crop is in full flower except where justified e.g. when recommended product use is pre-flowering.

Trial conditions

Place the colonies in or on the edge of the flowering crop on which exposure will take place. In the case of applications during flowering, the colonies are placed in position approximately 2-3 days before the trial to ensure that bees are foraging mainly in the test plot on the day of treatment, as bees tend to begin foraging in areas immediately adjacent to their hives. The trial schedule should take into account the flowering (exposure) period of the specific test crop being used. In other cases, the timing for the placement of the colonies will depend on the specific trial objectives e.g. at the start of exposure in the case of systemic compounds. During spray applications, the test hives should be protected from spray drift.

Preparation of the bees

Use healthy, well-fed, queen-right colonies in normal condition that contain at least 10,000 to 15,000 bees, according to the season. Each colony should cover at least 10-12 frames, including at least 5-6 brood frames (nectar/pollen stores should not be excessive, especially where brood effects are a specific objective of the study). If colonies differ in size, ensure equitable distribution between treatments. Specific colony size and set-up may be adapted according to local beekeeping practice.

Design and lay-out of the trial

Treatments: product(s) to be tested and an untreated control; reference product(s) that present a known hazard to bees may be included, for comparison. As a toxic standard is normally not included, honeybee exposure should be otherwise demonstrated e.g. by evidence based on assessments of foraging bees before and after application (collecting pollen and marking bees in the field or at the hive may also provide useful information in this respect).

Plot size: The area of each plot required will depend on a number of factors e.g. the number and size of colonies, the crop type and seasonal timing, but should be large enough to provide sufficient forage to ensure appropriate exposure of the test bees. In the case of the standard attractive crops, 2500 to 5000 m^2 for *Phacelia* and approximately 1 ha for rape and mustard are appropriate. This should be considered in relation to the total number of bees (proportion of the foraging population) exposed. In the case of *Phacelia*, plots may need to be irrigated to ensure that the crop remains sufficiently attractive. Plots should be well separated to avoid bees foraging on the wrong plot (2-3 km depending on local conditions) but should be as homogenous (e.g. microclimate, exposure and surrounding landscape) as reasonably practicable. The distance between plots should be recorded. The plots should not be close to other flowering crops or non-cultivated areas which are significantly attractive to bees. As a guide the same separation distance as for the test plots should be considered, taking into account the size and attractiveness of the other crops or non-cultivated areas. Bee attractive weeds in the vicinity of the test plots cannot be avoided but it may be useful to record them during the exposure phase when considered significantly abundant.

Replicates: although very desirable, replication is often not feasible because of the requirements for separation.

Number of colonies per treatment/plot: Use at least 4 colonies per treatment (related to plot size and attractiveness of crop). Additional colonies may be needed for specific purposes e.g. for pollen traps. No large apiaries should be present in the area around the trial plots and if bee colonies other than those used in the study are present in the immediate vicinity, they should be recorded.

Application of treatments

Test Product(s): use formulated products only.

Toxic standard/Reference product(s)

A toxic standard is usually not suitable for field trials. In specific cases a toxic standard known to present a high hazard to bees may be used. In those cases where a toxic standard is not included, it should be demonstrated otherwise that bees have been exposed. Reference product(s) that present known hazards to bees may also be included for comparison with the test product.

Timing of application

Application timing should depend on the study objectives. Thus, for a standard field trial based on acute toxicity, the treatments should be applied during the daytime when bees are demonstrated to be actively foraging on the test crop. This may be modified e.g. when testing systemic compounds applied pre-flowering (seed dressings and soil applied products) or for assessing mitigation measures. Treatments should be applied in as short a time period as technically feasible, ensuring that conditions during application on the different plots are reasonably similar. Ideally, there should not be any rainfall before the treatments have dried e.g. for about 2 hours after application.

Shortly before application the number of bees per m^2, and how the assessments are carried out, should be recorded. Where a toxic standard has not been used, a foraging density of ideally at least 5 bees/m^2 on *Phacelia* or 2-3 bees/m^2 on rape and mustard (for the crop areas given in section 1.5) should be recorded shortly before application in order to verify exposure. These figures should not be used as validity criteria on their own. Lower figures should be explained and considered with other evidence of exposure. When assessing exposure, it should be remembered that foraging density may be affected by the total area available but at the colony level it will be determined by the total number of bees foraging on the test plots. However, in other cases foraging levels need to be related to the specific conditions of the trial e.g. for less attractive crops and pre-flowering application of systemic compounds (where exposure is related to a more sustained period that takes into account the duration of flowering).

Application rates

The product should normally be applied at the highest rate recommended for the relevant field use. Lower application rates may be applied e.g. if the off-crop risk needs to be assessed (using drift rates of application) or when exposure on weeds in orchards are tested (ground deposition rates). Volume of application and nozzle type should be as recommended and should be reported. Normally a single application will be sufficient when using a standard attractive crop. Multiple applications (according to the GAP) may be appropriate in specific cases e.g. for sprayed compounds that have the potential to move to the flowers via foliar uptake.

Mode of assessment and recording

Meteorological data

Temperature and humidity should be recorded at appropriate intervals throughout the trial period either at the trial site or at the nearest official weather station. Rainfall and sunshine or cloud cover should also be reported.

Type, time and frequency of assessment

Type

The precise nature of the assessment regime used in a particular field trial will depend on its specific objectives. The following parameters should always be assessed: flight and/or foraging activity in the crop as given by the number of bees/m^2 (by monitoring a fixed area e.g. 1 m^2, or using transects in the crop, in both cases for a defined period); general behaviour of bees on the crop and around hives using a standardised

approach; mortality of bees (using dead bee traps and possibly also on water-permeable sheets placed in front of the hives and in the crop); colony status/development (including consideration of disease and *Varroa* levels) at test initiation and test termination. These should be regarded as the core endpoints, which are particularly relevant for the interpretation of all field trial results.

In some cases, according to the requirements of the study, it may be appropriate to also include additional assessments: pollen collection (e.g. by using pollen traps or by other appropriate methods); pollen and nectar storage; hive weight development; more detailed brood assessments; specific behavioural observations and determination of residues in relevant bee and crop matrices (e.g. dead bees, nectar, pollen, wax and/or honey).

Time and frequency

Pre-application assessment: at least twice for mortality and flight activity (once for in-hive assessments); one should be carried out immediately before application in the case of spray applications during flowering.

Post-application assessment: field observations e.g. mortality and flight activity should be conducted at several intervals, preferably daily but at least 0, 1, 2, 3, 5 and 7 days after application. In-hive assessments should be conducted up to 28 days on an approximately weekly basis (i.e. sufficient to cover one brood cycle). The precise assessment schedule will depend on the study objectives and will need to be sufficiently flexible to accommodate prevailing conditions (colony assessments in particular should not be carried out during unfavourable weather conditions). Additional assessments should be carried out if appropriate on treatment day. Assessments should in general be performed at approximately the same time of day (again, adjusted according to prevailing weather conditions if necessary), although in-hive assessments (e.g. brood and food storage) can be carried out at any time of day provided climatic conditions are suitable.

Assessments may be continued for longer intervals e.g. to assess colony development over additional brood cycles if initial effects are seen. They may also be extended to consider the possibility of delayed effects or delayed exposure from stored pollen/nectar but these are not standard requirements and should be considered in the context of the study objectives (residue analysis may indicate if residues are occurring in food stores). In such cases the hives used in a study may need to be removed from the test plots (i.e. after the end of flowering of the treated crop) in order to maintain them for further monitoring (e.g. condition of colonies including brood assessments). The hives of all treatment groups should be set up together at the same post-treatment location where no further pesticide exposure is expected (i.e. no flowering crops present), in order that they are not exposed to different location-specific factors. The collection of untreated pollen and nectar from non-crop plants by the test colonies at this stage cannot be avoided and reflects normal field conditions.

Results

Repeat tests where control mortality is excessively high and also where effects in the toxic standard treatment (if included) are low*****. Control mortality needs to be considered in the context that natural (background) mortality in colonies can be highly variable. Also, if mortality in individual colonies is excessive e.g. due to diseases or other non-treatment related factors, these may be excluded from the analysis rather than compromising a particular test group, where this can be justified. Information on exposure can be obtained from the assessments of foraging activity. Other information may also be used to provide additional information about exposure e.g. palynological analysis of pollen from forager bees, pollen traps or combs and residue analysis of nectar and/or pollen.

Mortality, behavioural and colony assessment data must always be provided and any other data which is relevant to the properties of the product being tested. Adjustments may be needed for differences between colonies in pre-treatment levels of some parameters e.g. mortality and foraging levels.

***** The higher tier testing working group of the ICPBR Bee Protection Group will assess available data in order to provide more specific guidance on these points.

If appropriate, statistical analysis should be applied using relevant methods, which should be justified. However, due to the limitations on replication in field studies and the inherent variability in most of the relevant endpoints assessed, it has to be recognised that statistical analysis may not be feasible (this should be justified). It should also be remembered that individual hives are not replicates but that treatment effects should be considered on a plot by plot basis. Whether statistical analysis is available or not, expert judgement will be needed to assess the biological significance of any effects seen in the context of each colony and the test conditions. This will also be needed to consider the relative importance of the various parameters assessed, in the context of impact on overall colony health and the specific aims of each study.

Conclusions

While it is considered that the current assessment of pesticide risk to honeybees conducted for EU regulatory evaluations is robust and effective, it is also recognized that a continuous process of review and development is appropriate. This allows feedback from the increasing wealth of experience that has been gained over many years of implementation to be used to improve the testing and assessment. In particular, this experience has identified areas such as brood effects and systemic activity where increased emphasis may be needed, in part due to developments in the methods of plant protection. In this context, the ICPBR Bee Protection Group set up a working group to review the higher tier testing methodology provided in the EPPO standard PP1/170. It was considered that this should provide sufficient information to allow appropriate tests to be conducted and evaluated. However, it was also recognized that there are a number of different routes from the Tier 1 risk assessment level that can trigger higher tier testing and so it is also important that there should be sufficient flexibility to accommodate the specific needs of individual tests. The proposed revision of honeybee higher tier testing presented in this paper reflects the considered view of the 10th ICPBR Symposium and will be submitted to EPPO for consideration in their review process. It should however be recognised that refinement and improvement of the test guidelines is an ongoing process using feedback obtained and a consensus approach within the ICPBR Bee Protection Group. In this regards the higher tier testing working group will report back with any further recommendations considered appropriate e.g. in relation to acceptability thresholds for control and toxic standard mortality.

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