Proposal of the ICPBR Bee Brood Group for testing and assessing potential side effects from the use of plant protection products on honey bee brood

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Introduction

There have been recent developments in toxicity testing of plant protection products on honeybee brood. These need to be assessed in terms of methodology and for suitability for inclusion in a sequential risk assessment scheme. According to EU Council Directive 91/414 EEC, regulatory testing and risk assessment on bee brood is required only when bees are exposed to IGR (Insect Growth Regulators) like pesticides. However, bee larvae may also be at risk by exposure to other types of substances. Concerns have been raised that the current testing and risk assessment schemes for honey bee brood fully address issues. As a consequence, a working group (participants are the authors of this paper) was constituted at the ICPBR meeting in York in October 2005. The remit of this working group was to evaluate recent methodological developments on honey bee brood testing and risk assessment and to integrate these into the current risk assessment where appropriate.

Background

Larval development or brood success is a vital part of the survival and/or productivity of honeybee colonies. As an overall protection goal it has to be assured that there are no unacceptable effects on bee brood impacting colony vitality. However, no specific trigger values for certain endpoints and for effects on bee brood have been established or are commonly recognized. Expert judgement is still an essential tool and basis of risk assessment.

Analysis

The spectrum of plant protection products that are relevant for bee brood testing are IGRs and other substances showing a higher intrinsic toxicity to larvae than to adults. Compounds with a high intrinsic toxicity to larvae as well as to adults are already covered by the current risk assessment and testing scheme. In order not to overlook unintentional side effects on larvae, PPPs showing pronounced larvicidal activity/effects on juvenile stages of insects (based on available screening and efficacy data and the results of non-target arthropod testing) should also be considered.

Recommendations

Two recent methodological developments - the *in vitro* laboratory bee larval test (Aupinel et al., 2005) and the bee brood semi-field test (OECD Guidance Document 75) - have been additionally considered by this ICPBR working group for integration into the risk assessment.

In terms of methodology, the ring testing of the *in vitro* laboratory larval test is still ongoing. However, once ring testing of this method is completed it may be considered as a tier I method in order to test pesticides for intrinsic larvicidal effects. In order to implement this test in the risk assessment scheme under consideration of the relevant endpoint, and for determination of relevant exposure figures and definition of appropriate TER values (Toxicity/ Exposure Ratio), a validation versus higher tier testing will have to be conducted.

The existing brood testing method (Oomen et al., 1992) method was designed to test for intrinsic larvicidal effects and is based on an unrealistically severe exposure of honeybee colonies to the tested compound. Therefore, it is considered as a type of intermediate tier brood test employing more realistic conditions than a laboratory test, but not as a semi-field or field test. For the time being the Oomen et al. method should remain as one option for testing of bee brood. Although this method was never formally validated, it has in almost 15 years of use proven to be a reliable tool to detect intrinsic larvicidal properties of compounds.

The semi-field brood tunnel test (OECD Guidance Document 75, based on Schur et al., 2003) provides a more realistic worst-case by exposure of the bees to a treated crop. This method was validated for spray products by ring-testing and had been accepted as an OECD Guidance Document in 2007. It can be used in the tiered testing scheme as higher tier test and should be integrated into the revised version of EU Directive 91/414 EEC as a test for bee brood evaluation.

In any case, field trials should remain the highest tier within the sequential honeybee risk assessment scheme for testing of brood effects. In order to address specific brood effects, available evaluation methods on brood development should be integrated into the field trial design. As established in the tiered honeybee risk assessment scheme, results from lower-tier studies are superseded by higher tier results, and lower-tier studies can be omitted, if higher tier testing is carried out initially.

Conclusion

The established sequential tiered risk assessment scheme for honeybees has proven to be successful concerning the protection of honeybees. The aforementioned proposals concerning evaluation of potential effects to bee brood should be integrated as a refinement of the current EPPO risk assessment scheme. The aforementioned recommendations of the ICPBR Bee Brood Group were established in consistency with the recommendations of other ICPBR working groups (Risk Assessment for Systemic Compounds, and Higher-Tier Testing) and give some guidance for a harmonized risk assessment scheme at the European level.

References

Aupinel P, Fortini D, Dufour H, Tasei JN, Michaud B, Odoux JF and Pham Delègue MH, Improvement of artificial feeding in a standard in vitro method for rearing Apis mellifera larvae. Bull Insectol **58 (2)**: 107-111 (2005).

Directive 91/414/EC Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market, Official Journal L 230 , 19/08/1991 pp. 0001 – 0032.

EPPO standard PP 3/10(2): Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees, revised in 2002-09. Bulletin OEPP/EPPO Bulletin **33**: 141-145 (2003).

Oomen PA, De Ruijter A and Van der Steen J, Method for honeybee brood feeding tests with insect growth – regulating insecticides. Bull OEPP/EPPO Bull **22**: 613-616 (1992).

OECD (2007): Guidance document on the honeybee (Apis mellifera L.) brood test under semi-field conditions. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 75, ENV/JM/MONO (2007) 22.

Schur A, Tornier I, Brasse D, Mühlen W, Von der Ohe W, Wallner K and Wehling M, Honey bee brood ring-test in 2002: method fort the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions. Bull Insectol **56 (1)**: 91-96 (2003).