Section 2 – Honeybee Brood

2.1.P Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate?

Johannes Lückmann[1], Verena Tänzler[2]

[1]Rifcon GmbH, 69493 Hirschberg, Germany

[2]ibacon GmbH, 64380 Rossdorf, Germany

E-Mail: johannes.lueckmann@rifcon.de, verena.taenzler@ibacon.com

* on behalf of the ICP-PR Bee Brood Working Group and the bee brood working group of the German AG Bienenschutz

DOI 10.5073/jka.2020.465.043

Abstract

According to current European regulations on the risk assessment of plant protection products, the risk on honey bee larvae or honey bee brood has to be addressed. If the assessment indicates, that a potential risk cannot be excluded based on data derived from laboratory studies, two higher-tier options are given by the EFSA bee Guidance Document to refine this under more realistic conditions: the Oomen bee brood feeding test and brood studies performed according to the OECD Guidance Document 75. Both study types focus on the brood termination rate (BTR) as the key endpoint. While the Oomen brood test investigates the brood development after the acute or chronic administration of a test item spiked sugar solution to unconfined colonies, brood studies according to OECD GD 75 are performed under semi-field confined exposure conditions and examine potential effects on the bee brood after the overspray of a bee attractive flowering crop. However, the evaluation of historical data from semi-field studies according to OECD GD 75 showed a strong variability of the BTR of pre-imaginal stages developing from marked eggs (BTR_{eggs}) in the control. As an alternative, field studies according to EPPO 170 which comprise bee brood evaluations according to OECD GD 75 were considered to produce more reliable termination data.

The statistical analysis of available control data shows that Oomen feeding studies and bee brood studies performed under field conditions lead to significantly lower BTR_{eggs} of ≤ 20% compared to semi-field bee brood studies for which a mean BTR of about 30% is observed. Moreover, studies with unconfined colonies show a high proportion of control replicates with BTR_{eggs} ≤30% and ≤40% indicating a higher reliability compared to semi-field studies. A comparison of the possibilities and limitations of the three methods shows the strength of each method. In Oomen studies, the exposure of the brood and of the hive bees only can be regarded as artificial. However, the test concentrations can be adjusted to specific needs and to different feeding durations of at least one (acute) or 9 days (chronic). Furthermore, the absence of 'caging effects', the low dependency on climatic or crop conditions, the potential to test also herbicides which control dicotyledonous plants (since no crop plant is adversely affected by its mode of action) and an exposure period of at least nine days in chronic Oomen studies are crucial advantages. In contrast, the exposure scenarios of the two other methods are much more realistic and especially for semi-field studies a worst-case situation. Moreover, they also include exposure via pollen and exposure levels and durations, which strongly depend on the application rate and the flowering period of the treated crop. Whereas a dilution of plant protection product residues cannot be excluded during the exposure period in studies with unconfined colonies due to the shift to untreated flowering plants in the surrounding, this is not given for semi-field studies.

Keywords: bee brood testing, honey bees, semi-field, field, brood termination rate

Introduction

Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk on honey bee larvae or honey bee brood. According to the EFSA bee Guidance Document (EFSA 2013), both, the Oomen bee brood feeding test (Oomen et al. 1992) as well as the OECD GD 75 (OECD 2007) are given as the two higher tier options to refine the risk on honey bee brood. Both methods focus on the brood termination rate (BTR, unsuccessful development of pre-imaginal stages deriving from marked eggs or larvae) as the key endpoint. While the Oomen brood test investigates an artificial and worst-case acute or chronic oral exposure scenario to a test item spiked

feeding solution (Lückmann & Schmitzer 2019), studies according to OECD GD 75 depict a realistic worst-case test method to assess effects of plant protection products (PPPs) on honey bee brood in a treated, bee attractive crop under semi-field confined exposure conditions.

The evaluation of historical data from semi-field studies according to OECD GD 75 showed a strong variability of the control BTRs of marked eggs (BTR_{eggs}, in the text hereafter called BTRs) (Becker *et al*. 2015, Szczesniak *et al*. 2018). Therefore, field studies according to EPPO 170 (EPPO 2010) comprising the OECD GD 75 bee brood evaluation were regarded as an alternative to get more reliable BTR data, which was already envisaged by Becker et al. (2009). First results indicated that control BTRs deriving from OECD GD 75 studies conducted under field conditions were lower compared to BTR values obtained under semi-field conditions (Lückmann & Becker 2016).

Updated control BTRs, considering now also data of acute and chronic Oomen feeding studies as well as newly available BTRs from OECD GD 75 semi-field studies and from EPPO 170 field trials including bee brood evaluation according to OECD GD 75 are summarized and presented. Finally, possibilities and limitations of the methods are discussed.

Material and Methods

For the analysis control BTRs of marked eggs of acute and chronic Oomen studies, OECD GD 75 semi-field studies and EPPO 170 field studies including bee brood evaluation according to OECD GD 75 were compared (Tab. 1). The majority of the studies was carried out under GLP in Germany, Switzerland and France (Alsace). The studies were performed between 1997 and 2017 (Oomen, acute feeding), 2013 and 2019 (Oomen, chronic feeding), 2011 and 2019 (OECD GD 75, semi-field) and 2012 and 2018 (EPPO 170 & OECD GD 75, field). Data were provided and/or performed by Adama, BASF SE, Bayer, BioChem agrar, Dow AgroSciences, DuPont, Eurofins, ibacon, IES, RIFCON, Sparta Research and Syngenta.

As residuals were not normally distributed (Shapiro-test, $p<0.001$), for the statistical analysis a Kruskal-Wallis test (non-parametric) was performed revealing a significant difference ($p<0.001$). A Dunn's multiple comparison test was used as post-hoc test (two-sided, $\alpha= 0.05$).

Table 1: Number of studies and control replicates (colonies) for each study type

Study type	Number of studies [n]	Number of control replicates (colonies) for marked eggs [n]
OOMEN, acute feeding	27	85
OOMEN, chronic feeding	8	31
EPPO 170/OECD GD 75 (field)	7	39
OECD GD 75 (semi-field)	123	508

Results

The results show that Oomen feeding studies and bee brood studies performed under field conditions displayed mean BTRs between 15.8 and 19.9%, which are approximately 50% lower compared to BTRs obtained under semi-field conditions of 30.5% (Tab. 2, Fig. 1). Moreover, BTRs from studies with unconfined colonies were statistically significantly lower compared to BTRs from OECD GD 75 semi-field tests and show lower variability among replicates. And finally, studies with unconfined colonies, i.e. Oomen and field brood studies showed a high proportion of control replicates (colonies) with BTRs ≤30% and ≤40%.

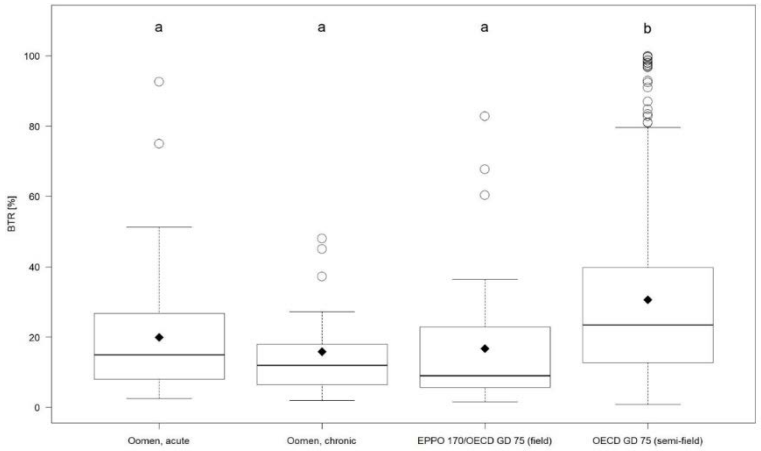

Fig. 1 Box plots of control BTR_{eggs}
(Dunn`s multiple comparison, $p<0.001$; diamonds = mean, solid line = median)

Table 2: Descriptive statistics of BTR_{eggs} in the control replicates (colonies)

Study type	Mean BTR_{eggs} ± SD [%]°	Min. BTR_{eggs} [%]	Max. BTR_{eggs} [%]	Proportion of replicates with BTR_{eggs} ≤30% / ≤40% [%]
OOMEN, acute feeding	19.9 ± 16.5 a	2.5	92.6	80.0 / 87.1
OOMEN, chronic feeding	15.8 ± 12.8 a	2.0	48.0	87.1 / 90.3
EPPO 170/OECD GD 75 (field)	16.7 ± 18.3 a	1.5	82.7	89.7 / 92.3
OECD GD 75 (semi-field)	30.5 ± 24.7 b	0.9	100	61.4 / 75.4

Discussion and Conclusion

The findings showed that studies with unconfined colonies resulted in lower control BTRs and lower variability between the replicates indicating a higher reliability of the test systems compared to brood studies under semi-field conditions. Thus, the BTRs of the study types with unconfined colonies were in a similar range compared to those which were obtained in the 'Reference data project' (von der Ohe *et a*l. 2015). There, the background BTR of honey bee colonies was studied at two colonies in 2014 and 12 in 2015. As in regulatory bee brood studies, the exact age of the eggs at BFD 0 was not known. The BTRs were 7.3% and 34.9% in 2014 and ranged between 2.0% to 28.4% in 2015, resulting in an overall mean BTRs of 12.0%. Two colonies, where the exact age of the eggs was known at BFD 0 due to caging of the queen for 24 hours in 2014, displayed a BTR of 7.3% and 87.6%. To extend the data base of the 'Reference data project', von der Ohe *et al.* (2015) also determined the BTRs of 18 colonies, where the population size was regularly estimated within the joint research project 'FitBee'. Based on this, the mean BTR displayed to be 28% (range: 1% to 40%).

Whereas both Oomen feeding test designs address the risk of PPP on honey bee brood and hive bees at defined, worst-case concentrations in sugar solutions (Lückmann & Schmitzer 2019), the OECD GD 75 semi-field test design reflects a realistic, worst-case exposure scenario to collected pollen and nectar, since honey bees are forced to forage on the PPP treated crop as the only food source in the enclosed system. On the other hand, field studies comprising bee brood evaluations according to OECD GD 75 investigate potential effects of a PPP on the bee brood, nurse and forager bees under realistic exposure conditions (Tab. 3). Under full field conditions forager honey bees can shift to untreated surrounding crops or flowering plants. Thus, a dilution of PPP residues cannot be excluded. Based on specific questions to be addressed by the study and taking the advantages and disadvantages of the respective study designs into account (Tab. 3), a set of methods are available to evaluate the potential risk on honey bee brood posed by PPPs.

Table 3: Possibilities and limitations of bee brood studies according to Oomen (acute and chronic), EPPO 170/ OECD GD 75 and OECD GD 75

Topic	**Oomen, acute & chronic**	**EPPO 170/ OECD GD 75 (field)**	**OECD GD 75 (semi-field)**
Exposure scenario	Artificial, worst-case concentrations; oral exposure of bee brood and hive bees	Realistic oral exposure of bee brood, hive and forager bees and contact* exposure of forager bees	Realistic worst-case oral exposure of bee brood, hive and forager bees and contact* exposure of forager bees
Exposure level and duration of exposure	Level can be adjusted to specific needs, e.g. max. field concentration acc. to intended GAP, residue levels in nectar, NOEC values derived from lab testing, etc.; constant for at least 1 (acute feeding) or 9 days (chronic feeding); longer duration depends on storage and consumption behaviour of bees	Level based on GAP; Duration of exposure depends on flowering period of treated flowers, storage of contaminated food in the hive and food consumption; decreasing residue level over the time	
Exposure of bees to a realistic concentration in pollen	-	+	+
Exposure of bees to a realistic concentration in nectar	+ (can be adjusted based on residue data)	+	+
Foraging on non-target plants/crop	+ (dilution of PPP residues possible but study should not be carried out during mass flowerings)	+ (dilution of PPP residues possible but there should not be other mass flowering crops and low flowering activity of non-crops in the proximity of the study fields)	+ (dilution of PPP residues after exposure phase in the tunnel possible)
Testing of herbicides intended for dicotyledonous plants	+	Herbicide mode of action may lead to methodological problems in feasibility (rapid fading of crop possible)	
'Caging effect'	-	-	+
Dependency on climatic and crop conditions	low	high	high
Reliability of the test system	high	high	moderate

+ = influence/relevant; - = no influence/not relevant; * if applied during day time during foraging activity

Acknowledgements

Many thanks to all the contract labs (BioChem agrar, Eurofins, ibacon, IES and RIFCON) and companies (Adama, BASF SE, Bayer AG, Dow AgroSciences, E. I. duPont de Nemours and Company, Sparta Research and Syngenta) for providing their data on honey bee brood testing. Thanks also belong to Dr. O. Jakoby (RIFCON) who performed the statistical analysis and M. Metz (RIFCON) for his remarks on the manuscript.

References

BECKER, R., C. VERGNET, C. MAUS, J. PISTORIUS, I. TORNIER, S. WILKINS, 2009: Proposal of the ICPBR Bee Brood Group for testing and assessing potential side effects from the use of plant protection products on honey bee brood. In: Hazards of pesticides to bees, 10th Internat. Symp. ICP-BR, Bucharest, Romania 2008, ed. by OOMEN, P.A., H. THOMPSON, Julius-Kühn-Archiv **423,** 43-44.

BECKER, R., J. LÜCKMANN, J. PISTORIUS, 2015: Effectiveness of method improvements of OECD GD 75 – Evaluation of the ICP-PR Bee Brood Working Group. In Hazards of pesticides to bees, 12th Internat. Symp. ICP-PR, Ghent (Belgium) 2014, ed. by OOMEN, P. A., J. PISTORIUS, Julius-Kühn-Archiv **450**, 83-92.

EFSA, 2013: EFSA Guidance Document on the risk assessment of plant production products on bees (Apis mellifera, Bombus spp. and solitary bees) (published on July 04, 2013, updated on 04 July 2014). - EFSA Journal **11(7)**: 3295, 268 p.

EPPO, 2010: EPPO STANDARDS PP1/170(4), Efficacy evaluation of plant protection products: Side-effects on honeybees. Bulletin OEPP/EPPO Bulletin **40**, 313-319.

LÜCKMANN, J., R. BECKER, 2016: Honeybee brood studies under field conditions: Is there a difference of the brood termination rate compared to semi-field studies? – Poster on the 26th Annual Meeting of SETAC Europe, 22-26 May 2015, Nantes, France.

LÜCKMANN, J., S. SCHMITZER, 2019: The Oomen bee brood feeding test – revision of the method top current needs and developments. Bulletin OEPP/EPPO Bulletin **49(1)**, 137-146.

OECD, 2007: Guidance document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions. Series of testing and assessment, Number 75, ENV/JM/MONO(2007)22, 27 p.

OOMEN, P. A., A. DE RUIJTER, J. VAN DER STEEN, 1992: Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bulletin OEPP/EPPO Bulletin **22(4)**, 613–616.

SZCZESNIAK, B., E. PILLING, S. BOCKSCH, R. BECKER, J. LÜCKMANN, 2018: ICP-PR Bee Brood Working Group – Variability of brood termination rates in reference to validity criteria and limited effectiveness of method improvement in honeybee semi-field studies (OECD GD 75). In: Hazards of pesticides to bees, 13th Internat. Symp. ICP-PR, Valencia (Spain) 2017, ed. by OOMEN, P.A., J. PISTORIUS, Julius-Kühn-Archiv **462**, 111-115.

VON DER OHE, W., M. JAHNKE, D. LÜKEN, 2015: Referenzwertprojekt 2014 – 2015. Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Institut für Bienenkunde Celle. Abschlußbericht. 21 S.

2.2.P Toxicity of oxalic acid on *in vitro* reared honeybee larvae

Lucia Sabová[1], Martin Staroň[2], Anna Sobeková[1], Dana Staroňová[2], Jaroslav Legáth[1], Rastislav Sabo[1]

[1]University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

[2]Institute of Apiculture Liptovský Hrádok, Gašperíkova 599, 033 80 Liptovský Hrádok, Slovakia

Correspondence: rastislav.sabo@uvlf.sk

DOI 10.5073/jka.2020.465.044

Abstract

Varroa destructor is considered as a serious pest of honeybees (*Apis mellifera*) and its resistance to acaricides has been reported since the early 1990s. Because large colony loses are yearly reported from over the world, new methods of treatment for *Varroa* mites are still in focus of many scientists. In our bioassay, we determined the lethal concentration 72 h LC_{50} of 2.425% oxalic acid solution following single spray exposure of honeybee larvae under laboratory conditions (Guideline OECD 237, 2013).

Keywords: honeybee larvae, oxalic acid, spray exposure, OECD 237

Introduction

Oxalic acid (OA) is a naturally occurring carboxylic acid used worldwide in apiculture to control *Varroa destructor*. It´s mode of action of OA is unknown, but the direct contact between them is required (Aliano *et al*. 2006). Some authors attributed its acaricidal action partly to a sensitivity of this species to acid pH (Maggi et al. 2016; Nanetti 2017). The instructions for administration of the authorised veterinary medicinal products with OA as an active ingredient recommend spraying,