Section II: Developments in laboratory, semi-field and field testing for honeybees

2.1 Developments in testing methods for use in risk assessment with the new EFSA guidance document

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Abstract

The new guidance of EFSA will result in a more complex, but a substantially more comprehensive risk assessment for pollinators. The guidance document suggests the implementation of a tiered risk assessment scheme. Each tier ensures that the appropriate level of protection, set by the risk managers, is achieved. For the lower tiers, a number of laboratory studies are required which include the use of non-validated test methods. The on-going activities of ICPPR in this area are recognised by EFSA. The guidance also includes a number of new requirements for the design of field studies. It is acknowledged that some of them are very challenging, especially to ensure the required exposure level and the statistical robustness. However, it is worth noting that those requirements are scientifically sound and are necessary to properly satisfy the protection goals. The guidance document provides recommendations that will assist in addressing those requirements. There is a need for field studies to be more exact and much more controlled in the future.

Keywords: EFSA, guidance, bee, pollinator, exposure, statistic

Introduction

The currently used risk assessment schemes for pesticides [1,2,3] are considered not able to address the risk to pollinators in a comprehensive way. This indicated the need to review the current risk assessment schemes and to develop new, more sophisticated ones. As a response to this regulatory challenge, the European Commission asked EFSA to develop guidance for pesticide risk assessment for bees. The mandate specified that the guidance document should consider:

- *Apis mellifera, Bombus* spp. and solitary bees
- Acute and chronic effects, including the colony survival and development
- The estimation of the long term effects due to exposure to low concentrations
- The development of a methodology to take into account cumulative and synergistic effects
- The evaluation of the existing validated test protocols and the possible need to develop new protocols, especially to take into account the exposure of bees to pesticides through nectar and pollen

From these requirements, it was clear that the new risk assessment schemes must be much more complex and comprehensive than any guidance previously used. For example, it became immediately clear to the dedicated working group of EFSA that acute honey bee tests alone were not sufficient as a starting point (i.e. not even for a screening step).

Setting protection goals is the remit of the risk managers in the EU. As agreed by them, the so-called specific protection goals were defined as tolerated effects on bee colonies up to 7% in terms of reduction of colony size (or reduction in wild bee populations). Forager mortality should not be increased by a factor of 1.5 for six days or by a factor of 2 for three days or by a factor of 3

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for two days compared with controls. The exposure assessment goal was defined as 90th percentile worst-case considering colonies (populations) at edges of treated fields in the area of use of the substance [4].

This new guidance document [5] was issued on July, 2013, but has not yet been adopted for use in regulatory risk assessments.

Structure of the risk assessment schemes and the required toxicity endpoints for lower tiers

The guidance document suggests the implementation of a tiered risk assessment scheme with relatively simple lower tiers moving to more complex higher tiers (screening, first tier, second tier, highest tier). Each tier ensures that the appropriate level of protection set by the risk managers is achieved. For the lower tiers (screening, first tier), a number of laboratory studies are required including the use of non-validated test methods such as a 10-day chronic test or a repeated exposure larval test on honey bees. Also, some even newer elements such as studying the development of the hypopharyngeal glands (HPG) and the potential accumulative effects are required. An overview on the required laboratory tests for honey bees is presented in table 1.

The situation for wild bees is less advanced since only a few promising test methodologies were available in the open literature. Nevertheless, some laboratory methodologies were recommended by the guidance document as outlined in table 2.

Where no validated protocols were available, first proposals for test protocols were included in appendices of the guidance document. These are based on potential methods outlined in the published literature. However, it is important that fully validated test protocols are developed in the near future. The on-going activities of ICPPR in this area are recognised.

Table 1 The required laboratory tests for honey bees

Test type	Outline (age / route of exposure / length of exposure / endpoint)	Method
Acute	Adult / oral and contact / single exposure / LD50	OECD 213, OECD 214, EPPO 170
Chronic + HPG	Adult / oral / 10-day exposure / LC50 (for chronic) and NOEC (for HPG)	Appendix O on the basis of: Decourtye et al. 2005, Suchail et al. 2001, Thompson p.c., CEB 2012
Larva	Larva / oral / 5-day exposure / NOEC	OECD 237, OECD draft guidance document on repeated exposure
Cumulative effects	Adult / oral / variable exposure length / qualitative	Appendix O applying the principles of the 10-days chronic test

Table 2 The required laboratory tests for bumble bees and solitary bees

Test type	Outline (age / route of exposure / length of exposure / endpoint)	Method
Acute (bumble bee and solitary bee)	Adult / oral and contact / single exposure / LD50	Appendix P and Q on the basis of OECD 213 and OECD 214
Queenless microcolony test of bumble bee	Adult + larva / oral / 60-day exposure or less / NOEC	Appendix P on the basis of Mommaerts et al. (2010) and Laycock et al. (2012)
Larval oral toxicity test of solitary bee	Larva / oral / developmental period / NOEC	Appendix Q on the basis of many publications as analysed in EFSA, 2012 [6]

Higher tiers – issues with the field study design for honey bees

Higher tiers include refinement of the exposure estimate (second tier) or the use of effect field studies (highest tier). As regards to the design of effect field studies, there are some new

considerations given in the guidance document compared to EPPO 170 [7]. Some of them have been intensively discussed by the stakeholders. Two issues in particular have been highlighted to be extremely difficult to achieve in reality. These are the recommendations regarding the exposure of bees in the effect field studies and the sensitivity of the study to reveal such a small effect as 7% reduction in colony size.

How to satisfy the exposure protection goal

The exposure assessment goal was defined as 90th percentile worst-case considering colonies at edges of treated fields in the area of use of the substance. In the area of use of the substance, each individual field will provide a different exposure situation. This is because the residues brought back to the hive will depend on several factors, such as the quality and quantity of feed items offered by the field, local weather conditions or the alternative bee pastures available in the surrounding area. The guidance document recommends a simplified method to estimate the range of exposures (focusing on the oral route of exposure). This should be done by residue measurements (pollen and nectar) from returning foragers in at least 5 representative fields in the area of use of the substance. In order to avoid bias, in the surroundings of these fields, the alternative bee pasture should be minimal. From the collected residue data, the 90th % highest (worst case) residue levels should be established (i.e. highest from the 5 locations).These data will be considered as a kind of benchmark that will be used to compare the exposure in the effect field study. Alternatively, residue data directly from the crops could be used. The advantage of this alternative solution is the independency from the landscape. Conversely, this also has the disadvantage that dilution will not be accounted for. Nevertheless, the requirement for the minimal alternative foraging area, in order to avoid considerable dilution in residues brought back to the hive, should always be considered in the effect field studies.

The approach discussed above is considered by many stakeholders as impractical since it is very difficult to find potential test fields with sufficiently low alternative foraging area and to ensure a low level of dilution. EFSA acknowledges these concerns. However, if the dilution is too high, the 90th %-tile exposure case will not be achieved with the result that the assessment goal agreed by the risk managers will be breached and the study will not cover many realistic situations.

A number of recommendations to improve this situation is included in the guidance document. The use of larger test fields, the use of an attractive test crop or the removal of the majority of food stock from the hives before the test, can all help to encourage the bees to focus their foraging activity on the test fields. Although not specified in the guidance document, therefore not part of the official opinion of EFSA, the following may also be considered: placing the hives in the middle of the field, choosing test sites in monoculture areas of another crop that is not attractive at the time of the study, over-spraying attractive alternative areas, growing *Phacelia* to flower in a period when relatively low alternative food is available in the landscape.

To further improve this situation, the guidance document recommends to always measure the residues from the crop and from bees entering the hive and additionally providing a description of the surroundings of the field. These data may potentially be used in future to establish default dilution factors for different landscapes. If these data are available, they may also be used in the short-term for a weight-of-evidence based risk assessment.

How to satisfy the statistical requirement

The protection goal for effects is defined as tolerated effects on colony size of up to 7% percent. Also, forager mortality should not be increased by a factor of 1.5 for six days or a factor of 2 for three days or a factor of 3 for two days compared with untreated controls. Whether a field study is able to cover these protection goals should be statistically underpinned. To support this, a statistical equation was included in the guidance document with some examples. The example, which focuses on the colony size, resulted in a requirement for a high number of repetitions (in terms of colonies and test fields). The feasibility of this requirement has been heavily criticised

since the publication of the guidance document. Obviously, the statistical power of a study is largely dependent on the variability of the main parameters. Normally, honey bee colonies and agricultural landscapes are very variable compared to the maximum tolerated effects of 7% percent. Increasing the number of repetitions is always a possible and valid solution in such cases. However, the guidance document recommends increasing the sensitivity and exactness of the biological observations instead of ad infinitum increasing the repetitions. The fact that the biological observations used in field studies previously conducted are not very precise is also a considerable source of variability. A number of recommendations is included in the guidance document that will assist in reducing variability from different sources. For example, the test fields (treated and control) should be as similar as possible in terms of size and surroundings. The bee colonies at the beginning of the test should be as similar as possible in terms of size, health status, genetic background or composition. Additionally, it is recommended that the test hives should be randomly allocated to control and treated groups. Although not specified in the guidance document, therefore not part of the official opinion of EFSA, the following may also be considered: to keep the distance between treated and control fields as small as possible (e.g. 4-5 kilometres), to use the same variety of the crop and ensure that the same agronomic practice is used (i.e. sowing time, plantation rate, weed control, etc.), to have control on a big apiary and handle the colonies continuously the same manner early before the start of the test, to collect data early before the start of the test and use this data for a selection of the test hives.

As regards to the more precise biological observations it is recommended to continuously measure the weight of the hives during the test and check the forager losses by tagging a number of them. Using automatic bee counters may also be a good idea.

Another solution would be to focus on forager mortality. A study which focuses on the forager mortality is feasible, as indicated by another example in the guidance document, but may have been overlooked by the critical stakeholders. Nevertheless, such a study will not automatically satisfy the protection goal for the colony strength. Therefore, if this solution is chosen, the link between the forager mortality and colony strength would need to be considered. Currently no fully accepted methods exist for this link. Population models may be used for this purpose, although currently no validated models are available. Reasonable modelling exercises, supported by direct observations on the colony strength, and expert judgement may satisfy the regulatory needs, even if the statistical requirements for the colony strength were not fully addressed.

Alternative solutions

The following points may be considered in future for risk assessment. However it is important to note that these points were not considered in the guidance document, therefore are not part of the official opinion of EFSA, nor have they been challenged in regulatory context:

- Conduct a large number of effect field studies on randomly chosen sites. It is possible that a number of studies, which are not considered to be sufficiently robust alone, could be used in combination for risk assessment, as the dataset as a whole may counterbalance the limitations of the individual studies
- Use field tests on wild bees as surrogate of honey bees (smaller foraging distance, single repetition is 'smaller' and cheaper)
- Fit for purpose and validated population models

Discussions and conclusions

The new guidance document prepared by EFSA includes sufficiently comprehensive risk assessment schemes for bee pollinators. The guidance document suggests the implementation of a tiered risk assessment scheme. The protections goals set by the risk managers are fully respected by each tier. For the lower tiers, a number of laboratory studies are required including the use of non-validated test methods such as a 10-day chronic test or a repeated exposure larval test on

honey bees. As an interim solution, it is recommended that methodologies developed by scientists and reported in the open literature are followed. Of course further developments and standardisations are awaited. The on-going activities of ICPPR in this area are recognised by EFSA.

There are a number of new requirements for the design of field studies compared to EPPO 170. It is acknowledged that some of them are very challenging, especially to ensure that the required exposure level and the statistical robustness are achieved. However, it should be noted that field studies as conducted previously, are not robust enough to detect effects with the necessary accuracy. Therefore, there is a need for field studies to be more exact and much more controlled in the future. The guidance document provides a number of novel recommendations that will assist in addressing these requirements. For example, considerations are outlined for more synchronised colonies and for more exact measurements of the biological parameters.

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