1.16 Sensitivity of honey bee larvae to plant protection products and impact of EFSA bee guidance document

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Abstract

In addition to other assessments, the 2013 EFSA bee guidance document requires the risk assessment of plant protection products on honey bee larvae. At the time the EFSA document was finalized, no data on honey bee larvae were available. In 2013 ECPA (the European Crop Protection Association) perfomed an impact analysis of the (then) new EFSA risk assessment and the reliability of the outcomes, using estimated endpoints derived from acute oral honey bee tests together with the usual extrapolation factors. Today, a number of honey bee larvae toxicity studies have been conducted according to the newly developed testing methods for single exposure (OECD TG 237) and repeated exposure testing (OECD GD 239). These experimental data have been used to update the ECPA impact analysis. Data on 114 active substances or formulated products were used, covering 166 worst case uses; (58 herbicides, 53 fungicides, 47 insecticides and 8 PGRs). The "pass" rates were determined according to the EFSA Bee guidance document and compared with the original outcome of the impact analysis from 2013 and with adult chronic toxicity data. When the findings of the impact analysis based on experimental data from 22 day larval tests was compared with the impact analysis from 2013 based on extrapolated data the two gave very similar results, thus indicating that the original assessment using acute data and extrapolation factors was suitably predictive.

Keywords: Honey bee larvae, impact analysis, risk assessment

Introduction

In July 2013, the European Food Safety Authority (EFSA) published a guidance document on the risk assessment of plant protection products on honey bees, bumble bees and solitary bees (EFSA 2013), which intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009 (EC 2009). An ECPA (European Crop Protection Association) impact analysis assessed whether the EFSA document brings the desired improvement to the risk assessment on bees, including bee larvae, and reliability of the outcomes (Alix et al. 2013). Since a complete lack of data on bee larvae at that time, the impact assessment was conducted using data from acute toxicity tests with adult honey bees, together with the usual extrapolation factors to account for difference in sensitivity from acute to chronic testing. In the meantime since 2013, a number of larvae toxicity studies have been conducted according to the newly developed testing methods for single exposure (OECD test guideline 237, 2013) and repeated exposure testing (OECD guidance document 239, 2016). The objective of this paper is to summarize all available experimental data industry has generated to comply with the regulation, to assess the "pass" rates according to the EFSA Bee document and to compare the outcome of experimental data with the original outcome of the impact analysis which used estimated endpoints. Available adult chronic test data were also considered to investigate if larval or chronic adult risk assessment was the more critical.

Methods and data sources (honey bee risk)

The analysis from Alix et al. (2013) considered 151 active substances covering 163 uses: 60 were herbicides comprising plant growth regulators (PGRs), 52 fungicides, and 51 insecticides comprising acaricides. Because at the time no data were available as test methods were yet to be developed, larval toxicity endpoint ($NOED_{larvae}$ – no observed effect dose) were estimated as

follows: 1/10th of adult's acute oral LD_{50} corrected for mean larval body weight (83 mg), e.g. acute oral LD_{50} of 100 µg a.s./bee ≙ NOED of 8.3 µg a.s./larva.

For the current analysis, experimental data from 114 active substances or formulated products were considered, covering 166 uses: 58 herbicides, 53 fungicides, 47 insecticides and 8 PGRs.

As study methods developed throughout the last years, studies on larvae were performed according to different methods and provided different endpoints: single exposure studies until day 7 (reflected by OECD TG 237), which results are expressed as "D7" endpoints, repeated exposure studies until day 8 ("D8" endpoints) and repeated exposure studies until day 22 (reflected by OECD GD 239) leading to "D22" endpoints.

For the risk assessment, 'exposure-toxicity-ratios' (ETRs) were calculated based on the application rate (AR, in kg a.s./ha) and the $NOED_{larvae}$. Whereas for the 'screening step' risk assessment only the application rate and an application-type dependent 'short cut' (SV) value was considered (ETR larva = AR x SV /NOED), the tier 1 risk assessment (RA) takes into account on the one hand crop dependent exposure factors (Ef) and on the other hand SV-values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar and crop attractiveness (ETR larva = AR x Ef X SV /NOED) (for details see EFSA 2013). Moreover, it distinguishes the risk for bees being exposed to different scenarios, from which risk of being exposed to the 'treated crop' and to 'weeds flowering in the field' were regarded as the most relevant. Calculations were done using the EFSA–tool (Excel spreadsheet), Version 3 (October 2015). Adult chronic pass rates were taken from Miles et al. (2017).

Results (honey bee risk)

Larval data evaluation analysis results:

- The compiled data comprised single and repeated dosing as well studies with 7/8 and 22 day endpoints, resulting in the overall screening step and tier I RA pass rates described in Table 1.
- In D22 studies (n=21) the D8 endpoint is equivalent to the D22 endpoint In 43% of the cases, while in 48% of the cases D22 endpoint is lower than D7/8 endpoints (Table 2).
- Lower potential pass rates have to be expected, at least for compounds showing toxicity (*i.e.* many insecticides) compared to compounds of low toxicity (*i.e.* many fungicides and most herbicides), according to the requirements (repeated exposure, D22 endpoint) of the EFSA Bee GD (Table 3).
- The risk assessment based on extrapolated larval data (Alix et al. 2013) and experimental chronic adult honey bee data (Miles et al. 2017) resulted in lower pass rates for all compound groups compared to larval data, with the exception of insecticides using a D22 larval endpoint (Table 3).
- As standardized test methods for larval non-*Apis* bees are not available, risk would be based on 1/10th of the HB endpoint as surrogate. In this case the pass rates of spray application uses would significantly decrease for bumble bees (< 5%, n = 162) and solitary bees (< 5%, n = 162).

Table 7 Overall pass rates of screening step and tier 1 RA for honey bee larvae

Use (n)	Pass rates from 2017 analysis [%]*		
	Screening step	Tier I	
		'treated crop'	'weeds in the field'
Insecticides (47)	21	40	43
Fungicides (53)	77	89	96
Herbicides & PGRs (66)	96	97	97
All (166)	69	79	82

* derived from all uses and including single exposure (lasting until D7) and repeated exposure studies (lasting until D8 or D22)

Table 8 Sensitivity of D8 and D22 endpoint in repeated exposure D22 honey bee larvae studies

Endpoint relation	Proportion [%] (n_{ges} = 21)
D8 ≙ D22	42.9
D8 > D22	47.6
D8 > D22	4.8
D8 data not available	4.8

Table 9 Pass rates using endpoints of single (D7) and repeated exposure (D22) larvae studies as well as adult chronic studies

Use	Pass rates [%]			
	Honey bee larvae			Adult honey bees
	Screening * (Alix et al. 2013)	Tier I (2017) ** ('treated crop' scenario)		Tier I (Miles et al. 2017)
		Single exposure (D7)	Repeated exposure (D22)	Chronic exposure
Insecticides	26	43	15	18
Fungicides	58	89	80	44
Herbicides & PGRs	47	100	100	46
All	44	81	63	36

* endpoint deriving from acute oral testing

** derived just from single exposure (lasting until D7) and repeated exposure studies (lasting until D22)

Summary and Conclusions

- The findings of the initial impact analysis conducted in 2013 were supported and confirmed to be predictive when compared to the findings based on real-life endpoints from 22 day larval studies.
- Risk assessments using experimental larval data confirm that the chronic risk assessment for adults is the key driver of honey bee risk in the EFSA Bee GD as stated in the original impact analysis.
- Based on the data with different larval endpoints it can be concluded that larval tests providing D7/D8 endpoints can be used in the risk assessment for non-toxic compounds.
- The high failure rate on insecticides for honey bees jeopardize their registration, as risk assessments cannot be refined by the (unworkable) higher tier studies required by the 2013 EFSA guidance.
- Almost all compounds and their respective products (>95%) will fail the bumble bee and solitary bee larval risk assessment, because valid laboratory methods on their larvae are not available and higher tier studies are long-term research projects.
- The need to develop internationally recognised guidelines remains. New guidance should be built on existing guidance, recent research results as well as experiences and recommendations of all stakeholders.

References

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3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
7th Symposium, Avignon, France, 1999
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Foto
Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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