European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

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Efficacy evaluation of plant protection products
Evaluation biologique des produits phytosanitaires

Side-effects on honeybees

Specific scope

This standard describes the conduct of trials for the evaluation of side-effects of plant protection products on honeybees.

Specific approval and amendment

First approved in 1991-09.
Aligned with revised standard text in 1998.
Revision (updated with ICPBR-recommendations) approved in 2000-09 and 2010-09.

Introduction

It is important that plant protection products should be authorized for use only in ways which do not pose an unacceptable risk of harm to honeybees (*Apis mellifera*). For this purpose, it may be necessary to provide evidence during the registration process to enable the safety of the product in question to be evaluated. This standard presents several different types of test (laboratory tests, semi-field cage tests and field trials) which can be used to provide such evidence.

The description of these methods is based upon the 'Recommendations for harmonization of methods for testing hazards of pesticides to honeybees', decided by the International Commission for Plant–Bee Relationships (ICPBR) at the Symposia on the harmonization of methods for testing the toxicity of pesticides to bees held in Wageningen, NL (1980), Hohenheim, DE (1982), Harpenden, GB (1985), Rez, CZ (1990), Wageningen, NL (1993), Braunschweig, DE (1996), Avignon, FR (1999), Bologna IT (2002), York, GB (2005) and Bucharest, RO (2008).

The laboratory tests examine oral toxicity and contact toxicity of the plant protection product. The semi-field cage test and the field trial study the effects of application of the product during bee flight. As well as providing a worst-case assessment under realistic conditions of exposure, the cage test can be designed to study certain hazards to honeybees which are not possible to study by field trials, such as the effects on bees foraging the honeydew from aphids. Laboratory tests are conducted with single bees or groups of bees, while semi-field and field tests are conducted with bee colonies on a crop.

While recognizing that no single test method can provide sufficient information to classify the side-effects of plant protection products on honeybees, it is also important to stress that all these tests are not required. Because field testing is time-consuming and costly, the laboratory tests or semi-field test may serve to classify many products as definitely harmless or harmful without having recourse to field trials. The decisions on which tests to perform and on whether to proceed from one test to another will depend on the characteristics of the plant protection product, its use pattern, and the tests already performed. These decisions can be derived from a logically constructed sequential decision-making scheme (Oomen, 1986). A joint EPPO/Council of Europe Panel on Environmental Risk Assessment of Plant Protection Products has developed such schemes, including one for honeybees (OEPP/EPPO, 1993, 2003, 2010). This guideline is designed to provide sufficient information to allow the appropriate tests to be conducted and evaluated, but also to be sufficiently flexible to accommodate the specific needs of individual tests (Alix & Lewis, 2010).

Laboratory tests

1. Experimental conditions

1.1 Principle of the trial

Oral and contact toxicity of test compounds to adult worker honeybees are assessed in the laboratory. Bees are exposed to different doses of the compound by way of feeding or topical application. Mortality values are used to provide a regression line and LD_{50}.

1.2 Trial conditions

Bees are kept in holding cages that are well ventilated and easily cleaned. Plastic cages should not be used unless they are disposed of after use, because of possible contamination. Re-use of

wooden cages should be avoided unless they are very well cleaned and sterilized. Cages should not cause control mortality. Bees should be stored at a temperature of 25 ± 2°C after treatment. Relative humidity during the test should be recorded.

Bees should be kept in darkness during the whole trial period, except during assessments.

1.3 Preparation of the bees

Uniform, young adult worker bees should preferably be used. Bees should be adequately fed and from a healthy and queen-right colony. Where applicable, the last treatment against Varroa should be identified and the timing recorded. The treatment should have ended at least 4 weeks before the start of the test. Bees should be collected in a standardized way. Collection in early spring or late autumn should be avoided. Bees collected either from frames without brood, or from the flight board at the hive entrance, are suitable. Bees may also be reared in an incubator, fed with fresh or well preserved pollen and sucrose solution. The method of collection used, the age and (if known) the race of bees, and date of the experiment should be reported.

Bees may be anaesthetized with carbon dioxide for testing of contact toxicity. The amount used and times of exposure should be kept to a minimum, but complete anaesthesia should be ensured. Application should not lower the temperature of the holding cage and the bees.

1.4 Design of the trial

Treatments: either formulated products or active substances are tested. A control treated with the dosing vehicle should be included, and an appropriate toxic standard to check consistency of results (e.g. dimethoate; Gough *et al.*, 1994).

Test units: bees should be dosed individually or in groups of at least 10. They should not be confined individually for more than 1 h.

Replicates: at each concentration, at least three groups of 10 bees should be used. For limit tests, the number of groups should be increased to 5.

Concentrations: a suitable range and number of concentrations should be used in order to provide a regression line and LD_{50}.

2. Application of treatments

2.1 Oral toxicity test

2.1.1 Test product(s) The formulated product or active substance should be used, in 200–500 g L^{-1} final concentration of sucrose solution. Formulations should be dissolved or dispersed without additional solvents if possible (but, if necessary, should be included in the control at the same concentration).

2.1.2 Mode of application Bees should be starved for up to 2 h before tests. A dose of 10 or 20 µL of test solution per bee should be supplied through single-use feeders. By group feeding, bees share the test solution between themselves and so receive similar doses. There should be a maximum period of dosing (e.g. 4–6 h) to avoid mortality due to starvation.

If, at the end of this period, there is still test dose remaining, the amount should be measured. This allows the precise dose taken by the bees to be determined, which is more accurate for the LD_{50} calculation and provides information on distastefulness/repellency.

Fresh sucrose solution should be provided after the dose has been taken, and changed daily if the test period exceeds 48 h.

2.2 Contact toxicity test

2.2.1 Test product(s) The active substance should be dissolved in acetone where possible. Other solvents should be used only if the active substance is insoluble in acetone. These solvents should have been shown to be harmless to bees. Formulated material should be applied in an aqueous dispersion using an appropriate wetting agent where necessary (if used, this should also be used in the control at the same concentration).

2.2.2 Mode of application Anaesthetized bees should be treated individually by topical application. A measured amount of product should be applied to the dorsal thorax of each bee. Fresh sucrose solution should be provided after application and checked daily (replenish if necessary).

3. Mode of assessment

The treated bees should be returned to the cages. The number of dead or affected bees should be counted at 24-h intervals for up to 48 h (additional assessments at shorter intervals may be useful in specific cases), or longer if mortality is still increasing, i.e. an increase of >15% in the 24–48-h period.

4. Results

Tests should be repeated where control mortality is above 15%. Mortality should be assessed after correction for control mortality. Appropriate statistical methods should be used to analyse the results and calculate the median lethal dose value (LD_{50}), expressed in µg of active substance per bee and/or µg product per bee (when conducting the risk assessment, both exposure and toxicity should be expressed in terms of active substance or product).

Semi-field tests

Semi-field tests (involving cages, tunnels or tents, consistently referred to here as cages) 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, they 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, evaluation of the hazard of applying plant protection products to honeybees foraging the honeydew secreted by aphids, or 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.

1. Experimental conditions

1.1 Principle of the trial

Honeybees 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.

1.2 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 through which the bees cannot escape, 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). Crops on which use of the product is proposed may be appropriate as a second tier of cage testing for direct exposure (not for off-crop assessment), if significant effects are seen or expected with the standard attractive crops and if these crops are less attractive than the standard ones. This will have implications for the design and interpretation of the study, e.g. a toxic standard may not be appropriate as the expected levels of exposure (foraging) 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) are suitable.

1.3 Preparation of the bees

Use one small, healthy queen-right colony per cage containing approximately 3000–5000 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. Feeding of the colonies during the trial may be necessary depending on the available forage, and water should be offered.

1.4 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.

2. Application of treatments

2.1 Test product(s)

Only formulated products should be used.

2.2 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 h 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 five bees per 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).

2.3 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 is tested (ground deposition rates), or in cases where products 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 during flowering will be sufficient, but multiple applications (according to Good Agricultural Practice) may be appropriate in specific cases, e.g. for sprayed compounds that have the potential to move to the flowers via foliar uptake.

3. Mode of assessment

Pre-treatment assessments should be sufficient to demonstrate stable background mortality, and to show that the bees have acclimatized 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 acclimatize the bees with plants previously treated at appropriate intervals.

Conduct mortality and behavioural assessments at least 2 days prior to treatment (to demonstrate that the bees are acclimatized), 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. Flight and/or foraging activity in the cages as given by the number of bees per m^2 should be recorded by monitoring a fixed area (e.g. 1 m^2) or using transects along the length of subplots (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 standardized approach. 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) should be counted.

The condition of the test colonies (including brood status) should be assessed at least once just before exposure (e.g. when moving the colonies into the cages or shortly before treatment) and at least once at the end of exposure. However, due to the confinement of the colonies, post-treatment assessments are of limited use unless the trial has been specifically designed to address this (e.g. OECD Guidance Document 75). 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. Temperature, humidity, rainfall and cloud cover at appropriate intervals should be recorded throughout the assessment period (in the cages where appropriate). Alternatively, data from the nearest official weather station may be used.

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), so 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.

4. Results

Tests should be repeated where control mortality is excessively high or where effects in the toxic standard treatment are low.[1] While there should be a statistically significant increase in effects with the toxic standard compared with 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.

[1]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, which will be presented in its future proceedings.

Mortality, behavioural and colony-assessment data should 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.

Original (raw) data should be available on request. 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 recognized 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 should 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 for 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.

1. Experimental conditions

1.1 Principle of the trial

Honeybee 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 exposed mainly to the flowering field in which the hives are placed. Test fields should be well separated to minimize 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. A toxic standard is usually not suitable for field trials (e.g. due to national restrictions on application of products harmful to bees), but 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.

1.2 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). Crops on which use of the product is proposed may be appropriate as a second tier of field testing for direct exposure (not for off-crop assessment), if significant effects are seen or expected with the standard attractive crops and if these crops are less attractive than the standard ones. 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.

1.3 Trial conditions

The colonies should be placed 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.

1.4 Preparation of the bees

Healthy, well fed, queen-right colonies in normal condition should be used containing at least 10 000 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, equitable distribution should be ensured between treatments. Specific colony size and set-up may be adapted according to local beekeeping practice.

1.5 Design and layout 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, at least 2500 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 homogeneous (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: at least four colonies per treatment (related to plot size and attractiveness of crop) should be used. 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.

2. Application of treatments

2.1 Test product(s)

Only formulated products should be used.

2.2 Toxic standard/reference product(s)

A toxic standard is usually not suitable for field trials (e.g. due to national restrictions on application of products harmful to bees), but 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.

2.3 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 h 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, ideally a foraging density of at least five bees per m^2 on *Phacelia* or two to three bees per 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).

2.4 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 is tested (ground deposition rates). Volume of application and nozzle type should be as recommended and should be reported. Normally, a single application during flowering 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.

3. Mode of assessment and recording

3.1 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.

3.2 Type, time and frequency of assessment

3.2.1 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 per 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 standardized 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 also to 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).

3.2.2 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), so 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.

4. Results

Tests should be repeated where control mortality is excessively high or where effects in the toxic standard treatment (if included) are low.[1] 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 data 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 should 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.

Original (raw) data should be available on request. If appropriate, statistical analysis should be applied using relevant methods, which should be indicated.

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 recognized 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 or not statistical analysis is available, 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.

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