

## **Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches**

### **Executive Summary**

The crop protection industry recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013 is not a realistically feasible way forward. It is based on extremely conservative assumptions, its study requirements lack clarity and are not workable and guidelines for a number of studies are unavailable or not validated. Industry therefore believes that a revision of the assessment scheme for use by regulatory authorities is needed. Building on an analysis of the proposed developments in the EFSA Bee Guidance Document, we suggest a proactive and practical approach.

This new approach is summarized in the following overview. It provides a comparable level of protection to the EFSA approach and is based on the current scientific state of the art for bee pollinator risk assessment. Key features of this approach are the focus on honey bees as a representative species, the definition of core data packages, concentration on main exposure routes and the proposal of more realistic assumptions for the risk assessment process.

Industry believes that this *Practical Approach* is both a realistic and protective way forward for bee risk assessment and would welcome the opportunity to engage in a technical discussion with Member States experts and EFSA on this topic in order to help establish a workable and protective solution as soon as possible.

## Introduction: EFSA bee guidance document and industry impact analysis

Following the publication in July 2013 of the new EFSA Guidance document on the risk assessment of pesticides on bees, Industry started an evaluation of the impact of the proposed screening and tier I risk assessments on the pass/fail rate of currently available active substances on the EU market. This analysis considers 151 active substances covering 163 uses: 52 were herbicides, 52 fungicides, 51 insecticides or acaricides and 8 other uses like plant growth regulators. Solid applications were also considered with 20 active substances representing 36 uses. The analysis also considers risk assessment for *Bombus* spp. and solitary bees. A series of worked examples were included in the analysis in order to illustrate concrete examples for herbicides, insecticides and a fungicide. Several suggestions are included in this analysis on how to improve and correct the risk assessment presented by EFSA in their guidance document.

The multiple exposure routes, numerous endpoints and calculations required by this EFSA document make the resulting guidance incredibly complex and over-burdensome for the user. In addition, the proposed guidance multiplies worst case assumptions for nearly all aspects (e.g. residues, level of exposure, food intake, calculation of trigger values), resulting in a risk assessment, which is over-conservative and not representative of the actual risk under real field conditions.

### Key findings of the ECPA impact analysis:

- ➔ Almost all substances and uses fail the screening step for chronic risk to larvae and chronic risk to adult honey bees for both spray and solid application types.
- ➔ For bumble and solitary bees very few substances pass the acute screening step and none pass for chronic risk assessments.
- ➔ Even known non-bee-toxic substances fail the risk assessment and would need higher tier refinement. In order to pass the assessment, the required doses that would have to be tested would be so high that they would not be technically (solubility) or practically (consumption by the bee) achievable.

This 100% failure at tier 1 is made worse by the lack of practicable or available testing methods for higher tier refinement:

- Many of the laboratory test methods required by the guidance document were either not available or not fully developed for regulatory purposes. Methods for larvae and chronic exposure for adult honey bees have been developed but these are technically challenging tests with limited global capacity for testing.
- Higher tier semi-field and field methods for honey bees proposed by EFSA are impractical (e.g. number of replicate field plots required) and would require the use of an excessive and disproportionate number of honey bee colonies (i.e. harm to bees).

- There is also an absence of robust and reliable guidance how to conduct higher tier testing options for bumble bees and solitary bees.
- In addition, there is currently insufficient testing capacity available in Europe to meet the large increase in both laboratory and field study requirements in the EFSA 2013 guidance document.

In its present form, the EFSA guidance will virtually make it impossible to register any new or existing insecticide, as well as many herbicides and fungicides. Industry believes that further work and significant revision are required to build a pragmatic, applicable and consistent guidance document within the regulatory framework and has invested much time and money in developing a practical alternative based on the same science.

### ***Ongoing collation of the recent application of the EFSA bee guidance document to AIR 3 assessments***

For the assessment of active substances at EU level, it was proposed by EFSA to perform a Tier 1 risk assessment for chronic risk to adult honey bees and honey bee larvae according to the scheme described in the EFSA 2013 guidance document for bees (refer to the technical report of Pesticides Peer Review Expert Meeting 133<sup>1</sup>). However, not all experts were present at this meeting and even for member states that were present there was no real agreement on the proposal. Attached is a file summarising the outcome of the EFSA conclusions of 31 substances evaluated since the meeting in 2015. A total of 29 substances have been evaluated according to EFSA 2013 and although industry made great efforts to apply the data requirements and also conduct (when necessary) high quality higher tier studies, EFSA concluded either that there was a high risk to bees or that a high risk to bees could not be excluded. However, in the majority of cases member state experts concluded there was sufficient evidence to conclude to an acceptable risk to bees.

This includes evaluations for 18 herbicides which are of low toxicity to bees and other arthropods and are applied either out of flowering or to crops which are not attractive to bees (e.g. sugar beet and cereals). Consequently, the application of the EFSA 2013 bee guidance has resulted in the situation which was predicted by the industry impact analysis and clearly a revision of the EFSA bee guidance is required.



EFSA conclusions  
2016 bees 23 05 2017

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<sup>1</sup> EFSA, 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

## Proposed Scheme:

### A. Tier 1 Laboratory Data requirements based on (EC) Regulation No. 1107/2009 and EFSA approach

Testing should focus on honey bee testing only since a full set of testing methods is available, which have been sufficiently tested and validated (see [Technical Section 1](#)).

For bumble bees and solitary bees methods are only in development stages (see [Technical Section 2](#)). However, an acute test for bumble bees is soon to become available via the OECD. Conservative exposure approaches for honey bees will provide a high level of protection for other bees until validated methods are available for the other species (see [Technical Section 2](#)).

#### Core data package for active substance

- Acute oral toxicity study for adult honey bees (OECD 213).
- Acute contact toxicity study for adult honey bees (OECD 214).
- Chronic oral toxicity for adult honey bees (Draft OECD Test Guidance).
- Acute toxicity to larvae (data requirement in North America and Brazil). However, Acute testing on larvae is not needed if repeated exposure test is available. (OECD 237)
- Repeated exposure toxicity to larvae (OECD Test Guidance 239).

#### Testing of formulations

The approach below is more in line with other international regulatory requirements.

- Focus on acute testing only (OECD 213 and 214).
- If the formulation is more toxic than the active substance (by a factor of 5), then additional laboratory chronic/larval testing should be carried out with the formulation following the recommendation of EFSA 2013.

#### Testing of metabolites

- Testing of metabolites should be driven by an examination of existing data on other organisms and biological screening.
- The higher exposure level of the parent will compensate for any higher toxicity of the metabolite and therefore the risk will already be covered in the majority of cases.
- Testing should focus on metabolites of insecticides as for example, a herbicide with virtually no toxicity to bees or other arthropods is highly unlikely to suddenly gain insecticidal properties during its degradation processes.
- Testing should focus on acute oral toxicity as a screening test and further studies (e.g. adult chronic or larval toxicity tests) only required if the metabolite is confirmed to be significantly more toxic than the parent (10x fold).

## B. Tier 1 Risk Assessment for Honey bees

### Exposure Routes (see [Technical Section 3](#))

- Focus on contact with residues and ingestion of nectar and pollen from the treated crop as main exposure routes.
- Consider succeeding crops only for highly persistent, highly toxic and highly systemic compounds.
- Consider large data set showing flowering weeds within the crop are not a relevant route of exposure (see [Technical Section 4](#)).
- Consider large guttation data set showing guttation present low risk at colony level (see [Technical Section 5](#)).

### Acute Risk Adult Honey bees

Follow a tiered risk assessment approach for the main exposure routes (pollen and nectar). The findings of the Impact Assessment conducted by industry (see [introduction](#)) and a collation of the recent use of the EFSA bee guidance document to AIR 3 assessments showed that the proposed EFSA tiered approach and trigger values for acute risk to honey bees gives a very similar risk assessment outcome to the current Hazard Quotient (HQ) approach with a trigger of 50, which have been repeatedly validated and showed to be protective for spray applied products. Consequently, both approaches for the main exposure routes (pollen and nectar) have shown that they could provide a workable and protective acute risk assessment scheme. In this context, the acute trigger values proposed by EFSA may be used for the risk assessment, however they do not invalidate the use of the current HQ values, the latter being in line with the current uniform principles.

### Chronic Risk Adult Honey bees

One of the most problematic parts of EFSA Bee Guidance is the Chronic Risk ETR trigger value of 0.03 and associated assumptions/calculations, which fail almost for all of products including herbicides and fungicides (see [introduction](#)).

- Two alternative options based on more realistic but still conservative assumptions are proposed see [Technical Section 6](#) for more details).

### Option 1: Modified EPPO 2010 Approach

This is based upon the method of EPPO 2010 risk assessment for systemic substances which is cited in the regulation. EPPO 2010 recommended the calculation of a HQ (equivalent to an ETR, exposure toxicity ratio) using the following equation:

$$HQ = \text{daily dose} / \text{NOEDD}$$

Where daily dose (DD) is based on the worst case sugar need of 128 mg/bee/day of a bee feeding exclusively from nectar containing a more representative 30% sugar using the following equation:

$$\text{Daily dose } (\mu\text{g a.i./bee}) = \text{A.R.} \times [0.128 \text{ g}/(1000 \times 0.3)] \times \text{RUD}$$

A.R. = application rate in kg a.i./ha

RUD = residue per unit dose from the EFSA bee guidance. Mean  $\text{RUD}_{\text{nectar}} = 2.9 \text{ mg a.i./kg}$  (foliar sprays), Mean  $\text{RUD}_{\text{nectar}} = 0.0458 \text{ mg a.i./kg}$  (seed treatment)

Sugar content of major crops types ranges from 32.4 - 59% (oilseed rape), 45.7 – 61.3% (Phacelia) and sunflowers up to 49%, so 30% is still a conservative scenario (see [Technical Section 6](#)).

EPPO 2010 suggests a chronic TER trigger (NOEDD/daily dose) of 1 as the entity to be protected is the test species. However, triggers could be calibrated using semi-field and field data as has been done for the acute risk assessment HQ.

### Option 2: Refined EFSA approach

In EFSA 2013 the Khoury model is used to translate and increase in forager mortality to the SPG as 1.27x in hive background mortality (5.3%) over 10 days. This means the maximum increment in mortality is:

Max increment =  $0.27 \times 5.3 = 1.43$  % mortality (i.e. equivalent to more 1 dead bee in 70).

EFSA then uses a linear interpolation model to set the chronic trigger value of 0.03.

A simple refinement can be applied as the EFSA guidance assumes that zero mortality can only occur at a dose of 0 µg/bee/day. Where the data from the chronic dosing study can be used to generate both a reliable LDD<sub>50</sub> and an LDD<sub>0</sub> the existing proposed EFSA chronic trigger of 0.03 (based on the LDD<sub>50</sub>) can be achieved directly using LDD<sub>0</sub>.

However, both the EFSA approach to chronic risk and the option above are based on an unrealistic linear model which greatly overestimates the required trigger and hence the level of protection achieved. This is because true dose-response relationships are nearly always sigmoidal. Neither approach takes into account the true nature of the dose-response relationship or the slope of the dose-response.

To take into account the true nature of the dose-response relationship the EFSA approach may be used but modified to take into account compound specific properties such as the dose-response model, slope of the dose response curve and the use of either LDD<sub>50</sub> or NOEDD as the former cannot always be measured due to palatability and solubility issues. From these data, compound specific Chronic ETR trigger values can be calculated that will still meet the EFSA SPG of 1 dead bee in 70 (see [Technical Section 7](#)).

### Risk to Larval Honey bees

**The risk assessment based on EFSA (2013) does not discriminate between toxic and non-toxic compounds (see [Technical Section 8](#))**

- This is driven by exposure assumptions that are much higher than in real life (e.g. low sugar content of nectar, residues in unprocessed food, no dilution in the hive).
- ➔ Two alternative options based on more realistic but still conservative assumptions are proposed (see [Technical Section 8](#) for more details).

### Option 1: Using more representative conservative nectar sugar content, feeding and residue assumptions

Use EPPO (2010) together with EFSA mean RUD and 30% sugar (ecologically relevant scenario), LD<sub>50</sub> and trigger values of 10 (acute) and trigger of 1 (chronic) using a NOED.

Sugar content of major bee-relevant crops types ranges from 32.4 - 59% (oilseed rape), 45.7 – 61.3 (Phacelia) and sunflowers up to 49%, so 30% is still a conservative scenario (see [Technical Section 8](#)).

An assessment factor of 10 for acute risk assessment and 1 for the chronic risk assessment should be used with this option (according to EPPO 170 for chronic assessment).

### Option 2: Use concentration values rather than dose in risk assessment

The main dietary exposure route of compounds to honey bee larvae occurs via pollen and nectar. In order to put the obtained larval toxicity endpoints into perspective, the NOEL endpoints (see option 1) can be directly compared to residue data in pollen and nectar determined in the residue trials, as recommended in Regulation (EU) No 284/2013.

A comparison of the exposure concentration value based on mean RUDs with the concentration NOEC from the acute and/or chronic larval studies should be made in the risk assessment. If the data indicate lower residues than NOELs, then this indicates a margin of safety between exposure concentrations in the relevant matrices under field conditions and larval toxicity endpoints of bee larvae.

### C. Tier 2 – Exposure Refinement Study Options (see [Technical Section 3](#))

The generation of residue studies only makes sense if there is a chance to reach residue levels that can help to pass the risk assessment. This implies the revision of the chronic risk assessment (i.e. trigger values) is a necessary preamble to the proposals below, as existing case studies show that these trigger values are so over-conservative and that refinement through residue trials is virtually impossible.

#### Option 1: Field test or tunnel test refinement of exposure

- The requirement of replicated residue studies in each zone and crop is impossible to achieve.
- The analysis of these biological data as well as residue trials could also help with the definition of crop groups within which residue levels are expected to be similar.
- The development of standards for residue studies performance as a function of environmental factors that influence residue content would be a basis towards a guidance.
- Similarly, guidance on the testing protocol would be needed prior to pursue with the generation of additional data.

#### Option 2: Exposure refinement modelling (see [Technical Section 9](#))

- Allows to generate worst case exposure scenarios, using observed biology traits as a basis.
- BEEHAVE model available.
  - Ongoing development of worst case realistic exposure scenarios to be used in Tier 1 and Tier 2.
  - Pesticide module in final development phase.
- Alternative to costly field exposure studies can be used to test many more scenarios than experiments, and cover uncertainties relative to geographical differences, agricultural practices etc.
- The potentialities of modelling could be further explored in a dedicated working group to accelerate the development of the scenarios mentioned above.

### D. Tier 2 – Effect Refinement Study Options (see [Technical Section 10](#))

The available EPPO/OECD approach allows:

- Differentiated Tier 2 testing of adult bees and broods building on established testing practices in the EU.
- Delayed effects can be detected.
- Evaluated parameters available not only for individual bees but also for colonies.
- In-hive behaviour is evaluated likewise.

- Know-how and experience in EU testing organizations available, and also in the industry.

**If risk to adult bees at Tier 1:**

- **EPPO 170 tunnel study**, 7-day exposure, and monitoring of colonies over up to 2 brood cycles, 4 replicates per dose, 20 tunnels in total (number of replicates being practicable).

**If risk to broods at Tier 1:**

- **OECD 75 tunnel study**, 7 days exposure and monitor of individual development of 100 eggs to adult. Colony monitoring as in EPPO 170 for 1 brood cycle, 4 replicates per dose, 20 tunnels in total (number of replicates being practicable).
- **Oomen study**, which can deal with concentrations and allow for adaptable feeding periods. Study can be linked to residue and modelling.

## **E. Tier 3- Refinement Options - Field studies in case Tier 2 still identifies a risk (see [Technical Section 11](#)).**

**Oomen et. al, 1992 / EPA FIFRA 40 CFR, Part 160 – Colony Feeding Study**

- Allow to control the level of exposure.
- Colony feeding studies allow long-term evaluation of bee health (overwintering success).
- New study type optimized since 2013 and accepted by the US-EPA.
- Location independent.
- Can be directly linked to nectar residue studies. Link to pollen exposure needs further investigation.
- Interpretation can be facilitated by the BEEHAVE model<sup>2</sup>.
- Protocol to be designed by ICP-PR based on current EPA methodology.

**Improved EPPO 170 Field studies:**

- Workable field study design.
- Realistic reflection of use-specific scenarios (e.g. spray applications at flowering).
- ICP-PR updating existing EPPO 170 Guidance.

## **F. Protection Goals – Honey bees**

In principle, the Specific Protection Goal (SPG) which was agreed in consultation with risk managers in the SCoFCAH (Standing Committee on the Food Chain and Animal Health) and EFSA to accept only “Negligible Effects” on honey bee colonies is fully supported by industry. It is the translation of “Negligible effects” into < 7% effect on colony size however that cannot be supported. This is because such a small effect has been shown to be impossible to measure under field conditions due to background hive mortality being frequently double that

<sup>2</sup> Becher MA, Grimm V, Pernille Thorbek P, Horn J Kennedy PJ, Osborne JL. (2014) BEEHAVE: a systems model of honey bee colony dynamics and foraging to explore multifactorial causes of colony failure. Journal of Applied Ecology 51, 470-482. DOI: 10.1111/1365-2664.12222



under normal bee keeping practise. The consequence of this inability to measure such a small effect during field studies is that it is impossible for industry or any other researcher to demonstrate compliance with the protection goal under field conditions of use.

One potential solution is a new honey bee colony model called “BEEHAVE” which was recently developed and published (see [Technical Section 12](#)), which is a more sophisticated model than the original Khoury model used by EFSA to derive their Protection Goal proposal and the associated trigger values reported in the EFSA Bee Guidance<sup>3</sup>. In addition, a published paper by Thorbek et al 2016 (see [Technical Section 13](#)) described how this model showed that a reduction in forager of up to 20% would be acceptable for normal colony development. This is almost 3 times the colony size protection goal given in the EFSA Bee Guidance. This observation is supported by practical beekeeping considerations. It is common practice that beekeeper will remove brood frames and adult bees (in effect a “>10 % decline”) to build new colonies and prevent swarming. This is practical evidence that a 7% reduction is not detrimental and is easily compensated by a colony.

It is recommended that Specific Protection Goals should be investigated further and refined using BEEHAVE Model once the new BEEHAVE Pesticide Ecotox Module (currently being developed) is published and validated.

## **G. Bumble bee and Solitary Bee testing and Risk assessment**

No testing or risk assessment should be conducted for bumble bees and solitary bees until a full set of validated methods are available.

In toxicological terms the honey bee is a good surrogate for other bee species and existing data reviews highlight this (see for example Arena & Sgolsatra 2014; Thompson 2016). Testing methodology for non-*Apis* bees is under development; however robust and reproducible methods will not be available in short-medium term. Therefore, there is a need to currently focus testing and risk assessment on honey bees which is the most reliable and scientifically robust one presently available. In terms of exposure the highly conservative exposure approaches used for individual honey bees to pollen and nectar should be protective for non-*Apis* bees (Guidance [Document] for Assessing Pesticide Risks to Bees [EPA, PMRA & CDPR, June 19, 2014]). This was also the consensus of a group of international experts at a recent workshop hosted by the US-EPA (Workshop on exposure of non-*Apis* bees, US-EPA, Washington DC, Jan 2017). See technical sections 2 & 3.

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<sup>3</sup> <http://www.efsa.europa.eu/en/efsajournal/pub/3295>

## Technical Sections 1 – 15

### Technical Section 1

#### Test Method Availability

At the time of publication of the EU data requirements (Regulations 283/2013 & 284/2013) there was a lack of validated methods which were suitable for use within global and EU frameworks. Over the past 5 years industry has made significant contributions towards the development and validation of new methods. This rapid development and validation of multiple methods is unprecedented in regulatory science for example OECD 202 (Daphnia reproduction test) took over ten years to be ready for use. There is a range of new tests for bees becoming available to meet regulatory needs. With the existing test these are now often commonly referred to as the honey bee “5-pack” and are considered to meet global regulatory needs. However, not all tests are fully validated and reliable, and meet the requirement for mutual acceptable of data (MAD) as an OECD test guideline.

Table 1: Honey bee data requirements and test methods

Data point(s)	Data requirement	Test Guideline/Guidance document	Notes
8.3.1.1.1 10.3.1.1.1	Acute oral toxicity study for adult honey bees	OECD test guideline 213	Well established test and validated for global use
8.3.1.1.2 10.3.1.1.2	Acute oral toxicity study for adult honey bees	OECD test guideline 214	Well established test and validated for global use
8.3.1.2	Chronic oral toxicity test for adult honey bees	Draft OECD test guidance	Method to be adopted as an official OECD test guidance in 2017
8.3.1.3	Acute toxicity to larvae (8 day study)	OECD test guideline 237	New test method which is performing well. Can be a data requirement in North America and Brazil
8.3.1.3	Repeated exposure toxicity to larvae (22 day study to adult emergence)	OECD guidance document 239	This is only a guidance document and not a fully validated test guidance due to lower reliability. Technical challenges in regards to running the controls to adult emergence. Many studies fail to meet the 22-day validity criteria and hence need to be repeated several times to achieve a valid test

Due to high demand and limited capacity there is currently a global shortage of testing capacity which cannot at present meet testing needs. Consequently, there is a need to prioritize substances for testing. The studies listed in Table 1 should be applied to active substance testing and it is the view of industry that the acute toxicity to larvae test not necessary if a repeated dose test is available. As chronic exposure of adults and larval exposure will be to active substance it is not considered necessary to routinely conduct these tests for products. Formulations and products should continue to be tested in acute toxicity tests. However data generation for a.s. may be done with formulated product (instead of a.s.) to overcome solubility constraints.

## Technical Section 2

### Test methods for non-*Apis* bees

Test methods for bumble bees and solitary bees are being researched and under development. There is a draft OECD test guidance for acute toxicity testing with bumble bees which will soon become available. There are also draft methods and ring tests on-going for solitary bees however; standardized and validated tests are still some way from being completed. Currently there are no chronic test methods for non-*Apis* bees although a preliminary bumble bee ring test is planned for 2017 based upon the acute oral draft method. No larval methods are available for either bumble or solitary bees and the current scientific view is that a bumble bee larval test is not technically possible. A solitary bee larval test, by virtue of the biology of the preferred species (*Osmia* sp.), would have a duration of over 1 year; this does not lend itself towards the development of a robust, routine and reliable test method.

### Honey bees as a surrogate for non-*Apis* bees

Consequently, at present testing and risk assessment should therefore focus on honey bees. In toxicological terms the honey bee is a good surrogate for other bee species and existing data reviews highlight this (Arena & Sgolsatra 2014; Thompson 2016). Therefore there is a need to focus risk assessment on honey bees which is the most reliable and scientifically robust one presently available. In terms of exposure the highly conservative exposure approaches used for individual honey bees to pollen and nectar should be protective for non-*Apis* bees. This was the consensus of a group of international experts at a recent workshop hosted by the US-EPA<sup>4</sup>.

#### Considerations for non-*Apis* bees

Honey bees (*Apis mellifera*) have been recommended and considered for regulatory testing of plant protection products in EU and other regions to cover bees in general since several decades. The EPPO testing methods (EPPO 2010a) and risk assessment scheme (EPPO 2010b) for honey bees were first approved in 1991/1992. Both documents were upgraded several times with the current versions published in 2010 covering methodologies for sprayed products and seed coating and soil treatment uses. Additionally, OECD test guidelines and guidance documents were adopted for honey bees (see other sections). This resulted in established tiered testing and risk assessment schemes for honey bees that were supposed to cover the group of bees in general. With the publication of the EFSA scientific opinion on bees in 2012 (EFSA 2012) *Bombus* spp. and solitary bees (ie *Osmia*) were suggested for additional regulatory bee testing next to honey bees.

Testing methods and risk assessment schemes for *A. mellifera* for plant protection products were established due to the economic importance of honey bees as livestock (production of honey and wax and crop pollination services) and the profound biology knowledge. This was supported by the fact that honey bee colonies easily provide test organisms for regular regulatory testing worldwide. Similar knowledge on the biology, rearing, testing and risk evaluation on the other more than 2000 bee species in Europe and elsewhere is lacking or incomplete. With the commercial use of *Bombus terrestris* colonies for greenhouse (i.e. tomato) pollination some more insight into their biology was gained by colony producers like Biobest and Koppert and related general research. At the same time first efforts were undertaken to investigate the combination of bumblebee use in greenhouses with the use of plant protection products. In 1994 and 2001 Van der Steen (1994, 2001) published experiences made with laboratory and higher tier method development for *B. terrestris* testing.

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<sup>4</sup> Workshop on “Pesticide Exposure Assessment Paradigm for non-*Apis* bees” held in January 10-12, 2017, at the United States Environmental Protection Agency (EPA), Arlington, VA, USA.

The commercial availability of *B. terrestris* colonies facilitated further research with bumblebees. Similar rearing successes for other non-*Apis* species were – so far – not established and therefore initial research on testing methods on the side-effects of plant protection products are limited to selected taxa and using variable and different methodologies (eg Landurer et al. 2005 for *Osmia*).

The lack of test methods for non-*Apis* bees and consequent lack of pesticide side-effect data for these species results in limited knowledge on the sensitivity of different bee species. Therefore, efforts to extrapolate from honey bees to non-*Apis* bees in pesticide sensitivity and risk assessments are rare (e.g. Thompson & Hunt 1999, Thompson 2001, Arena & Sgolastra 2014) with indications that bumblebees are less sensitive than honey bees.

To overcome the lack of standardized, validated and internationally accepted test guidelines for non-*Apis* bees an ICPPR Non-*Apis* Working Group with participants from regulatory authorities, research institutes, industry and contract research organizations got established in 2014. The ICPPR Non-*Apis* WG focused its efforts on the exchange of testing experiences and to develop proposals for laboratory and higher tier testing methods for *Bombus* and *Osmia*. In August 2016 draft proposals for new OECD test guidelines for acute oral and contact bumblebee testing with *Bombus* were published for public commenting. These OECD test guideline proposals may be finalized in the near future and then for the first time would allow standardized testing according to widely accepted OECD test guidelines. Similar efforts are also underway for the development of acute *Osmia* test guidelines. While the development of an acute contact *Osmia* test guideline seems to be possible there are still experimental challenges to be addressed for the development of an acute oral test ensuring standardized and consistent oral uptake of test substances. The ICPPR Non-*Apis* WG is also working on the development of protocols for semi-field methods for *Bombus* and *Osmia*, and a ring test is planned in 2017, but testing experiences made so far have shown that there are numerous challenges to resolve and that it requires much more efforts until guideline or guidance proposals can be made.

In summary, there are not yet internationally adopted test guidelines available for non-*Apis* species, ie *Bombus* and *Osmia*, not even for initial Tier 1 acute laboratory testing. The development of higher tier non-*Apis* test methods is in progress, but realistically it will take several years until guideline proposals will be developed for consideration as OECD test guidelines. Also, it has to be kept in mind that the availability of adult bees (pupae for hatching and subsequent adults for acute testing of *Osmia*) is time-limited to a few months in spring/early summer. Similarly getting or producing standardized *Bombus* colonies with defined numbers of worker bees and larval stages at a given time period during the year for semi-field testing requires further efforts. Therefore, regular regulatory non-*Apis* testing under laboratory or semi-field conditions is not possible now and may not be possible in the near future. Likewise, testing capacity at experienced laboratories will be limited for future non-*Apis* testing and therefore sufficient time for data generation would also be needed before any regulatory implementation of additional non-*Apis* consideration.

In conclusion, it is currently impossible to perform non-*Apis* testing according to internationally adopted test guidelines and to perform non-*Apis* risk assessments for plant protection products based on a validated tiered risk assessment scheme and based on sound science for registration of plant protection products. For the time being regulatory requirements for testing and risk assessment of non-*Apis* bees should be put on hold until better and appropriate science will be in place in the future.

## References

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## **Technical Section 3**

### **Exposure Routes and Refinements**

#### **Experimental approaches**

The impact analysis of the EFSA proposal for a guidance document on the risk of plant protection product on bees highlighted a high failure rate of active substances in the screening and tier 1 steps. The options to further refine the risk assessment at Tier 2 have been presented by EFSA (Szentcs, 2014) and include:

- Refinement of exposure values using field measured residues in pollen and nectar for crops and/or flowering weeds;
- Measurement of the actual sugar content in the crop(s) and/or flowering weeds (s) to which bees are exposed;
- Measurement of actual values of food consumption;
- Measurement of actual drift and dust off-field deposition rates and
- Various landscape factors such as presence of in-field weed, adjacent crops and attractiveness of succeeding crops.

This list may be amended with the need for options to refine the risk assessment for exposure to water (puddles, guttation surface water).

No detailed guidance on how these refinements can be achieved is given in the EFSA document. While the measurement of the expected residue content in nectar and/or pollen after treatment are active substance-specific and depend on the intended uses, the other aspects listed above, *i.e.* the measurement of the actual sugar content in the crop(s) and/or flowering weeds to which bees are exposed, actual values of food consumption, actual drift and dust off-field deposition rates are generic data, which should be preferably generated collectively rather than on a case-by-case basis for specific dossiers, as this would bring robustness and consistency to the risk assessment. It is therefore recommended to address them through collective research projects.

For similar reasons, considerations regarding the landscape factors that may reduce exposure relate to risk mitigation options the effectiveness of which would be addressed more robustly through generic data in collective projects.

Finally, bee exposure via water (puddles, surface water or guttation) is most often described at the individual scale. Effects have been reported at the individual level, which did not result in observable effects at the colony level. For these reasons, this exposure route is typically not considered as a major one compared to pollen and nectar by regulatory bodies. In addition, the options to reproduce these exposure routes experimentally on a standardized way are not provided in guidance documents.

The sections below therefore focus on refinement of exposure estimates relative to pollen and nectar and propose common views on how such tier 2 data could be generated in the context of a registration dossier.

#### Basic principle to generate residue data in pollen and nectar

To date no technical guidance is available yet for these residue studies, from OECD or EPPO. Therefore, the option described below represents an option when a tier 1 risk assessment indicated potential risks, typically for insecticides.

Practically, the refinement consists in performing residue trials where nectar and pollen are sampled for residue analysis of the active substance and where relevant its degradation products.

The trial is performed in a field plot or under tunnel when it involves bees in order to sample pollen and nectar collected from the forager bees or via nectar/pollen collection by hand using micro-pipettes and sieves etc. The trial is performed preferably on an attractive crop, typically *Phacelia tanacetifolia* or on an attractive crop on which the product is to be used. The active substance is applied as a representative formulation and at the maximum rate, or including the maximum application rate. The application is most often performed during flowering but may alternatively involve pre-flowering treatments, if more relevant to reproduce the intended uses. Pollen and nectar may be collected from the flowers and when bees are present, they may also be collected from the foragers.

#### Recommendations from EFSA in their guidance document

In their document, EFSA state that a minimum of 5 field trials are necessary for each crop and geopolitical zone. From this data set, the highest value from each trial in each zone is considered to be the 90<sup>th</sup> percentile residue suitable for risk assessment.

For a product used in a single crop in each of the three zones (northern, central and southern) a minimum of 15 field residue trials would have to be generated. This might increase for different application timings (e.g. before and post flowering) and for a product with uses on several crops, if proposals for extrapolation are rejected. On this basis, if used on only five different crops, up to 75 residue studies could be necessary.

For minor use crops which can be economically important, this may limit the availability of products and hence the continued viability of their cultivation. In addition, minor crops do not involve surfaces large enough to provide the necessary experimental plots and therefore extrapolations rules are needed for these crops.

The associated costs for such a requirement are therefore 0.5 Million euros per crop and per zone, for 5 trials, if no extrapolation rule is defined, for field trials and for tunnel trials if replicates of a tunnel trial are requested. This estimation does not include trials to quantify residues in succeeding crops or flowering weeds, where the ETR do not pass the trigger values.

The number of replicates of 5 trials per zone is here critical and seems disproportionate considering that application rates for a product have already been defined to allow a reproducible control of a pest, as documented in the biology section of dossiers. Therefore, the variability that these replicates are intending to address is expected to be much lower at the intended application rates.

#### Degree of refinement of the residue levels needed to pass the risk assessment

An analysis was conducted to assess the likelihood of success of refining the risk assessment using measured field residues. Table 1 below reproduces the percentage of uses that passed the screening or tier 1 risk assessment for chronic risk to adult bees, when exposed to a flowering crop, from residues in in-field weeds, field margins or an adjacent crop:

*Table 1: Percentage of uses passing the screening or tier I risk assessment for chronic risk to adult bees (spray applied products 163 uses), from the impact analysis previously performed*

Exposure Scenario	Honey bees	Bumble bees	Solitary bees
Flowering crop	18%	0%	0%
In-field weeds	33%*	0%	0%
Field margins	85%	29%	NC
Adjacent crops	85%	30%	NC

\*For early applications in tree and vine scenarios; NC = not calculated, as covered by the analysis performed for bumble bees.

As risk to bumble bees represents the worst case situation the analysis focuses on honey and bumble bees only. Solitary bees are covered by the bumble bee assessment.

From the findings presented in table 1, it can be seen that a refined risk assessment would be triggered for a number of substances and uses, in-field but also in some cases off-field.

The degree of refinement required to pass the chronic risk assessment can be calculated by dividing the  $ETR_{\text{chronic}}$  by the appropriate trigger value. As an example, for an active substance for which the chronic risk assessment ETR value to the honey bee at step 1 is 0.021, the level of refinement needed to pass the risk assessment by using measured residue values is the ratio between the chronic risk ETR and the chronic risk trigger values, here  $0.021/0.03 = 7$  (see impact analysis, case study on insecticide A, page 75).

The degree of exposure refinement needed to pass the chronic trigger was calculated for honey and bumble bees and is presented in table 2. In addition, the requirement refinement level was qualitatively classified according to the chances of practical success (less than 10x = good, 10x – 100x = medium, 100-1000x = low and greater than 1000x = negligible).

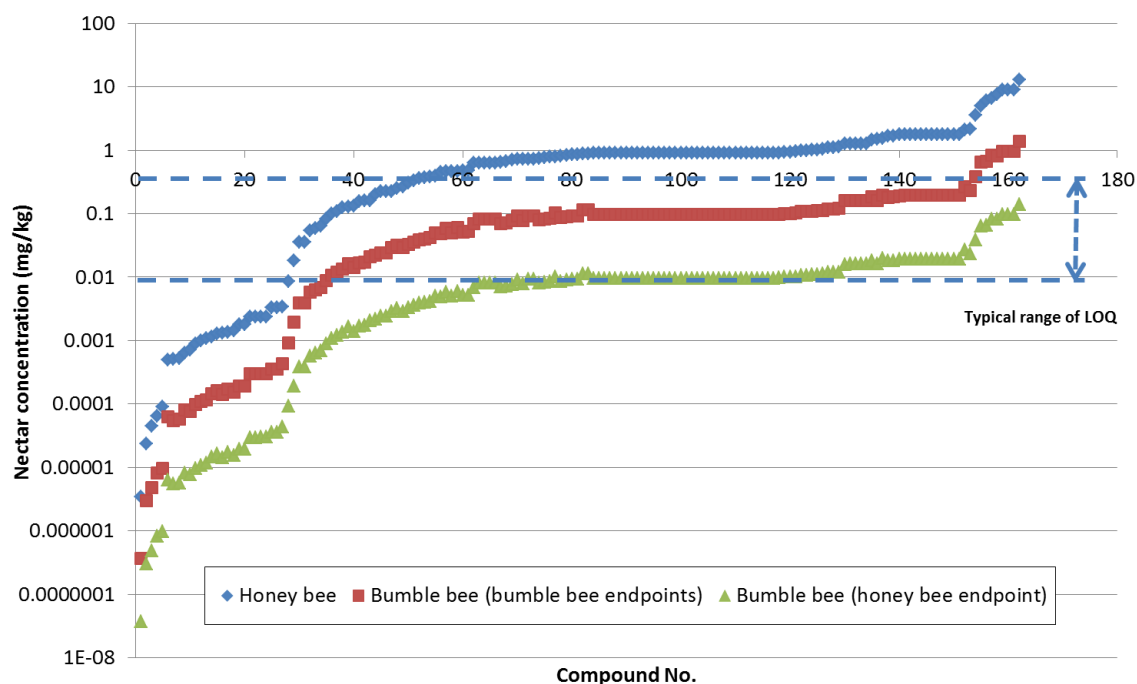
*Table 2: The percentage of substances requiring exposure refinement to pass the chronic risk assessment for honey bees and bumble bees and related chances of success (calculations for 133 and 162 uses, for honey bees and for bumble bees respectively):*

Degree of exposure refinement needed to pass chronic trigger	Chance of success	Honey bee risk assessment	Bumble bee risk assessment	Bumble bee risk assessment using honey bee endpoints
Less than 10x	Good	46%	19%	1%
Between 10x-100x	Medium	30%	38%	17%
Between 100x to 1000x	Low	1%	24%	38%
Over 1000x	Negligible	15%	19%	43%



Based on experience from exposure studies conducted in the past for a variety of purposes a degree of refinement of a factor of 10 at best could be achieved with field measured residue data, implying that the risk assessment can be refined for 46% of active substances in the case of honey bees, and 19% or 1% of active substances for bumble bees pending on the availability of dedicated toxicology endpoints. Larger degrees of refinements are not expected to be achieved at least for applications during or close to flowering.

In addition, the possibility to actually measure residue levels that would allow to meet the trigger imply analytical performances that may be impossible to reach, taking into account the nature of the matrices analysed and the volume of samples that can be collected for those matrices. Taking sample size out of a consideration, if residue levels need to be as low as 1 ppb (0.001 mg/kg) to pass the trigger value for an ETR then the LOQ of the analytical method needs to be 1 ppb or below. The necessary LOQ was calculated for each substance/use by using the given trigger, toxicity value and bee exposure level (by dividing by the appropriate short cut values in the EFSA guidance document). Figure 1 shows the distribution of the necessary field concentrations in nectar which would need to be detected.



*Figure 1: Distribution of Limit of Quantification (LOQ) values as pesticide concentrations in nectar required in field residue studies for the tier 2 refinement of honey and bumble bee risk assessments.*

As it can be seen on figure 1, a significant number of substances require LOQ several orders of magnitude lower than the typical range of 0.5 – 0.01 mg/kg. If we take an LOQ of 0.01 mg/kg as technically achievable for the majority of substances in crop materials, up to 47% of substances/uses (based on bumble bee risk using honey bee endpoints) would require analytical methods with sensitivity several orders of magnitude higher than the standard default values available.

### Conclusions and recommendations

A refinement of exposure estimates involving actual residue levels for the active substance and its relevant residues in pollen and nectar is in principle feasible but necessitates a thorough discussion with the RMS/zRMS as a preamble. Several needs have been identified that a preliminary to a more generalised requirement for refined residue measurements in pollen and nectar as tier 2 refinement, which are listed below:

- Agreement on a tiered approach as regards the crop to be used and extrapolations (discussion on a standard protocol and representative crops);
- Agreement to revise the trigger values so that tier 2 risk assessments are requested on a reasoned basis and not for non-toxic substances;
- A revision of the trigger values is also triggered to that refinement is actually realistically achievable;
- The revision of the number of replicates that takes into account the reproducible level of pest control at the application rates recommended, which contradicts the intrinsic variability of residue levels in a plant triggering 5 replicates in order to reach the 90th percentile;
- A discussion with experts in residue trials in order to identify crop groups within which residue levels are expected to be similar;
- The development of standards for residue studies performance as a function of environmental factors that influence residue content would be a basis towards a further guidance.
- Modelling is an alternative to costly field exposure studies and can be used to test many more scenarios than experiments, and cover uncertainties relative to geographical differences, agricultural practices etc. Modelling, such as BEEHAVE for example, may be of great help to generate realistic worst case exposure scenarios, using observed biology traits as a basis. The pesticide module of BEEHAVE is in its final development phase. The potentialities of modelling could be further explored in a dedicated working group to accelerate the development of the scenarios mentioned above.

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## Technical Section 4

### Industry data on occurrence of in-field weeds

<https://ojs.openagrar.de/index.php/JKA/article/view/5318>

## Appropriate use of available evidence - an example of exposure of bees to in-field weeds

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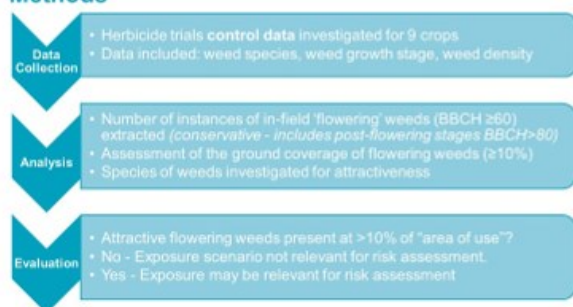
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### Introduction

Substances showing little or no toxicity to bees often fail the tier 1 risk assessment of the new EFSA bee guidance, based on a worst-case assumption of bees, feeding 100% on flowering weeds within the treated field. However, it is generally understood that weeds are not prevalent in arable fields. The EFSA guidance suggests, that if evidence is available to demonstrate that <10% of the area of use contains attractive flowering weeds, then the exposure route is not relevant in the 90<sup>th</sup> percentile case.

As part of an industry led initiative, we present and discuss the use of existing empirical evidence (i.e. occurrence and growth stage (BBCH scale) of weeds in control plots from herbicide efficacy field trials), to illustrate that the scenarios in the guidance document could be modified using currently available data. This could enable development of a more effective tier 1 risk assessment, whilst ensuring appropriate levels of protection are maintained.

### Methods



### Results

Crop	Total number of trials examined	Total number of weed recordings	% weeds recorded with BBCH ≥ 60
Wheat	1024	9113	0.86%
Maize	7669	38421	1.94%
Oilseed Rape	1022	3587	1.28%
Sunflower	388	1435	1.11%
Potatoes	182	1159	1.04%
Sugar Beet	156	5006	0.12%
Peas	650	5780	0.48%
Beans	203	1807	1.49%
Permanent Crops	233	553	36.2%
Total	11527	66861	-

Table 1: Data from control herbicide efficacy plots showing percentage of weeds above a flowering growth stage (BBCH 60).

### Results – BBCH ≥ 60 – 'Flowering Weeds'

- <2% of all weeds in arable fields were 'flowering'
- In permanent crops 'flowering weeds' accounted for ~36%

### Results – % Ground Cover

- The vast majority of in-field weeds, covered <10% of the area and were not 'flowering' (BBCH < 60), see Figure 1.
- For permanent crops (vines and orchards) the % of weed recordings ≥ 10% ground cover and BBCH ≥ 60 was only 20.5%.

### Results – Bee Attractive Weeds

- Initial analysis using dicotyledonous and monocotyledonous, as surrogates for attractive and non-attractive species:
- For permanent crops the number of weeds which were 'attractive', 'flowering' and ≥10% ground cover = 12%

### Conclusions

- Large, high quality dataset studied (>11500 regulatory trials)
- Unrealistic worst-case control plot data (chosen for high weed pressure & no weed control measures used)
- In-field attractive flowering weeds are not an appropriate scenario for arable crops (<2% of weeds recorded).
- In-field attractive flowering weeds may be an appropriate scenario for permanent crops (12% of weeds recorded).
  - Mitigation and refinement options are available (e.g. weed mulching, use of crop interception & field studies)

### References:

EFSA 2013. Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295 [268 pp.].

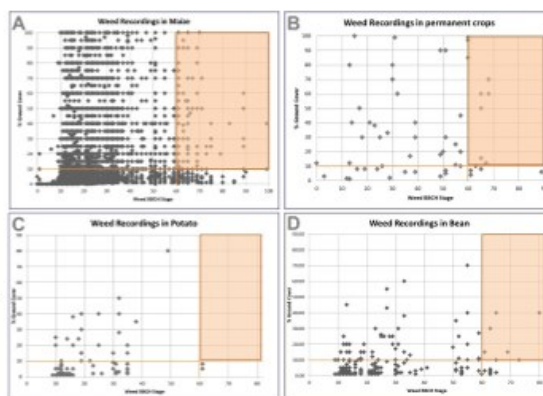


Figure 1: Example plots of % ground cover of weeds vs. Maximum BBCH stage of weeds recorded in maize (A), permanent crops (B), potatoes (C) and Beans (D). Orange box indicates those instances where weeds were found to be at a flowering growth stage (BBCH ≥ 60) and abundant at >10% ground cover.

### Further Work

- It is recommended that this study could be used to refine the exposure scenario in future updates to the guidance.
- Further examination of the attractiveness of weed species will help to refine this risk assessment.

## Technical Section 5

### Guttation

Over the past years many publications have indicated that since Girolami et al. (2009)<sup>5</sup> laboratory experiment where guttation was created under laboratory conditions and augmented with sugar and fed to bees the actual impact of exposure to guttation water to honey bees at the colony level is negligible. Much of this work has been conducted by members of the ICP-BR Bee Protection Group.

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Pistorius, J. Wallner, K. Joachimsmeier, I. Reetz, J. Schenke, D. von der Ohe, W. Illies, I. Maus, C. Block, T. Becker, R. (2012b). Review on activities in Germany to assess the occurrence, residues and possible risk of guttation for honey bees. 11th International Symposium of the ICP-BR Bee Protection Group, Wageningen (The Netherlands), November 2-4, 2011 Julius-Kühn-DOI: 10.5073/jka.2012.437.021/

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<sup>5</sup> Girolami, V., L. Mazzon, A. Squartini, N. Mori, M. Marzaro, A. Di Bernardo, M. Greatti, C. Giorio, and A. Tapparo. 2009. Translocation of Neonicotinoid Insecticides From Coated Seeds to Seedling Guttation Drops: A Novel Way of Intoxication for Bees. *Journal of Economic Entomology* 102(5): 1808-1815.

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## Technical Section 6

### Tier 1 Chronic Risk Assessment Proposal for adult honey bees

The industry impact analysis and the finding from recent EFSA journal (see Technical Section 1) both confirm the excessive conservatism of the EFSA (2013) approach for chronic risk. The factors contributing towards this high level of conservatism are

- Trigger of 0.03: the regulatory acceptable daily dose (RADD) must be 34x higher than the exposure level
- Endpoint: a LDD<sub>50</sub> value is used with the trigger however this means that a high endpoint is needed. For compounds of low toxicity testing high doses is often not practicable due to solubility and palatability issues. In these cases, it is often only possible to achieve a no observable effect daily dose (NOEDD) which is not suitable for use with the trigger which was calibrated for use with the LDD<sub>50</sub> value. Using the NOEDD in place of the LDD<sub>50</sub> will greatly overestimate the level of protection.
- Exposure: the chronic exposure is assessed at the 90<sup>th</sup> percentile level rather than mean which is the case for other dietary risk assessments (e.g. for birds and mammals).
- Food consumption rates: Exposure calculations are based on bees consuming nectar which contains only 15% sugar. This means to meet their maximum daily energy requirements the forager bee must consume approximately 8x its body weight per day (equivalent to a 70 kg human eating >3,700 apples/day). Observations of the range of nectar concentrations collected by honey bees (e.g. Couvillion et al 2014) and modelling approaches (Miles et al 2014) have shown that honey bee colonies cannot survive when nectar contain less than 30% sugar.
- The sugar content of major crops ranges from 32.4 - 59% (oilseed rape), 45.7 – 61.3 (Phacelia) and sunflowers up to 49% (Hedtke, 1998, Mandl, 2006, Pritsch, 2007).

Consequently, the conditions of risk assessment present a set of ecological conditions within which the test organism cannot survive. Furthermore; there is a testing requirement that for many substances the endpoint required cannot be measured (only NOEDD values). Industry has developed a number of options in order to establish a feasible chronic risk assessment for honey bee adults.

#### Chronic honey bee risk assessment option 1

This is based upon the method of EPPO 2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the endpoint so avoids the issues associated with the generation of LDD<sub>50</sub> values for substances of low toxicity, and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO 2010 recommended the calculation of a TER using the following equation:

$$\text{TER} = \text{NOEDD} / \text{daily dose}$$

Where daily dose (DD) is based on the worst case a sugar need of 128 mg/bee/day (Rortais et al 2005) of a bee feeding exclusively from nectar containing 30% sugar using the following equation:

Daily dose ( $\mu\text{g a.i./bee}$ ) =  $\text{A.R.} \times [0.128 \text{ g}/(1000 \times 0.3) \times \text{RUD}$

A.R. = application rate in  $\text{kg a.i./ha}$

RUD = residue per unit dose from the EFSA bee guidance. Mean  $\text{RUD}_{\text{nectar}} = 2.9 \text{ mg a.i./kg}$  (foliar sprays), Mean  $\text{RUD}_{\text{nectar}} = 0.0458 \text{ mg a.i./kg}$  (seed treatment).

EPPO 2010 suggests a chronic TER trigger (NOEDD/daily dose) of 1 as the entity to be protected is the test species. However, triggers could be calibrated using semi-field and field data as has been done for the acute risk assessment HQ.

### Chronic honey bee risk assessment option 2

This option uses the EFSA approach to set quantitative protection goals but is modified to take into account compound specific properties and the use of either  $\text{LDD}_{50}$  or NOEDD as the former cannot always be measured due to palatability and solubility issues.

In EFSA 2013 the specific protection goal (SPG) is set to a maximum of 7% reduction in colony size compared to the control. However, the effect on individuals is assessed in the laboratory study. Although industry believe the use of the Khoury model (Khoury et al 2011) and the assumptions made leading to the factors in the calculation of trigger are flawed however it is still possible to modify the EFSA approach to make a highly efficient risk assessment based on the assumptions of EFSA.

In EFSA 2013 the Khoury model is used to translate and increase in forager mortality to the SPG as 1.27x in hive background mortality (5.3%) over 10 days. This means the maximum increment in mortality is:

Max increment =  $0.27 \times 5.3 = 1.43 \%$  mortality (i.e. equivalent to more 1 dead bee in 70 over days).

Using a linear interpolation model the chronic trigger was set as:

$50\%/1.43\% = 34$  (0.03)

As a linear model is used this overestimates the requirement trigger as true dose-response relationships are sigmoidal rather than linear. It also does not take into account the slope of the dose-response.

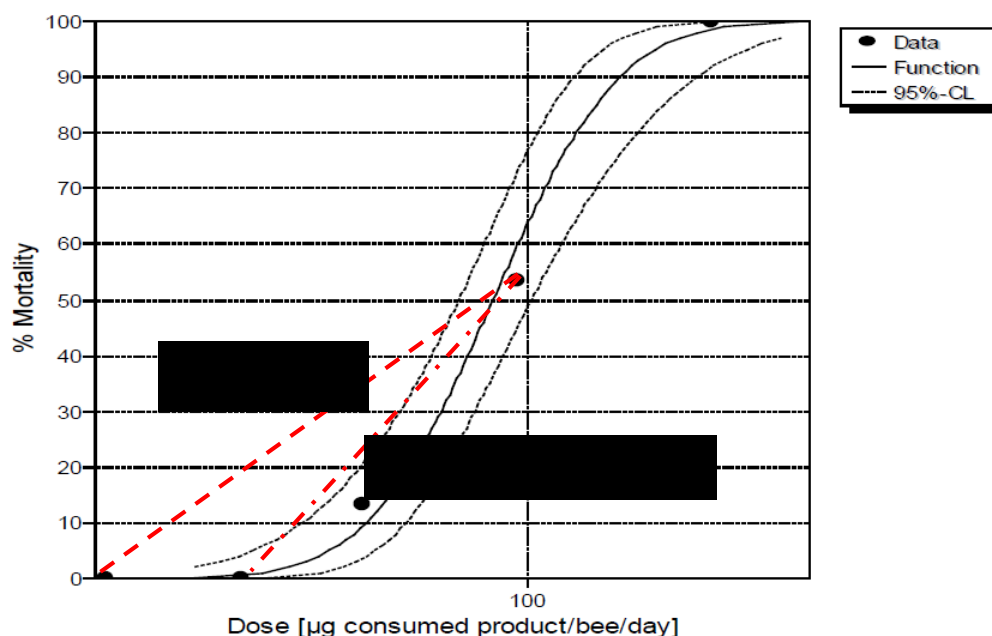
The EFSA guidance assumes that zero mortality can only occur at a dose of 0  $\mu\text{g/bee/day}$  which is clearly over-conservative. Where the data from the chronic dosing study can be used to generate both a reliable  $\text{LDD}_{50}$  and an  $\text{LDD}_0$  the existing proposed EFSA chronic trigger of 0.03 (based on the  $\text{LDD}_{50}$ ) can be achieved directly using  $\text{LDD}_0$ . Thus, the  $\text{LDD}_0$  from the 10 day dosing study is subtracted from both the daily dose and the  $\text{LDD}_{50}$  in the EFSA chronic ETR trigger equation. i.e.

$\text{ETR} = (\text{daily dose} - \text{LDD}_0) / (\text{LDD}_{50} - \text{LDD}_0)$



this approach is still conservative as the slope is always greater than the true slope of the sigmoidal dose-response curve

**Dose-effect curve showing the influence of the test item on mortality of the introduced test organism as observed after 10 d.**



Where there the  $LDD_0$  cannot be reliably determined from the data and the  $LDD_{50}$  value is available from a study there will also be information about the dose-response relationship, the model used, the goodness of fit and slope (b). Consequently, information about the slope and model can be used to set a compound specific trigger which meets the SPG derived mortality of 1.43%. For example, if we assume a compound with a  $LDD_{50}$  value derived from a Log-Probit model with good fit and a slope of 1.43 the trigger of and ETR of 0.03 precisely meets the SPG of 1 dead bee in 70. This works because  $0.03 \times LDD_{50}$  is equivalent to the dose which will kill 1.43% of bees using the relationship between dose and effect of the Log-Probit model. However, the majority of substances for which  $LDD_{50}$  values can be measured experimentally have slopes greater than 1.43.

If the slope is slightly increased to 2 then meeting the trigger of 0.03 imply a higher level of protection. In this case it would indicate 1 dead bee in 862 bees (0.11%) which is an overestimation of 12x and the actual trigger required would be 0.076. At a slope of 3, the trigger of 0.03 leads to an overestimation of risk by 2,860x indicating why substances of low toxicity to bees cannot be shown to pass the EFSA 2013 chronic risk assessment. Table X below gives the ETR triggers required for a range of slopes and commonly used dose-response models so that the level of protection of 1 dead bee in 70 is met and hence lead to a conclusion of no unacceptable chronic risk to bees. Slope increments of 0.5 are presented so that the user should select the slope equal to or below the measured slope. For example, for a compound  $LDD_{50}$  value with a slope of 4.25 and a dose-response fitting a Weibull model the ETR to meet the SPG would be taken as 0.283; i.e.  $0.283 \times LDD_{50}$  = dose which would kill no more than 1.43% of bees. So if the calculated ETR is equal to or lower than this value



there would no unacceptable risk to bees as the protection goal would have been to have been met (or exceeded).

If a different model to those shown in Table 3 is used then it will be necessary to calculate the required trigger separately.

**Table 3: Look up table for triggers to meet SPG for chronic risk assessment**

Compound slope (b)	Trigger adjusted for slope to meet SPG of 1 dead bee in 70		
	Log-Probit	Log-Logit	Weibull
1	0.0065	0.014	0.0062
1.43	0.03	0.052	0.029
1.5	0.0356	0.0595	0.034
2	0.080	0.120	0.079
2.5	0.133	0.184	0.131
3	0.186	0.244	0.184
3.5	0.237	0.298	0.235
4	0.284	0.347	0.281
4.5	0.326	0.390	0.324
5	0.365	0.429	0.362
5.5	0.400	0.463	0.397
6	0.432	0.494	0.429
6.5	0.460	0.521	0.458
7	0.487	0.546	0.484
7.5	0.511	0.569	0.508
8	0.532	0.589	0.530
8.5	0.553	0.608	0.550
9	0.571	0.625	0.569
9.5	0.588	0.640	0.586
10	0.604	0.655	0.602

As mentioned above however; there are cases where is it not possible to achieve an experimentally measured LDD<sub>50</sub>. In these cases a NOEDD or LDD<sub>10</sub> could be used with an appropriate adjustment in the trigger. In these cases, as the level of mortality observed is zero or close to zero there is no need to take into account any dose-response other than a linear

relationship (as used in EFSA 2013). The appropriate trigger can be calculated using the same method as EFSA 2013. If we assume the observed NOEDD is equivalent to the LDD<sub>10</sub> which is now common place in ecotoxicology (or we have the LDD<sub>10</sub>) then the trigger can be calculated as:

$$10\%/1.43\% = 6.99 \text{ (0.143)}$$

Consequently, when using a NOEDD (or LDD<sub>10</sub>) if the calculated ETR is equal to or below 0.143 then no unacceptable risk to bee is concluded. This option is still conservative and could lead to false positives as it suggests that the SPG is met at a dose which is 7x (i.e. 6.99x) lower than the endpoint which gave no effect.

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## Technical section 7

# Linking protection goals to trigger values using compound specific properties: Chronic risks to bees



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Science For A Better Life

## 1. Introduction

In the EFSA guidance document for the assessment of risk of plant protection products (PPP) to bees a number of new trigger values are proposed. One of concern due to its conservative nature is the honey bee chronic oral trigger of 0.03. In effect a substance is considered low risk if the LDD<sub>50</sub> is 34x higher than the estimated exposure. An impact analysis indicated that using this trigger almost all substances would not pass the screening or tier I risk assessment leading to higher tier evaluations even for substances of low toxicity. As the risk assessment for a single stressor (PPP) is over conservative it will make the assessment of risk due to multiple stressors meaningless.

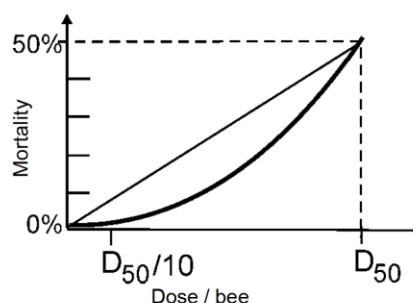


Figure 1: Linear (assumed) vs. sigmoidal (actual) dose response functions (modified after EFSA 2013)

## 2. Chronic trigger calculations according to EFSA GD

In EFSA 2013 the specific protection goal (SPG) is set to a maximum of 7% reduction in colony size compared to the control. Khoury model is used to translate an increase in forager mortality to the SPG as 1.27x in hive background mortality (5.3%) over 10 days. This means the maximum increment in mortality is:

Max increment =  $0.27 \times 5.3 = 1.43\%$  mortality  
(i.e. equivalent to no more than 1 dead bee in 70).

Using a linear interpolation model the chronic trigger was set as:  
 $50\% / 1.43\% = 34$  (0.03)

As a linear model is used this overestimates the required trigger as true dose-response relationships are sigmoidal rather than linear (Finney 1947). The area between the linear and sigmoidal functions (Figure 1) represents the overestimation of meeting the SPG. It also does not take into account the slope of the dose-response.

## 3. Trigger analysis and level of protection achieved

We analysed the methods and the underlying assumptions to calculate the actual level of protection afforded by a trigger of 0.03 to a range of plant protection products (PPP). In almost every case the level of protection achieved greatly exceeded SPG of <7%. This will differ for each model (e.g. Log-Probit, Log-Logit, Weibull etc.). For example mathematically, the trigger value of 0.03 meets the SPG for a given LDD<sub>50</sub> if the slope (b) of a Log-Probit dose-response relationship is 1.43. If the slope is greater the level of protection will exceed the SPG which is the case for the majority of compounds and generates a large number of false positives (see Table 1).

For many other substances it was observed that the measurement of a LDD<sub>50</sub> is not technically possible due to low toxicity and/or limited solubility (e.g. many herbicides and fungicides). In these cases only a no observable effect daily dose (NOEDD) can be determined. The use of a NOEDD also leads to an exceedance of the level of protection and false positives (i.e. low risk is indicated at  $1/34$  of the NOEDD).

To make a correction for the chronic trigger for a PPP where there is no LDD<sub>50</sub> but a NOEDD is available we can calculate a suitable trigger which will offer at least the same level of protection.

As the NOEDD is at a part of the dose-response where the relationship is rather flat we can use the calculation of EFSA using the linear interpolation model but assuming the NOEDD is equivalent to the LDD<sub>10</sub> which is now a common place approach in ecotoxicology:

Chronic trigger<sub>NOEDD</sub> =  $10\% / 1.43\% = 6.99$  (0.143)

Compound slope (b)	Individual effect chance at 0.03 threshold	Mortality using trigger of 0.03	Error/overestimate of risk	Trigger adjusted for slope to meet SPG
1.43	1 in 70	1.43%	None	0.03
2.0	1 in 862	0.12%	13x	0.082
3.0	1 in 407000	0.00025%	59850x	0.189
4.0	1 in 1788155175	0.00000006%	26,296,399x	0.285
5.0	1 in $7.55 \times 10^{13}$	0.000000000013%	1,110,160,000,000x	0.367

Table 1: Effect of slope on implied level of protection

## 4. Possible approaches / way forward

To ensure that the required level of protection is met:

### LDD<sub>50</sub> endpoints: (model and slope known)

- Use the information to calculate the appropriate trigger or use a look up table (see Table 2)
- Use information about the model and slope and calculate the individual effect chance (IEC)

### NOEDD or LD<sub>10</sub> endpoints: (when LDD<sub>50</sub> is not available)

- Use trigger of 0.143

## 5. Case studies

These approaches have been tested with a number of PPP. Below are some examples for foliar applied PPP according to the tier I chronic risk assessment of EFSA (2013):

Compound code	GAP (kg a.s./ha)	Endpoint (µg a.s./ha)	ETR	Slope	Compound /endpoint specific trigger	Individual chance of effect (ICE)
Herbicide 1	0.238	LDD <sub>50</sub> = 14.5	0.0952	3.468	0.234	1 in 5038
Herbicide 2	0.48	NOEDD > 4.4	0.633	N/A	0.143	≥1 in 70
Herbicide 3	0.01	NOEDD > 4.7	0.012	N/A	0.143	≤1 in 70
Fungicide 1	0.15	LDD <sub>50</sub> = 2.62	0.332	2.603	0.144	1 in 9
Fungicide 2	0.25	LDD <sub>50</sub> = 10.2	0.142	3.529	0.240	1 in 722
Insecticide 1	0.0075	LDD <sub>50</sub> = 0.53	0.08	3.080	0.195	1 in 2744
Insecticide 2	0.06	LDD <sub>50</sub> = 0.0137	25.4	11.997	0.657	1 in 1

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Compound slope (b)	Trigger adjusted for slope to meet SPG of 1 dead bee in 70		
	Log-Probit	Log-Logit	Weibull
1	0.0065	0.014	0.0062
1.43	0.03	0.052	0.029
2	0.080	0.120	0.079
3	0.186	0.244	0.184
4	0.284	0.347	0.281
5	0.365	0.429	0.362
6	0.432	0.494	0.429
7	0.487	0.546	0.484
8	0.532	0.589	0.530
9	0.571	0.625	0.569
10	0.604	0.655	0.602

Table 2: Look up table for triggers to meet SPG for chronic risk assessment

## 6. Conclusions

- We present a simple method to evaluate all PPP to the same level of protection by taking into account the type of endpoint (i.e. LDD<sub>50</sub> or NOEDD) and the slope of the dose response relationship which is compound specific
- The actual level of protection afforded by a given exposure toxicity ratio (ETR) as the individual chance of effect (ICE) can be calculated allowing for better informed decision making by risk managers
- The number of false positive and negatives in a risk assessment could be reduced by using specific triggers based on the properties of the test substance
- In complex models such as BEEHAVE which have the potential to assess both single PPP stressors and interactions with multiple stressors the type of endpoint and the shape and slope of the dose-response curve need to be taken into consideration.

## **Technical Section 8**

### **Larvae toxicity studies and Tier I risk assessment**

According to Regulation (EU) No. 284/2013, toxicity studies on honey bee larvae should be submitted as part of the application dossier for a plant protection product. The risk assessment scheme described in the currently agreed guidance document for the risk assessment for bees (the SANCO guidance document on terrestrial ecotoxicology – SANCO/10329/2002), however, does not specifically address honey bee larvae. EPPO (2010) includes a risk assessment scheme to larvae, for systemic products after soil and seed treatment that could also be adapted to foliar applications.

In contrast, the new EFSA guidance document for bees (EFSA, 2013) contains a risk assessment scheme for the chronic risk to adult honey bees and honey bee larvae. However, this guidance document has not been taken note of in the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF).

In the national approach of Belgium it was referred to the Pesticides Peer Review Expert Meeting 133 (EFSA, 2015) technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, bee section (EFSA supporting publication 2015: EN-924. 62 pp.). This approach is not compliant with the process of endorsing new guidance documents, as it does not involve the consultation of risk managers at the SCoPAFF (Member States and the Commission) and as such does not foresee an agreement of the risk managers on an implementation date after formal information of industry. Therefore, this document is not used in the following approach.

#### Methodology

There have been recent developments in toxicity testing of plant protection products on honey bee brood. These needs to be assessed in terms of methodology and for suitability for inclusion in a sequential risk assessment scheme at tier 1 level.

Two recent regulatory documents (OECD GL 237 & OECD GD 239) based on the methodological development of *in vitro* laboratory bee larval tests have been finalised and are considered for use into the Tier 1 risk assessment.

- 1) Acute: OECD GL 237 (7 day test, 1 dose at day 4). In the guideline there are some variations regarding the day bees are assessed e.g. 8 days, 1 dose at day 3.
- 2) Chronic: OECD GD 239 (7/8 days test up to 21/22 days; repeated dose, 4 doses starting on day 3).

Concerning the repeated dose testing evaluated until hatch of the bees, it should be noted that this is technically a highly challenging study which may require several repeats to achieve an acceptable study and additional time is therefore required. There is also globally limited testing capacity within CROs.

## Exposure

The risk assessment based on EFSA (2013) does not discriminate between toxic and non-toxic compounds, which is driven by exposure assumptions that are much higher than in reality following agricultural use (e.g. residues in unprocessed food, no dilution in the hive).

Therefore, the main exposure routes (pollen, nectar) will be considered, at least in the Tier 1 bee larvae assessment.

Experimental data on residues in larvae and royal jelly (DeGrandi-Hoffman et al. 2013, Purdy, J. 2014, Thompson et al 2014) (see [Technical Section 11](#)) are now available that confirm the low level of exposure of larvae to residues.

## Risk assessment

Larval development or brood success is a vital part of the survival and/or productivity of honey bee colonies. As an overall protection goal it has to be assured that there are no unacceptable effects on bee brood impacting colony development or vitality. As already mentioned no specific trigger values for certain endpoints have been established or are commonly recognized in the current tier 1 risk assessment. ETR, as described in the EFSA Bee GD, are considered as very conservative protection goals and lead to a considerable number of false positives.

There are two options proposed by industry for the honey bee larval risk assessment:

### Option 1

One feasible risk assessment option would be the use EPPO (2010) together with EFSA RUD or ETE and a 30% sugar content with mean RUD, an endpoint of a NOED and a realistic trigger value. The sugar content of major crops ranges from 32.4 - 59% (oilseed rape), 45.7 – 61.3 (Phacelia) and sunflowers up to 49% (Hedtke, 1998, Mandl, 2006, Pritsch, 2007). As a consequence, the sugar content in the risk assessment needs to be adapted to a more realistic level for all exposure routes. With an overall sugar content of 30% for all exposure routes including flowering weeds the risk assessment is reflecting the ecologically relevant scenario and remains still conservative enough to cover the worst-case. A realistic trigger value of 1 according to EPPO (2010) for the chronic adults risk assessment is suggested. Concerning the RUD as a minimum alternative a median RUD could be considered, too. An example calculation is provided in the next paragraph.

### Calculation and risk assessment for Larvae

A worst-case of potential exposure via residues in pollen/nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme, based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst-case data from Rortais *et al.*, 2005 as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae:

Worker larvae consuming 59.4 mg sugar in 5 days Assuming 30% sugar content of nectar the worst-case consumption with worker larvae is:

$59.4/0.30 = 198$  mg nectar in 5 days.

In addition worker larvae are considered to consume 2 mg pollen during their development phase (EFSA 2013).

Thus considering the mean RUD values for nectar and pollen in EFSA 2013 exposure can be estimated either for the whole development period of 5 days or per day.

E.g. foliar application at 0.25 g a.s./ha

Nectar dose:  $0.25 \times 2.9 \times 198/1000 = 0.14$  µg a.s./larva (0.03 µg a.s./larva/day)

Pollen dose:  $0.25 \times 6.1 \times 2/1000 = 0.003$  µg a.s./larva (0.0006 µg a.s./larva/day)

Total exposure ETE = 0.143 µg a.s./larvae or 0.03 µg a.s./larva/day (as a default worst-case residue at 0.25 g a.s./ha)

This can be compared to the larval NOED of 'x' µg a.s./larva, which is equivalent to a NOEDD 'x'/5 µg a.s./larva/day (based on 5 day development period).

$TER = NOEDD (\mu\text{g a.s./larva/day}) / ETE (\mu\text{g a.s./larva/day})$

Both acute and chronic test methods should be permitted for use in the risk assessment. For acute studies a NOED or a NOED per day may be used by dividing by 4 to account for the number of days of exposure.

An assessment factor of 10 should be used for the acute risk assessment (based on an LD<sub>50</sub> endpoint) and an assessment factor of 1 for the chronic assessment should be used (according to EPPO 170).

## Option 2

The main dietary exposure route of compounds to honey bee larvae occurs via pollen and nectar. In order to put the obtained larval toxicity endpoints into perspective, the NOEL endpoints (see option 1) can be compared to residue data in pollen and nectar determined in the residue trials.

A comparison of the exposure concentration value (1 mg a.s./kg) with the concentration NOEC from the larval acute or chronic studies should be made in the risk assessment. The main dietary exposure route of compounds to honey bee larvae occurs via pollen and nectar. In order to put the obtained larval toxicity endpoints into perspective, the NOEL endpoints (see option 1) can be compared to residue data in pollen and nectar determined in the residue trials.

If the data indicate lower residues than NOELs (comprising a margin of safety between exposure concentrations in the relevant matrices under field conditions and larval toxicity endpoints of bee larvae) a low risk can be concluded.

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## Technical Section 9

# Simple modelling approaches to refine exposure for bee risk assessment based on worst case assumptions



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## Introduction

In 2013 EFSA published its guidance document for bees. Industry performed an evaluation of the impact of the proposed tier I risk assessments on the pass/fail rate of currently available active substances on the EU market. The majority of substances fail the screening step for risk to larvae and chronic risk to adult honey bees including many known non bee-toxic substances. One proposed refinement step is to generate more accurate estimates of exposure. However, experimentally refining exposure is costly and time consuming (up to €1.5 million/crop).

Current default estimates of exposure (EFSA, 2013) are based on 90<sup>th</sup> percentile concentrations of active substances in pollen and nectar in the field. Although suitable for acute risks, in field concentrations are not suitable for chronic assessment especially for honey bees which feed from colony stores before making foraging flights or for larvae which are fed from in-hive food stores via nurse bees. Consequently there are opportunities to refine exposure using aspects of honey bee behaviour and crop specific factors which can be modelled using BEEHAVE without the need for field studies.

## Dose calculations according to EFSA GD

### Example: Fungicide applied at 0.25 kg a.s./ha

Type of bee	Food consumption		Dose		Estimated total dose
	Sugar (mg)	Pollen	Nectar	Pollen	
Forager bee	128 mg/day (853 mg nectar/day)	Negligible	2.41 µg	N/A	2.41 µg/bee/day
In-hive bee	50 mg/day (333 mg nectar/day)	6.5 mg/day	0.942 µg	0.337	1.28 µg/bee/day
Worker larvae	59.4 mg / 5 days (392 mg nectar)	2 mg / 5 days	1.11 µg	0.10 µg	1.21 µg/larva

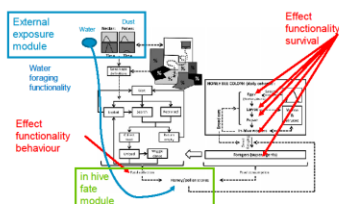
Using short cut values (SV) to derive 90<sup>th</sup> percentile values from the combined distributions for feed consumption and residue levels results in similar but slightly lower doses:

Forager bee:  $0.25 \text{ kg a.s./ha} \times 5.8 = 1.45 \text{ µg/bee/day}$   
In hive bee:  $0.25 \text{ kg a.s./ha} \times 3.78 = 0.945 \text{ µg/bee/day}$   
Worker larva:  $0.25 \text{ kg a.s./ha} \times 4.4 = 1.1 \text{ µg/larva}$

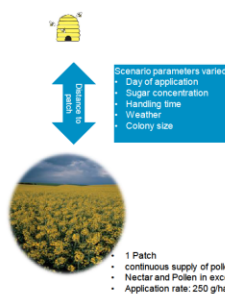
## Dose calculations with BEEHAVE<sub>Ecotox</sub>

### BEEHAVE<sub>Ecotox</sub>

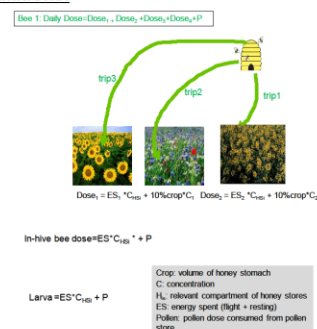
BEEHAVE model (Becher et al. 2013)  
with Ecotox-Module (Preuss & Thorbek, in prep)



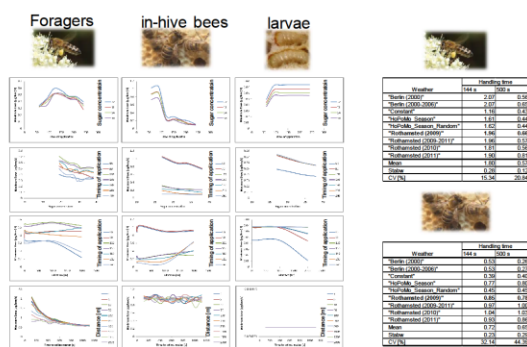
### Ecological Scenario:



### Dose calculations:



## Results - Nectar



- ⇒ Maximum dose per bee depends on the ecological scenario
- ⇒ Most important parameters is timing of application and handling time

## Results - Pollen

- ⇒ Maximum dose for pollen exposure is independent of all tested environmental variables under the assumptions:
  - that all available pollen is dosed
  - that pollen does not become limited

Maximum dose [µg/bee/d]	0.024	0.023	0.184
variation between environmental scenarios	1.4%	16.4%	0.8%

- ⇒ Dose depends on timing of application
- ⇒ Dose depends on handling time (crop specific)

## Conclusions

- ⇒ BEEHAVE<sub>Ecotox</sub> allows for a conservative exposure assessment under the intended use pattern (GAP)
- ⇒ BEEHAVE<sub>Ecotox</sub> could be used to develop crop specific scenarios (e.g. flowering oil seed rape, post emergent cereal herbicide uses) so that risk assessment is more ecologically realistic
- ⇒ The output doses are equivalent to a Tier 2 exposure assessment and could be used with laboratory derived endpoints (Tier 1 effect assessment)
- ⇒ Exposure could be easily calculated for crop specific scenarios by use of BEEHAVE<sub>Ecotox</sub> or simple look up tables



## **Technical Section 10**

### **Tier 2 Effect study refinement Options**

If the risk is not acceptable at Tier 1 according to the proposed scheme from industry, a higher tier assessment should be performed based on available semi-field and field studies. The following are available methods allowing to demonstrate an acceptable risk at the semi-field level and options to refine the honey bee risk assessment.

#### Tunnel study following EPPO 170 Guidance

A semi-field study following OEPP/EPPO Guideline No. 170(4), 2010 is intended to assess the mortality, foraging and to a certain extent colony effects of a pesticide applied to a honey bee attractive crop in a cage, tent or tunnel. The pesticide can be applied before flowering, during flowering onto foraging bees or during flowering before or after bee flight. Treatments should consist of a water-treated control, the test item applied at representative application rates for the proposed label, and a toxic reference e.g. dimethoate. The mortality of the bees and condition of the colonies should be examined before application; mortality, foraging activity and behaviour should then be assessed for approximately 7 days after application. The test method allows a worst-case exposure to the main dietary exposure route pollen and nectar under realistic exposure conditions and should include exposure verification. Samples of forager bees (for preparation of nectar from honey stomachs), pollen (collected via pollen traps or from pollen loads of forager bees) and in-hive samples for pollen and nectar should be collected during the study for subsequent residue analysis to demonstrate adequate exposure during the study.

#### Tunnel study following OECD 75 Guidance

The OECD 75 tunnel study is intended to assess the brood, mortality and to a certain extent colony effects of a pesticide applied to a honey bee attractive crop in a tunnel with a clear focus on honey bee brood development. Treatments should consist of a water-treated control, the test item applied at representative application rates for the proposed label, and a toxic reference known to have effects on brood e.g. fenoxycarb. The mortality, foraging activity, behaviour of the bees and condition of the colonies should be examined before and after application. Photographic assessments of the brood development of single cells should be conducted over one brood cycle starting after the test item application. The termination rate, brood index and compensation index should be determined. The conditions of the colonies should be assessed before the application and subsequently over two brood cycles. The test method allows a worst-case exposure to the main dietary exposure route pollen and nectar under realistic exposure conditions and should include exposure verification. Samples of forager bees (for preparation of nectar from honey stomachs), pollen (collected via pollen traps or from pollen loads of forager bees) and in-hive samples for pollen and nectar should be collected during the study for subsequent residue analysis to demonstrate adequate exposure during the study.

#### Oomen *et al.*, 1992 (Colony feeding) Study

The Oomen et al. (1992) study is intended to assess the brood and mortality effects of a pesticides applied at relevant exposure concentrations in sucrose solutions fed directly into hives placed in the field. Treatments should consist of a control of 50% sucrose solution, the test item dissolved in 50% sucrose solution and a toxic reference known to have effects on brood e.g. fenoxycarb, again dissolved in 50% sucrose solution. The treatments should be applied each day over a 9 day period. Bee colonies should be placed at the field site at least 4-5 days before start of feeding. The mortality and behaviour of the bees should be checked over at least 4 days before and up to 40 days after start of feeding (until end of 2<sup>nd</sup> brood cycle). The condition of the colonies should be assessed once before, and after start of feeding until the end of 2<sup>nd</sup> brood cycle. The brood development should be checked once before and four times after start of feeding. The termination rate, brood index and compensation index should be determined. The test method allows a worst-case exposure on colony level to a nectar surrogate (comparable to laboratory conditions), but neglects the exposure to pollen. Exposure verification should be included as well. Samples of stored nectar and honey from combs and stored pollen (bee bread) from combs should be taken once before and three times after start of application in all treatment and reference item groups and the control for subsequent residue analysis to confirm exposure. The free flying colonies can be considered as a major advantage in order to assess potential effects on a colony level. If colonies are in a suitable condition, overwintering success may also be considered as an additional endpoint.

The established sequential tiered risk assessment scheme for honey bees comprising the semi-field has proven to be successful concerning the protection of honey bees. All the aforementioned methods can be used in the regulatory process as all of them have their specific strength and advantages, but their limitations, too. In order to broaden the possibilities in the honey bee risk assessment the established test designs are still under further development, enhancement or improvement.

If the overall results from the semi-field studies indicate a risk according to the established sequential tiered risk assessment scheme including expert judgement, then higher tier field studies should be conducted to refine the risk assessment.

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## **Technical Section 11**

### **Tier 3- Refinement Options - Field studies in case Tier 2 still identifies a risk**

#### **Utility of the EPA method: 6 weeks colony feeding studies as a surrogate for field studies**

A useful alternative to full scale field studies is the large colony feeding study according to EPA FIFRA 40 CFR, Part 160, which is used to derive honey bee colony endpoint for active substances and used for US-EPA honey bee risk assessments. The study provides a colony level endpoint which can be applicable for use in risk assessment in a variety of situations without the need to test multiple crops or regions. In the study, the exposure levels can be controlled and effects linked to exposure studies in different crops. Further interpretation can be facilitated by use of the BEEHAVE model (see [Technical Section 13](#)). This study is a useful alternative to full field trials in which parameters can be better controlled. The protocol is currently being scrutinized by the field study working group of ICPPR with view to preparing some formal guidance.

Healthy queen-right honey bee colonies in normal conditions according to the season are used and are placed in an open-field / free-foraging test design, are held and repeatedly fed in a rural field environment with sucrose solution over a period of 6 consecutive weeks. Depending on the size of the colony between 1 and 2 L of syrup are placed in the colonies twice a week over the six week feeding period. Six weeks is chosen as a worse-case flowering period to cover exposure in more than one crop.

Untreated control consists of 12 replicates; for the biological and statistical evaluations. Two control variants may be used; C1 where bees receive no feeding solutions and C2 which will be fed untreated feeding solutions. If there is no difference between these groups they can be combined for statistical analysis.

Up to five test item treatment groups may be included (T1, T2, T3, T4, T5), each group with 12 replicates. All solutions (including the controls) are freshly prepared before each feeding event and subjected to chemical analysis.

The study is laid out in 12 replicate apiaries each with 7 colonies for biological assessments (C1, C2, T1, T2, T3, T4, T5) + 1 monitoring colony (no feeding of sucrose solution) which is used to sample nectar and pollen for multi-residue screening analysis + pollen for palynological analysis (characterize influx from environment).

In-hive pollen & nectar samples are collected as well as bee-samples from all colonies, to characterize their in-hive -test item and Varroa/Nosema-status. The exposure duration is 6 weeks followed by a post-exposure phase which can last until overwintering in the following year.

Assessment: mortality, food consumption, brood- and food development, colony strength are made prior to exposure and at least twice during the 6 week exposure phase. At least 3 post exposure phase assessment should be included.

By comparing the endpoint from colony feeding studies to those in the field, with possibly assisted interpretation by use of BEEHAVE (linking in-colony exposure to in-field) the risk to bees under a variety of use patterns can be evaluated and safe uses defined.

#### Traditional field studies

If the lower tier assessment indicates a potential risk to honey bees, which is not resolved by non-testing refinement options or semi-field testing then higher-tier testing in field trials is recommended. For products considered to be a high risk moving directly from lower-tier to field trials is an option.

Field testing provides an opportunity to investigate potential acute and chronic effects at the colony level following PPP exposure under conditions of actual use. Field studies allow for flexibility and should be designed specifically to address potential issues and uncertainties arising during lower-tier testing and risk assessment. As such a single test design is not proposed and field test designs must be carefully considered in order to address the areas of concern. Studies should therefore be considered on a case by case basis using expert judgement and it is also recommended to consult on test design with relevant regulatory authorities prior to study conduct.

General guidance on field trials is available, the test guideline EPPO PP 1/170 (4) (2000/updated 2010) is recommended.

- EPPO (European and Mediterranean Plant Protection Organization), 2010. PP 1/170 (4): Side-effects on honey bees. 40, 313-319

Contract research organisations (CRO's) have good experience in conducting studies based on the EPPO 1/170 recommendations, as this guidance has been applied as a standard approach to field testing for many years. The design is adaptable and covers both acute and chronic effects, longer-term effects on over-wintering can also be encompassed. However, further improvements to EPPO 1/170 are under development and it is recommended to follow and consider updates (e.g. ICP-PR developments/recommendations) when published.

There are a number of key points for which further understanding are necessary in order to address effects on bees at the field level and in accordance with requirements of EU Regulation 284/2013 (point 10.3.1.6).

*'The test shall have adequate statistical power and shall provide sufficient information to evaluate possible risks from PPP on bee behaviour, colony survival and development (EU Reg.284/2013, 10.3.1.6)'*

- The protection goal in regards to adverse impact at the colony level is under discussion. The EFSA guidance (2013) sets a SPG of < 7% reduction in colony size, with forager mortality acceptable at a level which will not result in ≥7% reduction in colony size. The scientific viability of this SPG is not clear (see Bakker F, 2015) and additionally, a trial designed with sufficient statistical power to detect a 7% effect under field conditions is not practical or feasible based on both the scale of the trial required and levels of normal variation in background mortality are known to exceed this level. It is recommended that field trial design is carefully considered in regards to the statistical power of the chosen design in regards to the specific parameters of interest,

e.g. ability to detect acute effects or chronic effects on colony and colony survival with adequate statistical power (see Rundlof et al 2015, field trial design).

*'Sub-lethal effects shall be addressed, if necessary by carrying out specific tests (for example homing flight) (EU Reg.284/2013, 10.3.1.6)'*

- Specific evaluation of homing flight of forager bees is not considered within the EPPO 1/170 Guidance. Standard monitoring and recording methodology including forager mortality in the crop, at the hive (dead bee traps) and assessment of colony strength (bee numbers) encompass impact on homing ability. For standard studies this is considered adequate to cover impacts on forager bees.
- For specific compounds, if such sub-lethal effects on homing ability/disorientation are suspected, then consideration of homing ability in specifically designed higher-tier trials could be considered. Methods are currently under development using radio-frequency identification (RFID) and whilst this methodology is still in development, it can be considered a future tool for investigation of effects on forager bees' behaviour, disorientation and mortality. Where it is considered necessary to evaluate this parameter then expert guidance on the experimental design and current science should be applied and relevant regulatory authority consulted. In particular the relevance of exposure of foragers at a distance to the colony relative to the protection goals proposed at the edge of the field is unclear.

Other key parameters impacting decisions for field testing and test design, these should be considered on a case by case basis depending on the specific issue to be investigated and the recommended end use(s) of the PPP:

Number of trials: Replication of trials by area and within trials, in regards to numbers of fields needs to be considered carefully both on the basis of statistical power of the trial and relevance of data between regulatory zones.

Location of trials: Trials should ideally be conducted within the zones where registration is anticipated, suitability of read-across between zones can be considered and has to be fully justified.

Crop type: Depending on the recommended uses the field trial crop could be conducted in a highly bee attractive surrogate, e.g. *Phacelia*, Oilseed rape, Mustard (recommended to cover field crops), or could be conducted based on the target crop. If effects or uncertainty still remain following studies in surrogate attractive crops, trials in the actual crop can be conducted as a further refinement step.

Controls: Control field and colonies should be included as standard and described in EPPO 1/170.

Exposure: Application should be made based on the worst-case for exposure according to the defined use of the PPP. Initial exposure can be measured by analysis of residues in the crop pollen and/or nectar (as applicable) and including bee matrices where applicable, and also *via* data on observation of foraging in the crop and through pollen source analysis. Longer-term analysis of hive products, stored pollen and honey should be considered where chronic effects are being investigated and if effects on colonies are monitored over-winter into the following season.

Long-term monitoring: Chronic effects should be considered by longer-term monitoring of the colonies by covering a minimum of 2 brood-cycles. Monitoring over-winter is also possible with further assessment of colony development in the following spring, it is noted that over-wintering data can be difficult to interpret due to the potential for additional multiple stressors.

Specific designs: The field trial should be considered as a flexible tool for investigation of specific issues or exposure regimes, e.g. effects on application pre-flowering. Whilst other routes of exposure are under discussion, e.g. guttation and honeydew, standardised guidance on field effects under reproducible conditions of exposure is not available therefore test designs should be discussed with expert guidance and with relevant regulatory authority in advance.

## **References**

Bakker F (2015) Design and analysis of field studies with bees: A critical review of the draft EFSA guidance. Integrated Environmental Assessment and Management — Volume 9999, Number 9999—pp. 1–7. DOI: 10.1002/ieam.1716.

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Rundlof M, Andersson GKS, Bommarco R, Fries I, Hederstrom V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature letters doi:10.1038/nature14420

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## Technical Section 12

### BEEHAVE honey bee Colony Model

The development of the honey bee model, BEEHAVE came from a project: "Honeybee population dynamics: integrating the effects of factors within the hive and in the landscape" (Rothamsted Research, UK, 2009-2013) which was co-funded by BBSRC (<http://www.bbsrc.ac.uk>) (circa 85%) and Syngenta (circa 15%). The model integrates colony dynamics, population dynamics of the varroa mite, epidemiology of varroa-transmitted viruses and allows foragers in an agent-based foraging model to collect food from a representation of a spatially implicit landscape. It is freely available online ([www.beehave-model.net](http://www.beehave-model.net)) and has undergone extensive sensitivity analyses and tests illustrate the model's robustness and realism. BEEHAVE offers a valuable tool for researchers to design and focus field experiments, for regulators to explore the relative importance of stressors to devise management and policy advice and for beekeepers to understand and predict varroa dynamics and effects of management interventions. EFSA evaluated the model based on their opinion on good modelling practice. The overall conclusion is that BEEHAVE performs well in modelling honeybee colony dynamics, and the supporting documentation is generally good but does not fully meet the criteria of the good modelling opinion. BEEHAVE is not yet usable in a regulatory context primarily because it needs a pesticide module. A few other minor points were raised and these are being addressed and a pesticide module is being developed and validated.

Becher MA, Grimm V, Pernille Thorbek P, Horn J Kennedy PJ, Osborne JL. (2014) BEEHAVE: a systems model of honey bee colony dynamics and foraging to explore multifactorial causes of colony failure. *Journal of Applied Ecology* 51, 470-482. DOI: 10.1111/1365-2664.12222

<http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12222/full>

## Technical Section 13

### Analysis of honey bee Protection Goals using BEEHAVE

The European Food Safety Authority reported a protection goal of negligible effect at 7% of colony size and then used the Khoury honeybee colony model to set trigger values for forager losses. However, the Khoury model is very simplistic and simulates colonies in an idealized state. A more realistic honeybee model, BEEHAVE, was used to explore pesticide risks. The results showed that forage availability interacts with pesticide-induced worker losses, and colony resilience increases with forage quality. The results indicate that the protection goal of 7% of colony size and triggers for daily worker losses were overly conservative. Forage availability is critical for colony resilience and that with adequate forage the colonies are resilient to even high levels of worker losses. However, it was recommended that when setting protection goals suboptimal forage conditions should be used to ensure conservatism and for such suboptimal forage, a total of 20% reduction in colony size was safe.

Thorbek, P, Campbell, PJ, Sweeney, PJ, Thompson, HM (2016) Using BEEHAVE to explore pesticide protection goals for European honey bee (*Apis mellifera* L.) worker losses at different forage qualities. *Environmental Toxicology and Chemistry*, Vol. 36, No. 1, pp. 254–264, 2017 <http://onlinelibrary.wiley.com/doi/10.1002/etc.3504/epdf>