INTERNATIONAL COMMISSION FOR PLANT-POLLINATOR RELATIONSHIPS

Bee Protection Group

15th INTERNATIONAL SYMPOSIUM

York, United Kingdom

October 18 – 21, 2022

HAZARDS OF PESTICIDES TO BEES

Location: Fera Science Ltd., York Biotech Campus (https://www.fera.co.uk/about-us/our-facilities/directions), Sand Hutton, York, Y041 1LZ, United Kingdom

History ICPPR-Bee Protection Group conferences:

1st Symposium, Wageningen, the Netherlands, 1980
2nd Symposium, Hohenheim, Germany, 1982
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
8th Symposium, Bologna, Italy, 2002
9th Symposium, York, UK, 2005
10th Symposium, Bucharest, Romania, 2008
11th Symposium, Wageningen, the Netherlands, 2011
12th Symposium, Ghent, Belgium, 2014
13th Symposium, València, Spain, 2017
14th Symposium, Bern, Switzerland, 2019
15th Symposium, York, UK, 2022

Organizing Committee 15th Symposium

Dr. Jens Pistorius	Julius Kühn-Institute, Germany
Dr. Anne Alix	Corteva Agriscience, United Kingdom
Claire Boston-Smithson	Fera Science Ltd, United Kingdom
Selwyn Wilkins	Fera Science Ltd, United Kingdom
Dr. Thomas Steeger	U.S. Environmental Protection Agency, USA

Supported by

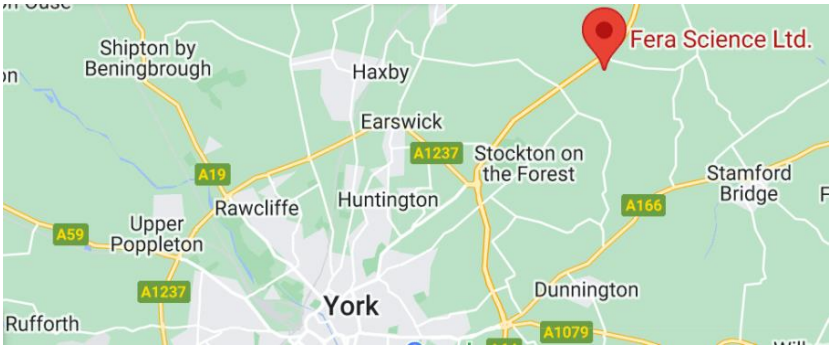
Sand Hutton, York, Y041 1LZ, United Kingdom

Contents

General Information

The Symposium will take place in:

Fera Science Ltd., York Biotech Campus
(https://www.fera.co.uk/about-us/our-facilities/directions),
Sand Hutton, York, Y041 1LZ, United Kingdom



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Own notes:

Preface

The ICP-PR Bee Protection Group Steering Committee welcomes you to the 15th International Symposium of the Bee Protection Group Hazards of Pesticides to Bees in York, UK. We would like to thank Fera Sciences Ltd. for generously hosting this event and particularly thank Selwyn Wilkins and Claire Boston-Smithson for their tireless efforts in organizing/coordinating the symposium.

Since the last symposium hosted by Agroscope Swiss Bee Research Center in Bern, Switzerland, the world has witnessed profound changes resulting from the COVID pandemic. The disease has served as a reminder for some and possibly as an epiphany for others that there are global challenges which supersede political and social boundaries and require a concerted response.

While the pandemic has prompted various responses, some of which focused on isolationism, the tremendous losses to our global community have most effectively been addressed through clear/timely communication, collaboration and cooperation based on sound science. While some continue to refrain "who would have thought this could happen", the more pertinent response may be "let's learn from this".

The challenges facing the global human community are not limited to the aftermath of COVID but include climate change and disparities in available resources to name a few. Similar challenges have been facing bees and have been characterized as the four Ps (*i.e.*, pests, pathogens, pesticides, and poor nutrition), which are intertwined with climate change. Perhaps the greatest lesson learned from COVID has been the recognition that the three Cs (*i.e.*, communication, collaboration, and cooperation) founded on strong science provides the most effective response.

The ICP-PR Bee Protection Group and its various workgroups have, even in the face of the pandemic, continued to advance the science with which to understand and mitigate hazards to bees from the 4Ps. The Steering Committee would like to take this opportunity to thank members of the BPG and its workgroups for their commitment toward promoting the science with which to inform regulatory decisions particularly given the personal and professional limitations placed on each of us over the past two years.

We are excited this year by the opportunity to interact with the Organization for Economic Cooperation and Development (OECD) and the United Nations' Food

and Agricultural Organization (FAO) and their interests in promoting sound science globally. As with meetings in the past, this symposium provides an opportunity to recognize and advance the science being developed and vetted by the workgroups and to understand the broader context in which data can be used to inform regulatory decision.

Once again, thank each of you for your contributions, perseverance, and dedication toward sound science.

Sincerely,

Jens Pistorius

Anne Alix

Tom Steeger

Accommodations

Hotel	Reference and contact details for delegates to booking
The Grand, York	yourstay@thegrandyork.co.uk +44 (0)1904 380038 Ref to quote: ICPPR22
The Principal	Reservation office open during office hours Monday – Friday only. Reservationsroyalyork@ihg.com +44 (0)1904 688615 Ref to quote: ICPPR
Malmaison York	Please call the Reservations Team on +44 (0)330 0160 380 to book your room. Ref to quote: ICPPR22
Park Inn by Radisson	Event Rate ICPPR22 • Email: eventplanning.york@parkinn.com please mention 'FERA ALLOCATION VIA MAKE IT YORK' when booking by email
Hotel Indigo	Bespoke booking link is here

Transportation

Getting around York

York is easily accessible from destinations across Britain. Direct trains run from London, Manchester and Edinburgh, getting you into the city in just two hours. Best explored on foot, the city also boasts excellent public transport connections, so it's easy to navigate once you arrive too.

- iTravelYork's handy guide gives you all the information you need on travel within the city
- It takes just 20 minutes to get from the outskirts to the city centre by bike, making cycling one of the easiest and most fun ways to access places to visit in York
- Looking to visit attractions outside of York? Coastliner operates regular services to nearby destinations including bustling Leeds and the quirky coastal town of Whitby

BY FOOT

Small in stature but big on personality, York's winding cobbled streets are best explored by foot. In fact, it takes just 20 minutes to walk from one side of the city to the other

BY BUS

First Bus run regular services throughout York and offer a range of money-saving tickets in addition to accepting contactless payments on all routes, for fast and convenient travel

BY BIKE

With a wide range of bicycle hire shops and bike tours available, you'll be spoilt for choice when it comes to discovering York attractions and landmarks on two wheels

In addition to the coaches that will transfer participants to and from the Fera campus at set times during each day of the conference, there are public coaches and taxis available that operate on regular schedules.

Social Event on Wednesday

Coaches will take you to Castle Howard - The Howard story is one of ambition, public service, liberal politics, and artistic endeavours. Although building work began in 1699, the construction of Castle Howard took over 100 years to complete, spanning the lifetimes of three Earls.

Sitting in the Howardian Hills, an Area of Outstanding Natural Beauty, the wider estate reflects a diversity of activities and environmental stewardship is at the

heart of this. Surrounded by almost 9,000 acres of farmland, woodland, rolling hills, lakes and rivers Castle Howard's natural environment is as beautiful as the house itself. Environmental stewardship is at the heart of the wider management of the estate. This includes schemes for the regeneration of hedgerows and field margins to encourage biodiversity.

The visit will include lunch in the Grecian Hall and include a visit to the House (free flow/self-guided) and two activities following which the coaches will return you to York in time to change for dinner.

Upon returning to York there will be a Viking-themed gala dinner in the Merchant Adventurers' Hall including a mead reception, a Medieval host to provide in-between course entertainment and Medieval Minstrels to provide traditional live music during the meal.

And then, there is York. . . to learn more, visit https://www.visitbritain.com/us/en/england/northern-england/york#

Program

Week at a glance

No.	Start	End	Title	Presenting Author
Day 1 Tuesday				
	11:30	12:30	**Welcome, registration, Lunch**	
	12:30	12:35	Introduction	Jens Pistorius, ICPPR BPG Board
	12:35	12:55	Conference O-pening	Andrew Swift, FERA, David Philips
	12:55	13:00	Organisational Issues	
Update from an exchange and collabporations with International Organisa-tions				
				Session chair: Jens Pistorius
	13:00	13:15	Leon van der Wal	OECD
	13:15	13:30	William Garthwaite	FAO
	13:30	13:45	Sofie Hoefkens	EU COM
	13:45	**14:00**	**Coffee and Tea Break**	
EFSA Bee Guidance				**Session chair: Selwyn Wilkins**
	14:00	14:35	Csaba Szentes	Introduction, Exposure (25+10)
	14:35	15:10	Brecht Ingels	Hazard
	15:10	15:45	Dirk Süßen-bach	Lower Tier RA
	15:45	**16:00**	**Coffee and Tea Break**	
	16:00	16:35	Working Group members	Specific issues
	16:35	17:10	Working Group members	Higher Tier
	17:10	17:40	Szentes + WG members	Summary and feedback
END OF DAY 1				
	17:45	19:30	Leon van der Wal	OECD EG-PTA meeting (members only)

No.	Start	End	Title	Presenting Author
Day 2 Wednesday				
ICPPR Working Groups				
	8:15	8:55	WG Chairs	Working groups Lab, SF, Field
	8:55	9:35	WG Chairs	Working groups Non-Apis
	9:35	**9:50**	**Coffee and Tea Break**	
	9:50	10:30	WG Chairs	Working groups Risk assessment
	10:30	11:10	WG Chairs	Working groups Bee Brood
	11:10	11:50	WG Chairs	Working groups Microbials
			Lunch and Excursion	
END OF DAY 2				

Day 3 Thursday				
Non-Apis				**Session Chair: Daniel Schmehl**
1.1	9:00	9:20	Daniela Grossar	A novel approach for acute single dose toxicity testing on a solitary bee, Osmia bicornis
1.2	9:20	9:40	Ana Cabrera	A chronic oral test protocol for orchard bees, Osmia spp. (Hymenoptera: Megachilidae)
1.3	9:40	10:00	Dan Schmehl	The surrogacy of Bombus impatiens (Hymenoptera: Apidae) for global use in a pesticide risk assessment
1.4	10:00	10:20	Ed Pilling	Sensitivity of a semi-field study design with solitary bees (Osmia bicornis)
	10:20	**10:40**	**Coffee and Tea Break**	
1.5	10:40	11:00	De Souza Rosa-Fontana	The Neotropical bee species Scaptotrigona postica as modelorganism for toxicological bioassays during the larval phase: a method for ring test

No.	Start	End	Title	Presenting Author
Risk assessment, Microbials				**Session Chair: Daniel Schmehl**
2.1	11:00	11:20	Verena Taenzler	Acute toxicity of pesticide mixtures to honey bees is generally additive, and well predicted by Concentration Addition
2.2	11:20	11:40	Karoline Wüppenhorst	Reviewing pesticide residues in larval food jelly of the Western honey bee Apis mellifera
2.3	11:40	12:00	Jakob Eckert	The pathway of residues from plant to honey bees – Factors influencing the exposure of honey bee brood
	12.00	**13.00**	**Lunch**	
2.4	13:00	13:20	Vanessa Roeben	Bee-longing together – Application of BEEHAVEecotox to predict semi-field studies
3.1	13:20	13:40	Abdulrahim Alkassab	Testing Microbial Pesticides in Bees –a comparative study on different bee species
3.2	13:40	14:00	Dan Schmehl	Factors that increase adult honey bee (Hymenoptera: Apidae) longevity in laboratory bioassays for microbial pesticide testing
Lab, Semi-field, Field				**Session chair: Silvio Knaebe**
4.1	14:00	14:20	Hervé Giffard	Current experimental advances from the French Methodological Bee Group. New improvement for future reprotoxicity tests.
4.2	14:20	14:40	Katharina Schmidt	How accurately can we measure Bombus colony parameters combining automated and manual methods?
4.3	14:40	15:00	Silke Andree Labsch	Assessing the Precision of state-of-the-art Bee Counters
4.4	15:00	15:20	Richard Gill	Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bees

No.	Start	End	Title	Presenting Author
4.5	15:20	15:40	Silvio Knaebe	Observation of Repellency Effects on Honey Bees and their Pollen and Nectar Collection Behaviour under Semi-Field Conditions with an automated bee counter
4.6	15:40	16:00	Jan Baas	BeeGUTS – a TKTD model for the interpretation and extrapolation of bee survival data
	16:00	**17:00**		**Poster session**
Lab, Semi-field, Field				**Session Chair: Mark Miles**
4.7	17:00	17:20	Mark Miles	Honeybee and bumblebee exposure to post-flowering applications of an insecticide in apple orchards
4.8	17:20	17:40	Anina C. Knauer	Nutritional stress exacerbates impact of a novel insecticide on solitary bees' behaviour, reproduction and survival
4.9	17:40	18:00	Paraskevi Kolokytha	From lab to field: a solid methodology for *Bombus terrestris dalmatinus* side effect studies
END OF DAY 3				

Day 4 Friday				
Monitoring, Organizational issues				**Session Chair: Jens Pistorius**
	09:00	09:45		Checkout
	09:45	10:35		ICPPR BPG Organisational issues- next conference etc.
5.1	10:35	10:55	Silvio Knaebe	Honey bee lifecycle assessment and homing success in field observations with the help of visual bee monitoring technology Poster
5.2	10:55	11:15	Silvina Niell	Monitoring of pesticide residues with beehives in different agroecosystems
	11:15	**11:35**		**12:00 Closing of Conference, Light Lunch**
END OF DAY 4- End of Symposium				

Program: Posters

1.		Session - Non-Apis bees
1.1.	Julian Fricke	Leafcutter bee Megachile rotundata semi-field test design
1.2	Schwarz, Janine M.	A more diverse pollen nutrition matters for developing solitary bees but does not mitigate the negative impact of pesticides
1.3	Knauer, Anina	Nutritional stress exacerbates impact of a novel insecticide on solitary bees' behaviour, reproduction and survival
1.4	Eugenia Soler	Method development for the acute contact test on the solitary bee *Megachiles rotundata*. – LD50 toxic reference
2.		**Session - Risk Assessment/ Microbials**
2.1.	Johannes Lückmann	Brood termination rate in honey bees in two consecutive brood cycles: a comparison
2.2.	Johannes Lückmann	BEEHAVE and brood termination rate - A modelling study how timing, magnitude and duration of effects determine colony strength
2.3.	Maryam Qureshi	Conceptual framework for the selection of higher-tier refinement options with focus on honey bee (Apis mellifera) brood
2.4.	Mark Milkes	Bumblebee (Bombus terrestris) versus honey bee (Apis mellifera) acute sensitivity - Final results of a CropLife Europe data evaluation
2.5.	Silvia Hinarejos	Compilation and statistical analysis of pesticide residue levels in pollen and nectar: refined Residue Unit Doses (RUDs) for Tier 1 dietary bee risk assessment in North America
3.		**Session - Laboratory/Semi-field/Field**
3.1.	Ratislav Sabo	The lethal and sublethal effects of synthetic miticide tau-fluvalinate (tech.) on adult honeybees
3.2.	Mareike Roeder	Comparison of Dead Bee Traps for Honey Bees
3.3.	Frederic Tausch	GLP requirements for using visual bee monitoring technology in ecotoxicological studies

3.4.	Hudson V. V. Tomé	Chronic larval and adult honey bee laboratory testing: which dietary additive should be considered when a test substance is not solubilized in acetone?

4.		**Session – Monitoring**
4.1.	Richard Odemer	Evaluation of bee counters - introduction of a new protocol for measuring the accuracy of daily losses.

5.		**Session - Microbials**
5.1.	Abdul Alkassab	Assessing the impact of microbial plant protection product mixtures on honeybee workers
5.2.	Silvio Erler	Bacillus thuringiensis ssp. aizawai - Observations on honey bees and distribution in colony matrices under field conditions

Abstracts: Oral Presentations

(in order of program)

EFSA Bee Guidance

Review of the EFSA bee Guidance document (draft, 2022)

Szentes, Csaba[1*]; Wassenberg, Jacoba[1]; Ingels, Brecht[2]; Süßenbach, Dirk[3]

[1] European Food Safety Authority, Pesticides, Parma, Italy

[2] Federal Public Service Health, Food Chain Safety and Environment, Department Plant Protection Products and Fertilizers, Brussels, Belgium

[3] Umweltbundesamt, Section IV 1.3 - Plant Protection Products, Dessau-Roßlau, Germany

*csaba.szentes@efsa.europa.eu

Abstract

EFSA's 2013 Guidance Document for the risk assessments for pesticides and bees has been reviewed and the first draft launched for a public consultation (summer 2022). Most of the aspects and methods for the characterisation of the exposure, the hazard, and for the lower- and higher tier risk assessments have been updated. The methods described in the new document are able to predict the effect of a pesticide on the colony/population in a more realistic way, while the protection goal as agreed by the risk managers is respected. Moreover, specific aspects were also reviewed; the new document includes comprehensive guidance for sublethal effects, for metabolites and for chemicals prone to time-reinforced toxicity. A series of presentations will explain the most important changes compared to the 2013 version, and the main characteristics of the reviewed guidance document.

Keywords: EFSA, Pesticides, Risk assessment

1. Session – Non-Apis bees

1.1. A novel approach for acute single dose toxicity testing on a solitary bee, Osmia bicornis.

Jeker, Lukas[1*]; Kimmel, Stefan[2]; Wenzel, Bettina[3]; Straub, Lars[1,4]; Grossar, Daniela[1*]

[1] Swiss bee research center, Agroscope, Bern, Switzerland

[2] Corteva Agriscience Germany GmbH, Munich, Germany

[3] Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland

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*lukas.jeker@agroscope.admin.ch, daniela.grossar@agroscope.admin.ch

Abstract

Robust laboratory-based guidelines for acute oral toxicity testing in solitary bee species are urgently needed to assess the risks of plant protection products and their active ingredients. Current attempts to develop such an interlaboratory testing system for solitary bees, are currently inadequate and face numerous obstacles. Among one of the major concerns is the inappropriate feeding methods. Thus, unlike the acute oral test systems for honeybees (OECD Guideline 213) and for bumblebees (OECD Guideline 247), such a guideline for solitary bees is currently lacking. Here, we propose a novel testing system for an acute oral toxicity test using Osmia bicornis. To both improve feeding success (oral dosage) and ensure that bees ingested the desired amounts of sucrose solution within a short period of time (e.g. 4 hours), we tested a novel feeding device and familiarized bees with the device during a pre-exposure training period. Compared to the commonly used Nicot cages, our new transparent cages had a larger volume and pipette tips as feeding devices. Feeding success (complete food intake) was very high (75-88%). This greatly improved acute oral dosing, and the use of the pipette tips reduced evaporation of the test substance. Mortality in the control group was low (11.8 %), monitoring of bee behavoiur and handling was simplified, which reduced stress on bees as well as decreased workload on respective assessments. Ultimately, our method appears a promising approach for reliably testing acute oral toxicity in solitary bees, yet additional studies are required to confirm and validate our findings.

Keywords: Solitary bee; *Osmia bicornis*; acute oral exposure;

1.2. A chronic oral test protocol for orchard bees, *Osmia* spp. (Hymenoptera: Megachilidae)

Cabrera, Ana[1*]; Exeler, Nina[2]; Schmehl, Daniel[2]; Pamela Jensen[1]

[1] Bayer CropScience LP, Environmental Safety, Chesterfield, USA

[2] Bayer CropScience, Environmental Safety, Monheim, Germany

* ana.cabrera@bayer.com

Abstract

The Pollinator Risk Assessment framework in North America and other regions is based on a tiered approach with the honey bee, *Apis mellifera*, as the representative organism. The protectiveness of the honey bee risk assessment for non-*Apis* bees has not been extensively validated due to limited availability of standardized methods. We developed a chronic oral test for orchard bees with *Osmia lignaria*, O. *cornifrons*, and O. *cornuta*. Our protocol includes elements from other chronic oral toxicity bee tests including the OECD 245 honey bee guideline and a validated protocol for bumble bees; these elements include the 10-d test duration, replication, and validity criterion for control survival. We measured the daily consumption of the feeding solutions and observed survival and other adverse effects. Evaporation controls were included to correct consumption estimates. On average, O. *lignaria*, O. *cornifrons* and O. *cornuta* body weight was 105 ± 12, 71 ± 8, and 129 ± 16 mg, respectivelly. Consumption in the control group was 49 ± 14, 85 ± 21, 157 ± 35 mg sucrose solution/bee/d for O. *lignaria*, O. *cornifrons*, and O. *cornuta*, respectively. Control survival was ≥ 85% for the three species evaluated. A fourth test was conducted with O. *bicornis* but outside the typical active season, which may affect the representativity of the results for this species. Dose-response tests with dimethoate, a positive control in bee toxicity tests, were conducted with each *Osmia* species and comparison of the resulting toxicity endpoints between honey bee and *Osmia* species will be presented.

Keywords: risk assessment, toxicity test, solitary bees, non-*Apis* bees

1.3. The surrogacy of *Bombus impatiens* (Hymenoptera: Apidae) for global use in a pesticide risk assessment

Schmehl, Daniel[1*]; Cabrera, Ana[1]; Jensen, Pamela[1]; Exeler, Nina[2]

[1] Bayer CropScience LP, Environmental Safety, Chesterfield, USA

[2] Bayer CropScience, Environmental Safety, Monheim, Germany

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Abstract

For over twenty years, the honey bee has been relied upon as the representative pollinator surrogate species for a pesticide risk assessment due to its global geographic distribution, ease of management, and validated test methods. More recently there have been questions on whether the risk of a chemical to the honey bee is truly representative for the other ~20,000 bee species globally. Honey bees have a eusocial life history comprised of tens of thousands of individuals, which is in contrast with the majority of bees that are semi-social or solitary. Bumble bees are a well known group of over 250 species that are important in agriculture and being considered as a representative semi-social bee in risk assessments. The majority of method development has been conducted in Europe on the buffed-tailed bumble bee (*Bombus terrestris*). While this species is used reliably for acute (OECD guidelines 246 and 247) and chronic toxicity bioassays, its performance is less predictive in a microcolony (brood test) or colony-level study. Here we present toxicity data for the Common Eastern Bumble Bee (*Bombus impatiens*), the commercially-available species of bumble bee in North America. We demonstrated consistent and predictive performance as individuals and in groups across the laboratory and field levels. Exposure of B. *impatiens* to the reference toxicant dimethoate yielded a toxicity profile that is comparable to B. *terrestris*, suggesting that B. *impatiens* endpoints are suitable and valid in cases when bumble bee data are required for use in a pesticide risk assessment.

Keywords: risk assessment, toxicity, surrogate, microcolony, bumble bee

1.4. Sensitivity of a semi-field study design with solitary bees (Osmia bicornis)

Franke, Lea[1], Klein, Olaf[1], Knäbe, Silvio[1], Pilling, Ed[2]

[1] Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

[2] Corteva Agriscience, Cpc2 Capital Park, Fulbourn, Cambridge CB21 5XE, United Kingdom

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Abstract

To be able to define Specific Protection Goals for bees, it is important to have a scientific database on the kind and magnitude of effects, which can be observed in higher tier studies (field and semi-field). High variability in field data is often an issue, leading to the question, which level of effects can be statistically detected. In the recently published revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) no protection goal was defined, because there is a lack of data (EFSA 2022).

One possibility to describe effects that can be observed are Minimal Detectable Differences (MDDs). They are used to describe the size of an effect in a test item treatment group, which can be statistically detected compared to a control group.

Based on a protocol published by the ICPPR Non-Apis working group (Franke et al 2021), two semi-field studies with the solitary bee species *Osmia bicornis* were conducted under Good Laboratory Practice (GLP). MDDs were calculated for the endpoints derived in these two studies and were compared to the published MDDs of the ring-test data.

The sensitivity of the semi-field test design in general and the sensitivity of individual endpoints, such as flight and nesting activity (as measure of acute effects), brood cell production and cocoon production per nesting female (as measure of effects on reproduction), will be discussed.

References

EFSA (European Food Safety Authority), Auteri, D., Arce, A., Ingels, B., Marchesi, M., Neri, F. M., . & Wassenberg, J.,2022: Analysis of the evidence to support the definition of Specific Protection Goals for bumble bees and solitary bees (Vol. 19, No. 1, p. 7125E).

Franke, L., C. Elston, C., Jütte, T., Klein, O., Knäbe, S., Lückmann, J., Roessink, I., Persigehl, M., Cornement, M., Exeler, N., Giffard, H., Hodapp, B., Kimmel, S., Kullmann, B., Schneider,

C., Schnurr, A., 2021: Results of 2-year ring testing of a semifield study design to investigate potential impacts of plant protection products on the solitary bees *Osmia bicornis* and *Osmia cornuta* and a proposal for a suitable test design. Environ. Toxicol. Chem., 40 (2021), pp. 236-250.

1.5. The Neotropical bee species S*captotrigona postica* as model-organism for toxicological bioassays during the larval phase: a method for ring test

Rosa-Fontana, Annelise[1*]; Dorigo, Adna[1]; Nocelli, Roberta[2]; Malaspina, Osmar[1]

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Abstract

Efforts to investigate if *Apis mellifera* is an appropriate representative species for the neotropical native bees on risk assessments (RA) has been requesting by the Brazilian regulatory agency. Recent advances in the scientific literature proved that toxicological bioassays on the larval stage of bees are essential, and that the use of the standardized method for honeybee larvae in stingless bees is unfeasible. *Scaptotrigona postica* was proposed as the most suitable Neotropical native species to be used as model organism for exposure to pesticides during the larval phase. The protocol was developed from adaptations to OECD 237 and 239 for A. *mellifera*. Five different *in vitro* larval rearing methods were carried out, and the most successful one was established. Parameters used for its validation were: mortality and emergence rates; progression of the larval stages; and morphometrical endpoints. The proposed protocol was tested using the active ingredient dimethoate. The oral LC_{50} were (in ng a.i./larva): 172.48 and 156.33 for 24 and 48 h, respectively. The method proved feasible, and the protocol was presented in two workshops held in Rio Claro, Sao Paulo, in April (physically) and September (online) 2022. The next step is to formalise the standardization throughout the national territory. The same 13 laboratories joined to the ring test for adult stingless bees will be invited, as well as the joining of new institutions will be welcomed. A summary of the parameters used for the method will be given and further recommendations will be presented.

Keywords: *in vitro* larval rearing, pollinators, ring-test, stingless bees

Proc FAPESP 2017/21097-3

2. Session – Risk Assesment

2.1. Acute toxicity of pesticide mixtures to honey bees is generally additive, and well predicted by Concentration Addition

Verena Taenzler[1]; Arnd Weyers[1]; Christian Maus[1]; Markus Ebeling[1]; Steven Levine[2]; Ana Cabrera[2]; Daniel Schmehl[2]; Zhenglei Gao[1] & Ismael Rodea-Palomares[2]

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Abstract

Understanding the frequency of non-additive effects of pesticides (synergism and antagonism) is important in the context of risk assessment. The goal of this study was to investigate the prevalence of non-additive effects of pesticides to honey bees (*Apis mellifera*). We investigated a large set of mixtures including insecticides and fungicides of different chemical modes of action and classes. The mixtures included represent a relevant sample of pesticides that are currently used globally. We investigated whether the experimental toxicity of the mixtures could be predicted based on the Concentration Addition (CA) model for acute contact and oral adult bee toxicity tests. We measured the degree of deviation from the additivity predictions of the experimental toxicity based on the well-known Mixture Deviation Ratio (MDR). Further, we investigated the appropriate MDR thresholds that should be used for the identification of non-additive effects based on acceptable rates for false positive (alpha) and true positive (beta) findings. We found that a deviation factor of MDR = 5 is a sound reference for labeling potential non-additive effects in acute adult bee experimental designs when assuming a typical Coefficient of Variation (CV%) = 100 in the determination of the LD_{50} of a pesticide (a factor of 2x deviation in the LD_{50} resulting from inter-experimental variability). We found that only a 2.4% and a 9% of the mixtures evaluated had an MDR > 5 and MDR < 0.2, respectively. The frequency and magnitude of deviation from additivity found for bees in this study are consistent with those of other terrestrial and aquatic taxa. Our findings suggest that additivity is a good baseline for predicting the toxicity of pesticide mixtures to bees, and that the rare cases of synergy of pesticide mixtures to bees are not random but have mechanistic basis.

2.2. Reviewing pesticide residues in larval food jelly of the Western honey bee Apis mellifera

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Abstract

In risk assessment, honey bees are used as a model organism to evaluate the effects of pesticides on pollinators. Honey bees forage on pollen and nectar, which are the nutritional basis for the nurse bees to produce the food jelly they feed to the larvae of all castes and sexes. It has been proven in several studies that pesticide residues can be found in different bee related products like wax, beebread, or honey and thus a further transfer into the larval food jelly might be possible. Here, we aim to summarize and analyze the current literature dealing with residue analysis of pesticides in food jelly. Furthermore, we assess the amount of contaminants remaining in jelly, to evaluate factors influencing their occurrences, and to deduce risk for larvae. Most of the studies focus on the detection of residues in royal jelly, while only one focused on worker jelly. It was demonstrated that 30 out of 176 analyzed pesticides were detectable in a range of 0.005 to 3860.25 ng/g in different royal jelly samples. The application and exposure method are the main factors influencing if residues remain detectable in food jellies. All detected concentrations were predominantly below toxicological values for bee larvae, but sub-lethal effects should not be neglected. Nevertheless, there is still information missing about the contamination pathway of pesticides, dilution or accumulation factors within the hive, degradation time in bee-related matrices, and impact on larval physiology, which should be completed to allow for sufficient protection levels of honey bees.

Keywords: royal jelly, contamination flow, larval development

2.3. The pathway of residues from plant to honey bees – Factors influencing the exposure of honey bee brood

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Abstract

Following the currently established risk assessment schemes for honey bees, the effects of plant protection products on honey bee larvae have to be investigated. However, field realistic exposure levels of honey bee brood remain largely unconsidered and are driven by worst case assumptions and the physical properties of the active substances (*i.e.*, solubility in larval diet). The aim of several semi-field and colony feeding studies was to trace the residue levels throughout the different matrices such as flowers, nectar, pollen, worker jelly and royal jelly following an application of a tank mixture on a highly bee attractive crop. To account for the different application rates of the active substances, a calculation of residue-unit-doses (RUDs) was used to characterize the decline of residues. The resulting exposure estimation of young honey bee larvae considers the different octanol-water partition coefficients of the active substances, residue decline, filtering and dilution factors, contrasting exposure conditions of honey bee brood in semi-field and colony feeding studies and castes of developing larvae.

2.4. Bee-longing together – Application of BEEHAVE$_{ecotox}$ to predict semifield studies

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Abstract

Factors affecting honey bee health are manifold, such as diseases, parasites, plant protection products (PPPs), environmental and socio-economic factors. In this

presentation we will briefly introduce the BEEHAVE$_{ecotox}$ model and show how the model can be applied to simulate and better understand (semi-)field studies. The model is a suitable and validated tool that mechanistically links exposure and effects and predicts PPP exposure both outside and inside the hive.

Keywords: Modeling, honey bee, ecotoxicity

Stichwörter: Modeling, Honigbiene, Ökotox

Introduction

Insect pollination is an important ecosystem service and pollinators play an essential role in providing important pollination services to most wild plant species and cultivated crops. Thus, pollinators and as such honeybees, are a crucial part of the environmental risk assessment of pesticides in the European Union. In this context, mechanistic modeling offers a powerful tool to predict the exposure and effects on bees in the field. Recently, Preuß et al. presented the BEEHAVE$_{ecotox}$ model, which mechanistically links the realistic exposure in the field, e.g., through foraging on nectar, pollen, and water, with subsequent effects on different levels of the bee colony. The model is designed with a modular framework in mind and can be parametrized using standard laboratory studies. For the regulatory risk assessment BEEHAVE$_{ecotox}$ can be used to extrapolate from laboratory to semi-field and field studies. Furthermore, it offers the possibility to study the effects in different crops and regions.

Material and methods

We use the BEEHAVE$_{ecotox}$ model as presented by Preuß et al. The model is implemented as an extension of the honeybee colony model BEEHAVE in NetLogo (Wilensky, 1999; Becher et al., 2014). BEEHAVE$_{ecotox}$ consists of 4 modules: the exposure module, the water foraging module, the in-hive fate module, and the effect module (Figure 1).

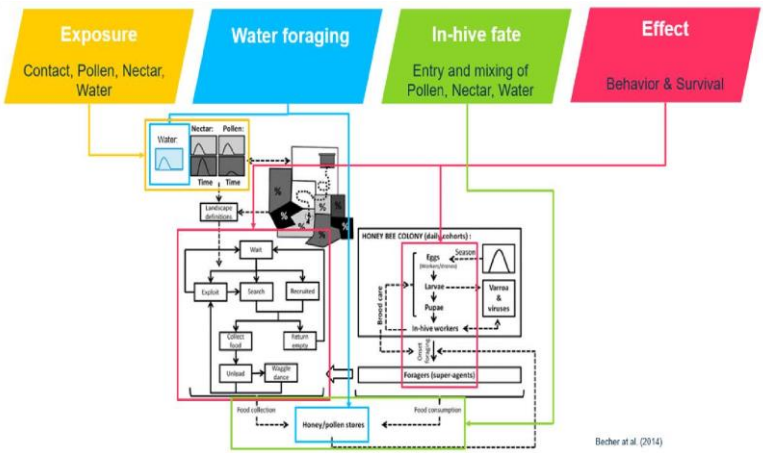

Figure 1 Flow-chart of BEEHAVE (Becher et al., 2014) and the BEEHAVEecotox additions. Black: original model. Orange: Landscape exposure module. Blue: water foraging module. Green: in-hive exposure module. Red: effect module for survival of different cohorts

The model was setup to represent the conditions of different (semi-) field studies in terms of number of adult bees, brood, honey and pollen stores, forage availability, and weather conditions. For this case study an insecticide application was simulated and the effects on the colony strength were assessed.

Results

The results show that the model is able to predict the colony strength of the simulated hives well. This highlights that the model can predict the effects soley based on available standard lower-tier risk experimental data. Observed discrepancies can be explained by missing empirical data on important environmental variables, such as food availability, which affect the nectar and pollen resources in the hive and can cause cascading effects.

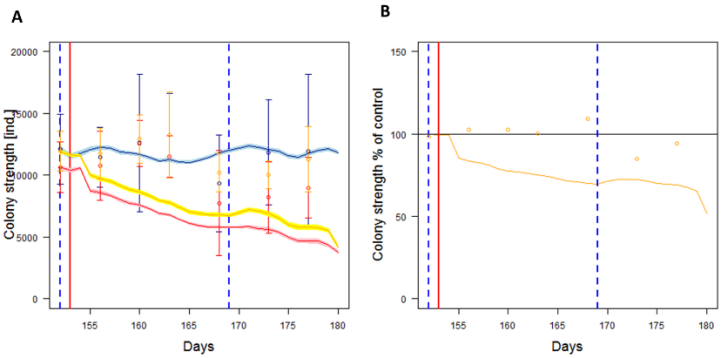

Figure 2 A: Measured (dots as an average with SD of three hives) and simulated (lines as an average with 95%

CI) colony strength in absolute numbers over time for control (blue), a toxic reference (red) and an insecticide (orange) for semi-field study. B: Relative impact of the insecticide on the colony strength compared to the control (dots and lines as an average of three hives for measured and simulated colony strength). Blue vertical lines indicate the start and end of the tunnel exposure phase. The red vertical line indicates the application day.

References

BECHER, M. A.; V. GRIMM; P. THORBEK; J. HORN; P. J. KENNEDY & J. L. OSBORNE, 2014: BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. Journal of Applied Ecology. 51 (2), 470–482, DOI: 10.1111/1365-2664.12222.

PREUSS, T.G., A. AGATZ, B. GOUSSEN, V. ROEBEN, J. RUMKEE, L. ZAKHAROVA, P. THORBEK, (under review): The BEEHAVEecotox model - Integrating a mechanistic effect module into the honeybee colony model. Environmental Toxicology and Chemistry.

WILENSKY, U., 1999: NetLogo. Center for Connected Learning and Computer-Based Modeling, Northwestern University.

3. Session – Microbials

3.1. Testing Microbial Pesticides in Bees –a comparative study on different bee species

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Abstract

Several microbial plant protection products (PPPs) have been developed as alternative to chemical PPPs, since growing concerns regarding the adverse effects of chemical PPPs on environment and non-target organism have been reported. In contrast to chemical PPPs, usually a higher application frequency of microbial based products is required which may result in a potential increase in their environmental dispersion. Although the mode of action of some microbial-based products has been extensively studied, several knowledge gaps related the interactions between non-target insects, including bees, and the applied microorganisms still exist. Based on the differences in colony and nest temperatures of various bee species and the preferred growth temperatures of the applied bacteria and fungi, we investigated the response of bee species (*Apis mellifera, Bombus terrestris*, and *Osmia bicornis*) to the exposure to different microbial PPPs under laboratory conditions. The bees were exposed acutely or chronically (over 10 d) to products containing either *Bacillus thuringiensis* subsp. aizawai or *Beauveria bassiana* at temperatures of 18°C, 26°C, and 33°C. Behaviour, food uptake and mortality were recorded daily 15-20 days. Our results show that the temperature may play an important role in the response of bees after exposure to the microbial PPPs. In general, tested bees were more sensitive to the tested B. *thuringiensis*-based product than to the B. *bassiana* based product. B. *terrestris* showed higher sensitivity to the tested B. *thuringiensis*-based product than other bee species, whereas O. *bicornis* were more sensitive to the tested B. *bassiana*-based product than other bee species. In conclusion, additional studies under field conditions are

needed to assess the infectivity and possible pathogenicity of such microbial PPPs for different bee species.

Keywords: temperature, microbial pesticide, *Apis mellifera*, *Bombus terrestris*, *Osmia bicornis*

3.2. Factors that increase adult honey bee (Hymenoptera: Apidae) longevity in laboratory bioassays for microbial pesticide testing

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Abstract

The interest in and use of biological materials (*e.g.* biostimulants, biopesticides) in crop production is increasing globally at a rapid pace. Part of the interest is that these technologies are viewed as safer alternatives to conventional chemicals and provide value in a holistic integrated pest management approach. While establishing the safety of these materials is as important as for conventional chemicals, there are important distinctions between them. For example, micro-organisms need to be evaluated for their pathogenic potential. The current EPA honey bee test guideline for assessing the pathogenicity potential of a microbial pesticide (OCSPP 885.4380) requires a 30-day observation period after dosing, but this test duration is difficult to achieve. A reliance upon shorter 10-day duration studies based upon OECD guideline #245 may not capture signs of pathogenicity, as some known bee pathogens take up to two weeks to elicit signs of an infection. Additionally, microbial-based test material can be difficult to deliver within a syringe feeder due to potential clogging or difficulty in maintaining homogeneity. The goal of the present study is to identify the factors that may increase adult longevity in laboratory cage bioassays, including age, cage type, number of bees, the presence of wax, honey, or water, and time of year were investigated. Factors that led to consistently high survival may inform an optimized test design for assessing the potential pathogenicity of a microbe to honey bee adults.

Keywords: risk assessment, microbials, pathogenicity, laboratory bioassay, honey bee

4. Session – Laboratory/Semi-field/Field

4.1. Current experimental advances from the French Methodological Bee Group. New improvement for future repro-toxicity tests.

Giffard, Herve[1] & al. (Chauzat, Marie Pierre[2], Fourier, Julie[3], Leblond, Sandrine[4], Aupinel, Pierrick[5], Aletru, Frank[6], Brunet, Jean Luc [5], Laporte, Jean Michel[7], Vidau, Cyril[3])

[1] Testapi, [2] Anses, [3] ITSAP, [4] BASF, [5] Inra, [6] SNA, [7] Syngenta

Abstract

This presentation follows that of Bern in 2019 since the improvement has not progressed during the pandemic.

The French Methodological is committed to provide guidance and protocols to assessors about local or international methodologies. Public and private researchers work together with beekeepers, industrials and CRO's in the aim of providing adapted protocols to the honeybee.

Laboratory LD_{50} tests and Semi-Field experiments were set up during the 70s' and review regularly under CEB 230, while new guidelines were initiated because of needs for new assessments.

The Brood test in laboratory conditions (Inra 2005), the chronic toxicity over ten-days (Itsap 2009) and the homing flight test (ITSAP 2011) were initiated before being extend at OECD level. The behavior of forager honeybees under tunnels as well as the measurement of HPGs (Hypopharyngial glands) are still under CEB230 methodology only.

Over the short-term effects in laboratory and mid-term effects in field or semi-field, the professional beekeeper organization requires for long-term effects of phytopharmaceuticals on colony development. It is also a requirement from the EFSA guidance document. In this aim it was discussed to apprehend the lifespan of bees, drones and queens. As it is a too large investment for a single methodology, we now focus on the drone fertility for a first step. Later on the lifespan of forager honeybees would be checked as a hypothesis of the decrease of the honey production if it is reduced by several days. Moreover the duration of queens will induce multiyear observations and difficulties to run under GLP.

Drone fertility.

The objective is to determine a NOEC on the spermatogenesis of the drones (quality and quantity).

There was two possibilities for the exposure and assessments of the drone development, in laboratory conditions and/or in semi-field conditions. After discussions within experts and beekeepers the current design uses laboratory conditions for the exposure and assessments of the drone development as the most efficient method to collect sexually mature drones.

Frames of drone wax are introduced in dedicated colonies in order to provide the expected brood with sufficient drone cells. Then drones and newly emerged bees are introduced in different queenless nuclei for adaptation in at least 3 modalities (control, positive reference and test item).

In laboratory conditions the exposure begins with the feeding of nurse bees (syrup at different concentrations + water and pollen ad libitum) during 20 days similarly to LD_{50} exposure.

Actually the protocol is not yet finalized but the collection of mature drone is efficient and the validity criteria are still under discussion. A guidance document is still expected (in 2023?), then it could be transferred for ring-testing at OECD level. Results may help to determine if an expected concentration of chemicals in realistic exposure has an effect on the sexual maturation of honeybee drones.

4.2. How accurately can we measure *Bombus* colony parameters combining automated and manual methods?

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Abstract

Bumblebees are important pollinators of agricultural crops, therefore methods for the evaluation of effects of pesticides haves been proposed in some regulatory schemes. Recently validated testing methods have been developed for individual adult bumblebees (OECD 246/247) but the development of higher tier studies

that would allow the assessment of colony development has proven to be more challenging. Existing data reveal a very high inter-colony variability, even under identical test and exposure conditions. Therefore, various approaches have been developed with some success to overcome this issue. Yet, it is still technically challenging to accurately measure key parameters in the field without disturbing colony development. Therefore, we have jointly been developing an approach to compare "conventional" assessment methods with novel automated, camera-based methodologies to survey some of these parameters. In this work, we present the comparison of measurements done in two trials, each lasting the entire colony life cycle. In trial 1, we monitored four colonies foraging freely and in trial 2, we collected these measurements in parallel on 6 colonies per treatment group (control and 2 concentrations of a toxic reference) for different parameters. Our data contribute to a better understanding of between-hive variability in bumblebees, and the influence of different assessment methods on the outcome of the measurements.

4.3. Assessing the Precision of state-of-the-art Bee Counters

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Abstract

Automatic recording of bee flight activity at hive entries can provide valuable information regarding the health of the hives and has been used in many studies. However, no clear guidance regarding the calibration of such counters is available. We have recorded counts of bees entering and exiting hives during semi-field trials for honeybees (*Apis mellifera*), which were designed to conduct pollinator risk assessments of crop protection products. In this work, we want to share our experiences, and initial results regarding counter calibration. We compare the recorded bee activity from photoelectric counters to the number of bees counted by experts and find that counters provide a higher precision, especially at high bee activity. Furthermore, we describe our setup and show results from a 'robbers test' performed in 2021 and find that the ratio of incoming and exiting bees is accurate within ±5%, for 31 out of 34 tests. Finally, we present a first snapshot of a

comparison between the light-barrier counters used in our studies and a video-based method.

Introduction

To assess the risk of potential side-effects of new insecticides on pollinators, different types of studies need to be conducted. Semi-field trials are one type of study in this framework, where beehives (honeybees; Apis mellifera) are kept in large (here: 50 m^2) net-tents to assess side-effects on the colony level. The assessments are performed by experts and follow EPPO guideline No. 1/170 (4). Additionally, automatic hive monitoring systems, especially bee counters, can provide valuable additional insights.

The development of devices that automate counting of bees that pass through the hive entrance dates back roughly 100 years. In an extensive work, Lundie (1925) discusses different approaches to build an apparatus that automatically counts exits and returns of bees, including detailed descriptions of the associated challenges like minimizing disturbances of the colony or various reasons for inaccurate counts. Today, many researchers have worked on different technologies to automatically count bees, using different technologies like pure mechanical solutions, photoelectric counters, or video and AI based counters (Knaebe 2020). An extensive review of the different developments has been published by Odemer (2021). Although bee counters have frequently been used in studies, the knowledge of their precision and methods to calibrate them are limited. One method to evaluate the accuracy of the ratio between incoming and exiting bees is known as 'Robbers tests' and has been introduced by Struye (1999). In this work we discuss our experiences regarding bee-counter calibration. Although the experiments have been conducted in net-tents, the same methodology could be used in the field.

Material and methods

Data collection

All presented data sets have been collected during bee studies in net-tunnels conducted at the Experimental Station Gut Höfchen (Burscheid, Germany). During each trial, healthy nucs (sister queens; several thousand worker bees) from a professional beekeeper were placed in the tunnels. Except for the 'robbers test', the tunnels contained a bee-attractive, flowering crop (either *Brassica napus* in April

or *Phacelia tanacetifolia* in July/August). Hive monitoring systems which include bee counters (photoelectric sensor) provided by beehero[1] have been installed at the hives to monitor incoming and exiting bees at the hive entrance with 10 minutes resolution. During a study in July/August 2020 two additional tents with hives have been placed next to the running trial and the bee flight activity has been recorded and analyzed with a video and AI based method provided by apic.ai[2]. All data presented in this work is from hives that have not been exposed to chemical treatments.

Specifically for this work, in addition to the sensor data, bees entering and exiting the hives have been counted manually by experts. In 2019 test counts by varying people have been taken, during which exiting and returning bees have been counted at the same time, and the total count (exiting plus returning bees) has been recorded. In April 2020, measures to increase the precision have been taken and counting at the hive entry has been performed by one expert who counted and recorded leaving and returning bees separately in two consecutive minutes.

To better understand the accuracy of the light-barrier bee counters we performed 'robbers tests' in April and May 2021, using a similar set-up as introduced by Struye (1999). We performed these tests in net-tents and covered the floor of the tents with plastic tarpaulins (see Fig. 1) to ensure that the food source provided to the bees was the only available food source. The covered floor also enables counting of dead bees that remained on the tent floor in the evening after each 'robbers test'. A box containing a bowl with summer honey (see Fig. 2) was used as a bee attractive food source. Prior to the experiments the bees were trained to find the food sources. During a first series of tests each tent was equipped with one hive and one food source, and we installed bee counters in front of both. Each test started in the early morning when the bees start flying and ended in the evening when all bees have returned to the hive. During the test bees enter the food source, 'rob' food, and bring it back to their hive. After each test and for

[1]Since 2021 the hive monitoring systems are provided by beehero (https://www.beehero.io/). Most devices used for this work are older generations of the setup (bought in 2019 and 2020), sold under the company names Canetis or Arnia remote hive monitoring™ (Arnia Limited, UK).

[2]apic.ai GmbH (https://apic.ai/)

each tent dead bees that remained in the box with the food were counted as well as bees lying on the tent floor. We note that, while counting dead bees in the food source can be done precisely, counting dead bees on the tent floor can be subject to human errors. During a second series of tests, we moved two to three hives into the same tent to increase the flight activity at the food source. For these runs (as we cannot ensure that the bees are always returning to their initial hive) only the data from the light barriers at the food sources have been analyzed. We derived the accuracy of the bee counters at the food sources as

$$accuracy_{food} = \frac{count_{food,out} + bees_{food}}{count_{food,in}}$$

and for the counters at the hives as

$$accuracy_{hive} = \frac{count_{hive,in} + bees_{food} + bees_{tent}}{count_{hive,out}}$$

where countfood/hive, out/in refers to the count of exiting/returning bees at the respective counter and beesfood/tent refers the dead bees counted manually at the food source or at the tent floor after each day.

Figure 1 Setup of the 'robbers tests' in 2021. Left: covering the floor with plastic tarpaulins to ensure that the bees will only find the provided food source. Right: Setting up a test with one hive and one food source (the photo has been taken before the hive has been moved into the tent).

Figure 2 Bowl with honey used as a food source in the 'robbers test'. We used summer honey, which is very bee attractive.

Data preprocessing

The (light barrier) counter timestamps have been rounded to full 10 minutes. In a few cases, usually if counters had to be reset, the accumulated count is reset to zero causing negative counts in a specific time bin. Such values have been removed from the data. Furthermore, missing or removed values have been interpolated, however this only applies to a very small number of values (for example one interpolated value is included in Fig. 3). The expert counts of exiting and returning bees have been merged to the light barrier counts on the time grid with 10 minutes precision. If, for the same hive, more than one manual count lies in the same time bin we take the average. As the expert only counted for one minute, for the results shown in Fig. 3, the data has been scaled by a factor ten to ensure comparability to the light barrier counts. Similarly, for the comparison with the results provided by apic.ai, who provided results in 'bees per minute', the counts from the light barriers have been scaled accordingly to simplify the visual comparison (Fig. 6).

Results

Comparison to manual counts

For both humans and automated counters, counting bees becomes more challenging at high bee activities. Humans start missing bees if the rate of exiting or incoming bees becomes too large and for the light barriers counting becomes more challenging if the gaps between the passing bees get very small. Fig. 3

shows data from April 2020 and compares the sensor count at the hive entry to the manual expert count. Each dot in the Figure corresponds to data recorded in a 10 minute time bin at a specific hive. The plot shows the 'total' (incoming plus exiting) bee count. To guide the eye, two lines have been added: the black line is the diagonal that would be expected for a 'perfect' (and noise-free) count, while the blue, dashed line is a simple linear fit to the data. Fig. 3 shows that the data fits our expectation at low bee activities (the scatter is expected, for example scaling the one-minute expert counts to 10 minutes will add noise to the plot, especially in the case of changing weather conditions), however at high activities we see that the manual count becomes significantly lower compared to the light barriers, indicating that at these activities the human starts missing bees. We note that the human expert only counts bees that have taken off, while the light barriers will also detect bees that walk out of the hive and directly turn around.

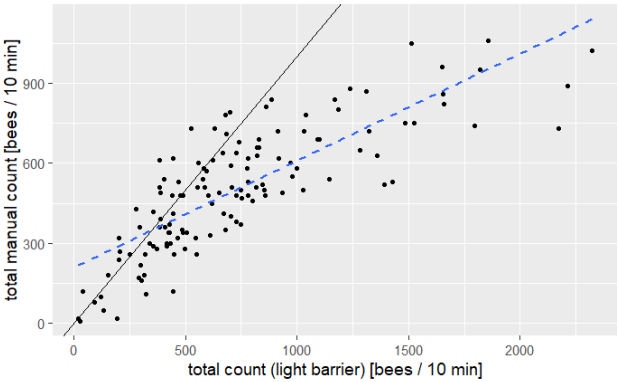

Figure 3 Data from a trial in April 2020. Total ("in+out") count from the light barriers compared to manual counts at the hive entry, scaled to correspond to 10 minutes of accumulated data. To guide the eye, the black line is the y=x diagonal, while the blue, dashed line is a simple linear fit to the data.

Robbers test

We tested 16 light barriers during 'robbers tests' that had been purchased for trials in 2019 and 2020 as well as three new ones purchased in 2021. We note that the older light barriers had already been used during studies and showed some wear. Fig. 4 exemplarily shows the data collected during one test, which takes one day. The upper panel shows the count of bees entering the food source (orange)

and bees leaving the food source (blue), with 10 minutes time resolution. The lower panel shows the accumulated counts for 'in' and 'out'. In this example 15,488 incoming and 15,400 leaving bees have been counted leading (after an irrelevant correction for 3 dead bees) to an accuracy of 0.99.

The number of bee flights per test varied substantially depending on a combination of the weather, the number of hives in the tent, the bees getting better at robbing the food source, and on whether the data was recoded at the hive or at the food source. Four old light barriers that repeatedly showed poor (errors larger than 5%) results have been removed and excluded from the data set and a few runs could not be used due to recoding issues and had to be repeated. The result of all remaining 'robbers tests' is summarized in Fig. 5, where the accuracy derived from different tests is plotted against the total number of flights recorded at the respective light barrier (defined as $(count_{in} + count_{out})/2$). The number of flights ranges between 784 and 36,444 (at the food sources), and between 9,708.5 and 99,612 (at the hives). For the correction factors (dead bees) for the counters at the food source, $bees_{food}$, we found values between 0 and 108 and for the correction factors for the counters at the hives, $bees_{food} + bees_{tent}$, values between 31 and 848. In the worst case the correction factor corresponds to 1.6% of the number of flights, usually the impact was lower (consequently, small errors on the bee count on the tent floor would have a very low impact). Each light barrier has been tested at least once at the food source, tests at the hives are repetitions, and the counters at the hives have been exchanged less often. In the final data set 31 out of the remaining 34 runs runs have an accuracy of 1 ± 0.05. A linear fit has been added to the data in Fig. 5, which indicates that, in the range of values we could test with our setup, the accuracy did not depend on the total flight activity.

As mentioned above for the tests in 2021, we used a bowl with honey in the food source, which comes with the disadvantage that the bees and consequently also the light barriers get very dirty, which can reduce their precision. During a repetition of the tests in 2022 (analysis still pending) we exchanged the bowl with honey for a complete honeycomb, which reduced the dirt significantly. The bees will still mark the entry to the food source, which could limit the functionality of the counter, however during an actual trial with a bee-attractive, flowering crop, this would not be an issue. Cleaning and checking the light barriers carefully before each trial will also increase the quality of the study. We not that the 'robbers test' only gives us the error on the $count_{in}/count_{out}$ ratio. Systematic errors affecting the count of exiting and returning bees would not be detected.

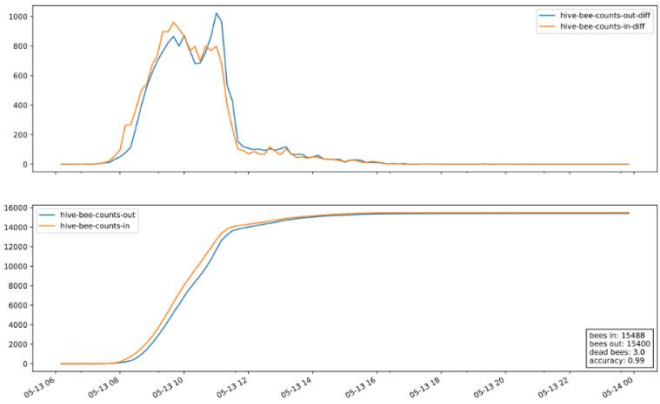

Figure 4 Robbers test for one counter, the count is from the light barrier at the food source. The upper panel shows the bee count per 10 minutes for incoming bees (orange) and leaving bees (blue). The lower panel shows the aggregated counts.

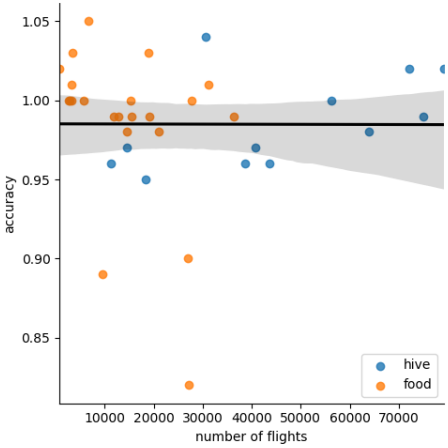

Figure 5 Overview of the results of our 2021 'robbers tests': the accuracy of the different tests, plotted against the total number of flights ($(count_{in} + count_{out})/2$). Four old light barriers that repeatedly showed large errors have been excluded. Blue or orange color indicates whether the count has been taken at a hive or at a food source. As expected, the activity at the hives is higher. For the range covered in our experiments, the accuracy does not decrease with higher bee activities.

Comparison to count data provided by apic.ai

Fig. 6 shows a first comparison between flight activity data which has been recorded with the light barriers vs. data that has been recorded and analysed using videos and AI based counters by apic.ai. The Figure shows the count of incoming bees recorded at six beehives in August 2020. The upper panel shows the data from four hives that have been monitored with light barriers (lines in different shades of blue) and raw data (counts extracted from the videos) from the two hives analyzed by apic.ai (black and grey line). In the lower panel, the black line shows the final result provided by apic.ai, which includes a correction of the raw count, for one of the hives (same hive as the black line in the upper panel). The grey lines are the 95% confidence intervals.

The raw data in the upper panel is comparable in terms of bee activity, the data from the hive displayed in black fits to the data set from the light barriers, the data from the hive displayed in grey shows a slightly higher count. Several smaller structures, for example in the early mornings, appear in both data sets. On August 6th, 7th, and 8th some of the hives observed with the light barriers show a dip during the day, which is less prominent in the apic.ai data. The final result provided by apic.ai (lower panel) is higher than the count from the light barriers, in the order of a factor two during the daytime for the hive displayed in black.

The data recorded with the two different methods is not perfectly comparable as each counter was connected to a different hive and the activity of the colonies can differ. Also, there was a delay regarding the points in time when the equipments have been set up and the counters are built very differently (for example the length of the tunnels the bees pass through), which could have an impact on bee behaviour. However, based on previous experiences, a factor of two or more in difference is not expected, especially as both hives monitored by apic.ai show a higher bee activity compared to the light barriers.

In this comparison, the video-based counting shows its strength as absolute errors on the counts can be quantified and reduced. With increasing certainty from the results of video-based methods, we will learn if a correction is also necessary for the light barriers. The light barriers have the advantage that the devices are easier to handle, the analysis is a lot simpler, and hence scaling, for example to lager numbers of hives is more feasible.

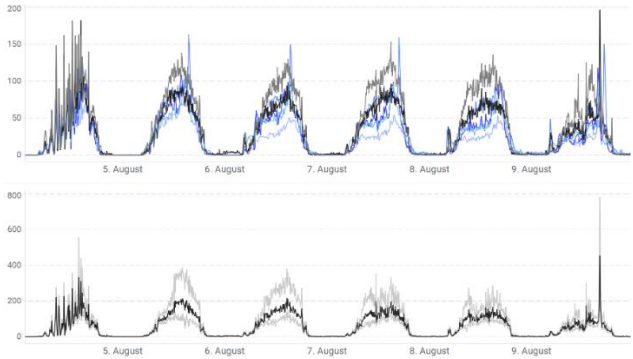

Figure 6 Count of incoming bees from six hives recorded during a trial in summer 2020. Six days have been selected for readability. The upper panel shows the data from four hives that have been monitored with light barriers (lines in different shades of blue) and raw data from two hives analyzed by apic.ai (black and grey line). In the lower panel, the black line shows the final result provided by apic.ai, which includes a correction of the raw count, for one of the hives (same hive as the black line in the upper panel). The grey lines are the 95% confidence intervals.

Acknowledgements

We would like to thank Daniel Burkow for proofreading and helpful suggestions.

References

European and Mediterranean Plant Protection Organization, Pp 1/170 (4): 563 Side-effects on honeybees, Bulletin OEPP/EPPO Bulletin 40 (2010) 313– 564 319.

A. E. Lundie, The flight activities of the honeybee, United States 568 Department of Agriculture, Department Bulletin 1328 (1925).

S. Knaebe; F. Tausch.; K. Schmidt; G. Gonsior, A feeding experiment with a sublethal neonicotinoid dosage: Effects observed with traditional assessment methods and a novel AI supported bee counter. Proceedings of the SETAC Europe 30th Annual Meeting Open Science for Enhanced Environmental Protection. 2020.

R. Odemer, Approaches, challenges and recent advances in automated bee counting devices: A review. Annals of Applied Biology Volume 180, Issue 1, p. 73-89. 2021.

Struye, M. H., Possibilities and limitations of monitoring the flight activity 594 of honeybees by means of BeeSCAN bee counters, in: L. P. Belzunces, 595 C. Pélissier, G. B. Lewis (Eds.), Hazards of pesticides to bees, INRA, 596 1999, p. 269–275.

4.4. Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bees

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Abstract

For social bees, an understudied step in evaluating pesticide risk is howcontaminated food entering colonies affects residing offspring developmentand maturation. For instance, neurotoxic insecticide compounds in foodcould affect central nervous system development predisposing individualsto become poorer task performers later-in-life. Studying bumblebee coloniesprovisioned with neonicotinoid spiked nectar substitute, we measured brainvolume and learning behaviour of 3 or 12-day old adults that hadexperienced in-hive exposure during brood and/or early-stage adult devel-opment. Micro-computed tomography scanning and segmentation of multiple brain neuropils showed exposure during either of the developmen-tal stages caused reduced mushroom body calycal growth relative tounexposed workers. Associated with this was a lower probability ofresponding to a sucrose reward and lower learning performance in an olfac-tory conditioning test. While calycal volume of control workers positivelycorrelated with learning score, this relationship was absent for exposedworkers indicating neuropil functional impairment. Comparison of 3- and12-day adults exposed during brood development showed a similardegree of reduced calycal volume and impaired behaviour highlighting last-ing and irrecoverable effects from exposure despite no adult exposure. Ourfindings help explain how the onset of pesticide exposure to whole coloniescan lead to lag-effects on growth and resultant dysfunction

Keywords: *Bombus terrestris*, imidacloprid, micro-computed tomography scanning, mushroom body calyces, neonicotinoid, sublethal

Introduction

A growing number of studies have highlighted how foragers directly exposed to insecticide compounds can lead to sublethal effects on behaviour with possible knock-on effects to colony function. However, with insecticide residues detected inside colonies across the globe, we know less as to how pesticide-contaminated pollen and nectar brought back by foragers place developing individuals being reared and residing inside colonies at risk. For instance, in-hive exposure could affect the physiological development of brood and early-stage adults (a.k.a. callows—a cohort representing the future generation of the colony's workforce), predisposing these individuals to exhibit lower performance of tasks important for colony function as older adults. Here we test this hypothesis.

Material and methods

We investigated if bumblebees (*Bombus terrestris*) developing inside colonies provisioned with the neonicotinopid (imidacloprid) treated nectar substitute showed impaired learning behaviour as adults when undertaking a olfactory association PER assay.

Using micro-computed tomography (µCT) scanning, we tested whether this was associated with reduced volumetric growth of brain regions during early-stage development.

Implementing a factorial experiment, we provisioned colonies with treated food at different development stages to compare the responses of workers that experienced in-hive exposure during either their brood development stage, early-adult stage, or both stages (Fig. 1).

Comparing responses between these three treatments (pre-eclosion, post-eclosion, or continual exposure, respectively) relative to unexposed workers (control), we investigated which developmental stage was more vulnerable to exposure in terms of later adult performance and physiology.

By tracking worker development, we tested two controlled age cohorts of adults at 3 and 12 days old, each of which we attempted to limit variation in prior experience and sensory input.

Our comparison of young (3-day) versus older (12-day) workers within and between treatments allowed us to: 1) distinguish the effects of exposure from variation caused by potential innate effects of age (experience independent change); 2) test whether developmental plasticity (in behaviour or tissue growth) allows

any potential impact from brood exposure to be recovered during the unexposed adult phase.

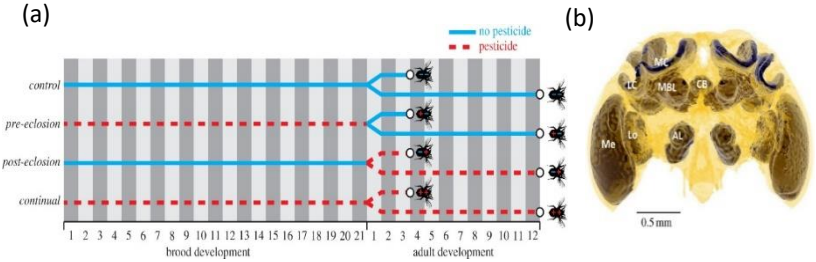

Figure 1. Panel a) Graphic showing the developmental and exposure periods of individuals inside colonies for the four colony treatments (*control*, *pre-eclosion*, *post-eclosion* and *continual*) and the eight cohorts of workers tested. 'Brood development' represents the larval and pupal stages of workers, with 'Adult development' representing the number of days after eclosion from the pupal case. White circles and individual bee symbols depict removal of these controlled aged adult workers at 3 or 12-days after eclosion for immediate involvement in the behavioural assay followed by decapitation for µCT scanning of the brain; Panel b) 3D rendering of a studied bumblebee brain using our µCT imaging method. Focal neuropils considered in this study are shown in dark purple, surrounded by remaining brain tissue in transparent yellow.

Results

Linking impaired learning behaviour with pesticide induced reduction to the volume of the mushroom body calcyes of the brain.

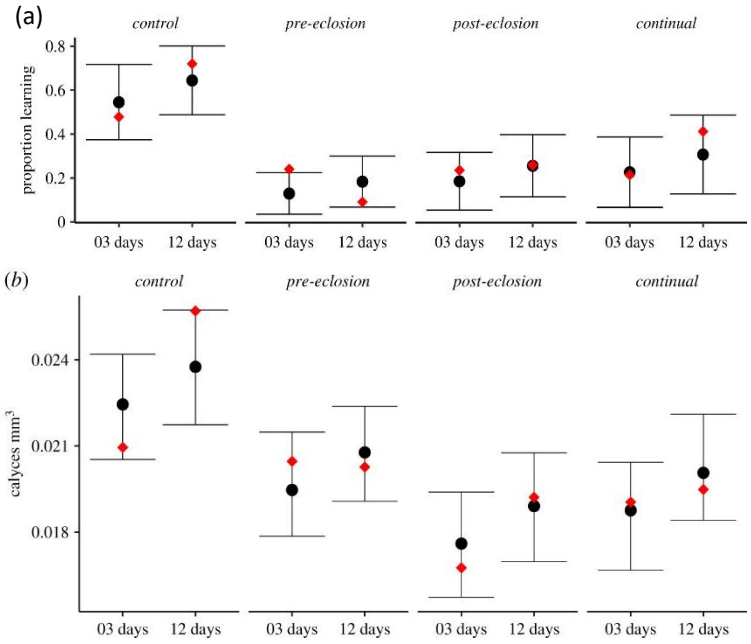

Figure 2 Panel a) Proportion of learners between treatments. Sample sizes of 3- and 12-day worker cohorts was: control = 23 and 25, pre-eclosion = 25 and 33; post-eclosion = 17 and 27; continual = 14 and 17. Panel b) Relative volumes of bumblebee worker mushroom body (a) calyces, Sample sizes of 3- and 12-day worker cohorts was: control = 9 and 8, pre-eclosion = 11 and 11; post-eclosion = 10 and 10; continual = 11 and 8. The intersecting circular points represent estimated model means taken from model back-transformation (binomial GLM) with bars depicting associated ±95% confidence limits. Red diamond corresponds to the mean value taken from therawresponse data.

Bees with bigger relative calycal volumes are better learners, but pesticide exposure during development counteracts this.

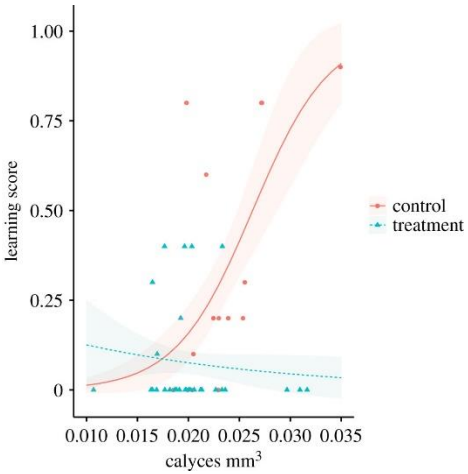

Figure 3. Relative mushroom body calycal volume plotted against the respective worker's learning score. Workers from all three pesticide treatments were pooled (blue triangles, n= 29; pre-eclosion = 11, post-eclosion= 12, continual= 6) and compared against controlworkers (red circles, n= 15), with fitted lines (blue dashed = pesticide treatment; red solid = control) representing binomial model (GLM) estimates and shaded areas representing the 95% confidence intervals.

Conclusions

Our findings of early exposure affecting later adult behaviour can provide an explanation for why reduced colony growth has been detected two to three weeks after the onset of neonicotinoid exposure in previous studies. If future generations of workers are predisposed to be inefficient functioning cohorts, this could lead to a density-dependent build-up of colony-level impairment increasing the risk of colony collapse. Our results suggest that even if newly eclosed workers were to delay the age at which they start any specific task performance, such a strategy could be futile given we saw a little adult recovery in behaviour from 3 to 12 days of adulthood from pre-eclosion colonies. Our method of provisioning colonies with a treated nectar substitute may also represent a conservative level of exposure given that developing brood are more dependent on pollen for tissue growth than adults, and that concentrations of neonicotinoid residues in pollen are typically higher than found in nectar. Importantly, our findings are unlikely to be exclusively applicable to: (i) workers, as newly reared males and queens are

also at risk with possible implications for mating and hibernation; (ii) neonicotinoids, as many neurotoxic pesticides including cholinergic insecticides (e.g. sulfoxamines, butenolides) can build up inside bee colonies and induce sublethal effects on individual and colony-level traits.

4.5. Observation of Repellency Effects on Honey Bees and their Pollen and Nectar Collection Behaviour under Semi-Field Conditions with an automated bee counter

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Abstract

apic.ai and EAS Ecotox are partners regarding the improvement of a visual bee monitoring technology in the research project OCELI (FKZ 281C307B19). A proof-of-concept semi-field study was performed using the apic.ai monitoring systems with computer vision technology. They were used to observe the activity and foraging of pollen at colony level and at the level of individuals.

Queen markers were attached to bees to identify them individually. The first cohort marked were foragers and the second cohort freshly hatched bees. Furthermore, classic assessments were performed: Colony assessments, weight assessment of the hives, flight and daily mortality.

The study ran in Germany in July/August 2022 with a total of 14 hives. Six tunnel were used for the control and 4 for each of the two treatments. Phacelia was the crop inside the 40 m tunnels. The hives stayed 14 days after application in the tunnels, until the end of flowering. Observation continued for a period of 14 days at a monitoring site, where bees could forage freely.

The aim of the study was to find out if the two different active ingredients have the same effect on activity and pollen foraging. Repellency effects were of particular interest. A further aim for the study was to contribute ground truth data on

the flight duration and frequency, as well as the age of first foraging and the question of specialization on the foraging of pollen. These data are also intended to be used to validate and improve the system model BEEHAVE.

References

EFSA (2013): EFSA Guidance Document on the Risk Assessment of Plant Protection Products on Bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013; 11(7): 3295

EU (2013): Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

EU (1997) Commission of the European Communities (EU) No 7029/V1/95: General recommendations for design, preparation and realization of residue trials V1/B/II-I; p.1-72

IMDORF, A. & GERIG, L. (1999): Lehrgang zur Erfassung der Volksstärke, Schweizerisches Zentrum für Bienenforschung. [Course in Determination of Colony Strength, Swiss Bee Research Center].

IMDORF, A.; BUEHLMANN, G.; GERIG, L.; KILCHMANN, V. AND WILLE, H. (1987): Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern, Apidologie 18 (2), 137 – 146. [A Test of the Method of Estimation of Brood Areas and Number of Worker Bees in Free-Flying Colonies].

OEPP/EPPO (2010): Efficacy evaluation of plant protection products – Side effects on honey bees. OEPP/EPPO, PP 1/170 (4), EPPO Bulletin 40, 313-319.

4.6. BeeGUTS – a TKTD model for the interpretation and extrapolation of bee survival data

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Abstract

There are different tests for testing the impact of chemicals on bees: the acute oral test, the acute contact test and the chronic oral test. For honey bees, OECD guidelines are avialable stating how these tests need to be conducted. The endpoint of the tests is an LD_{50}-value expressed in ug/bee, where the chronic test usually has the most conservative result. In current practise, the results of these tests are interpreted independently and the most conservative result is chosen for further evaluation. Unfortunately, in this approach it is not known how the different exposure regimes influence the result and what the time dependency of the LD_{50} values is.

Extrapolation and interpretation issues between exposure regimes and time can be solved by using a mechanistic approach where time is explicitly considered and effects are interpreted with time-independent parameters. The already developed and published GUTS modelling framework was used as a starting point and was adapted to take into account the physiology of the bees and the details of the different existing tests for bees. It showed that the different bee tests (acute oral, acute contact and chronic) could be interpreted within this framework with one set of parameters describing the toxicity of a compound for bees. The framework was then applied to other be species to compare sensitivity leading to new insights in bee sensitivity and bee testing.

Keywords: BeeGUTS, TKTD modelling, Bee sensitivity, LD_{50}, exposure

Introduction

For honey bees different tests were developed for the assessment of toxic effects of chemicals: the acute contact test, the acute oral test, and the chronic oral test. All tests have their specific OECD guidelines according to which a test needs to be

performed. For bees, an acute test usually lasts 48 hours, while a chronic test lasts 10 days. The end point of a test is an LD_{50}-value (the dose at which 50% of the organisms die at some specified point in time). If different test are available for a single compound, the standard procedurs is to take the lowest LD_{50} for further risk characterisation.

However, in this approach the time-dependency of the LD_{50} can be different for each compound tested, which is not explicitly considered. In addition the different exposure regimes might influence the LD_{50} which is also not taken into account. Therefore extrapolating results to different exposure scenarios or different points in time is virtually impossible. Even ranking the LD_{50} values for different compounds in terms of their toxicity or comparing species based on LD_{50}s needs to be carried out with great care as the time-dependency of the LD_{50} is generally not known (Baas et al., 2010) and different species might have a different response in the same test (Baas et al., 2022).

These extrapolation and interpretation issues can be solved by using a mechanistic approach where time is explicitly considered and effects are interpreted with time-independent parameters. The GUTS modelling framework (Jager et al., 2011) was used as a starting point and adapted to take into account the physiology of the bees and the details of the different existing tests for bees. So a standard model for the interpretation of effects of chemicals on survival for bees within a single modelling framework irrespective of the test was the aim of the research; this was called the BeeGUTS model (Baas et al., 2022). This modelling framework was also applied to other be species to compare their sensitivity in this novel framework.

Material and methods

Modelling framework

The central part of the model is the Toxicokinetic Toxicodynamic (TKTD) approach as was described for the GUTS modelling framework (Jager et al., 2011). The reduced GUTS model was modified to capture the specifics of the different bee tests and the physiology of the bee, by developing specific exposure profiles for the different tests.

The main assumptions in the model are that in an oral test the compound is taken up in the honey stomach, which is considered to be an inert vessel inside the bee from which the actural exposure takes plcae. In a chronic test the concentration

in the honey stomach is constant but in an acute oral test there is fast increase in the concentration in the honey stomach when the bees are fed contaminated food, followed by a first order decline when the bees are observed and fed non-contaminated food. For acute contact tests it proved that the concentration on the bee is not constant but declines over time (Zaworra et al., 2019; Haas et al., 2021) with a rather constant decline rate for different species and different compounds.

The input for the model is the survival data over time and the exposure profiles for the different tests for different species of bees, the output is the parameter values describing survival over time, see figure 1.

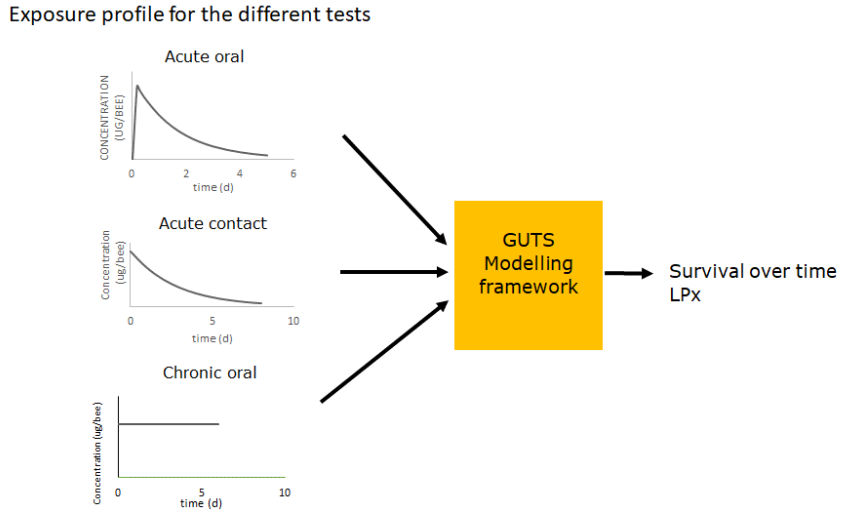


Figure 1 Overview of the BeeGUTS modelling framework.

Test results for honey bees

Raw survival data for honey bees for acute oral, acute contact, and chronic exposure were made available for 17 individual compounds by BAYER Crop Sciences. In addition literature data were used to complete the datasets. The starting point for the integration of the different bee tests is the chronic test. The raw data for this test contain 10 points in time and typically 5 or 6 exposure concentrations. This allows estimating the parameter values with (very) small confidence intervals. The acute tests with 2 usable points in time and 5 or 6 concentrations usually allow estimating parameter values; though typically the confidence intervals are large.

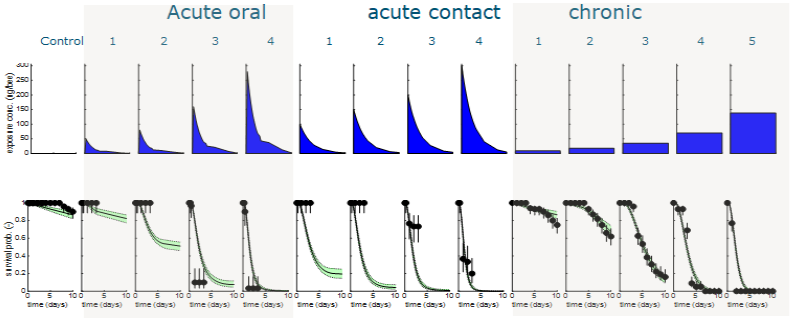
Test results for other bee species

The honey bee data were used as a starting point and wherever possible honey bee test results were supplemented with raw data taken from literature. The effect threshold (or by definition the LD0 for infinite exposure time) is derived with the model from the survival data. This time-independent parameter is an excellent starting point to compare the sensitivity of different species of bees (Baas & Kooijman, 2015).

Results

Integrating the different tests

The model was calibrated and validated according the EFSA guidelines on TKTD modelling (EFSA et al., 2018). It showed that the model can integrate the different test results including the time course of the observed effects with great accuracy for different pesticides with a different mode of toxic action. An example of the application of the BeeGUTS model for effects of dimethoate and thiacloprid on honey bees is shown in figure 2.



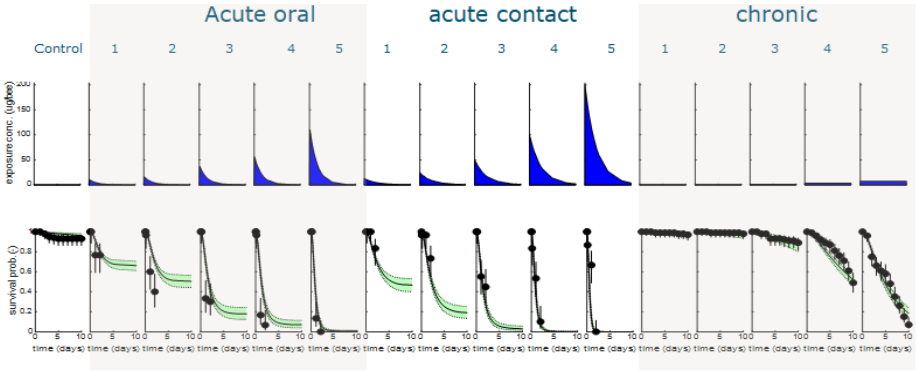


Figure 2 Results for dimethoate and thiacloprid, showing the the different test results can be integrated within one framework. The top panels show the time -dependent exposure concentration and the lower panels show the measured (dots) and modelled (line, with green 95% conf int) survival over time.

The model can be used in various ways:

- determine the actual toxicity of a compound for bees in terms of its Effect threshold, which is independent on time or exposure situation;
- identify test results that are incompatible with the overall test results and identify outliers. But most importantly, the extrapolation potential of a TKTD approach allows;
- an evaluation of the effects of field realistic time-dependent exposure profiles including (repeated) pulse exposures;
- Compare the sensitivity of bees based on the effect threshold, taking into account the physiology of the bee and the specifics of the exposure scenario

Comparing sensitivity of different species of bees

Complete and valid survival data could be found for 8 different compounds and 5 different species. The effect threshold is derived with the model from the survival data. The results are shown in figure 3 with the sensitivity of the honey bee set to 1.

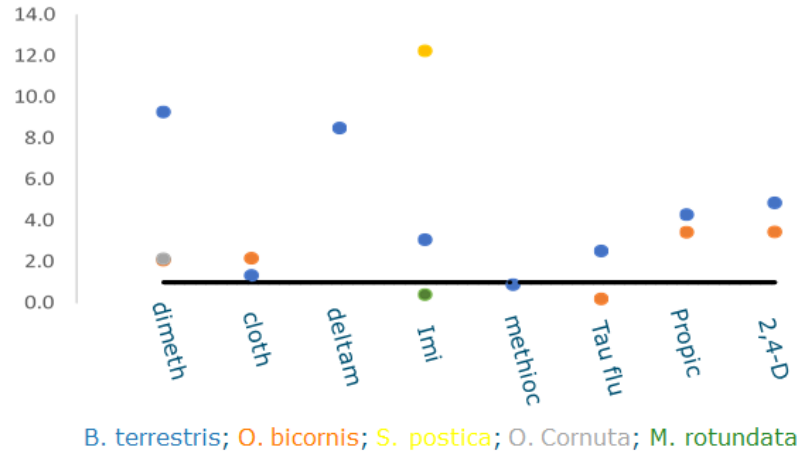


Figure 3 Comparison of bee sensitivity with the sensitivity of the honey bee set to a value of 1. Any dots below the line indicate a higher sensitive than honey bees and all dots above the line indicate a lower sensitivity than honey bees.

Figure 4 shows the same comparison, but now the data are corrected for the weight of the bees.

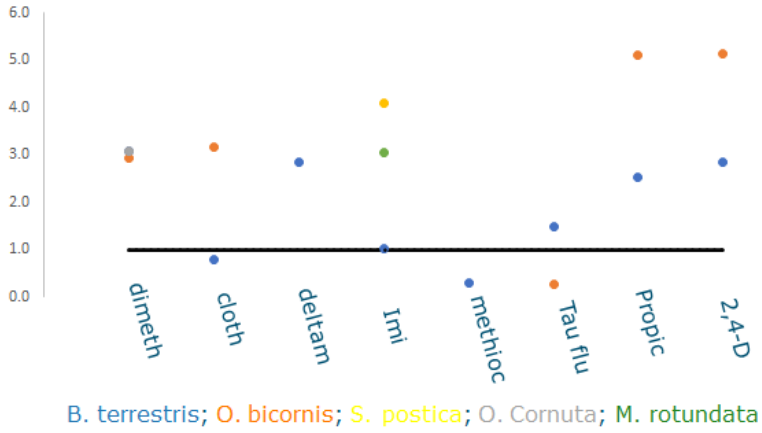


Figure 4 Comparison of bee sensitivity on a weight basis with the sensitivity of the honey bee set to a value of 1. Any dots below the line indicate a higher sensitive than honey bees and all dots above the line indicate a lower sensitivity than honey bees.

The species sensitivity analysis shows that the honey bee is consistently amongst the most sensitive bee species, in line with previous analysis based on LD_{50}s (eg (Arena & Sgolastra, 2014)). However since kinetic effects are taken out the differences in sensitivity of the bees are considerably smaller than those previously reported based on LD_{50}s.

The 48 hr LD_{50}, which is mostly used as a proxy for bee sensitivity for some compound has a number of drawbacks. Therefore a new approach was developed that allows integrating the different tests (acute oral, acute contact, chronic) within one consistent framework. Three parameters are needed to describe the hwole time course of toxic effects for the different tests, taking in account the physiology of the bee. The effect threshold is perhaps the most important parameters as this is a time-independent measure of the sensitivity of a bee. A species sensitivity comparison based on the effects threshold showed that the variation in the results is significantly smaller than previous comparisons showed and that the honey bee is consistently amongst the most sensitive bee species.

References

Arena, M., F. Sgolastra, 2014: A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology **23** (3), 324-334, DOI: 10.1007/s10646-014-1190-1.

Baas, J., B. Goussen, M. Miles, T.G. Preuss, I. Roessink, 2022: BeeGUTS – A TKTD model for the interpretation and integration of acute and chronic honey bee tests. Environmental Toxicology and Chemistry **n/a** (n/a), DOI: https://doi.org/10.1002/etc.5423.

Baas, J., T. Jager, B. Kooijman, 2010: Understanding toxicity as processes in time. The Science of the total environment **408** (18), 3735-3739, DOI: 10.1016/j.scitotenv.2009.10.066.

Baas, J., S.A. Kooijman, 2015: Sensitivity of animals to chemical compounds links to metabolic rate. Ecotoxicology **24** (3), 657-663, DOI: 10.1007/s10646-014-1413-5.

EFSA, Panel o.P.P. Products, t. Residues, C. Ockleford, P. Adriaanse, P. Berny, T. Brock, S. Duquesne, S. Grilli, A.F. Hernandez-Jerez, S.H. Bennekou, M. Klein, T. Kuhl, R. Laskowski, K. Machera, O. Pelkonen, S. Pieper, R.H. Smith, M. Stemmer, I. Sundh et al. , 2018: Scientific Opinion on the state of the art of Toxicoki-

netic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. EFSA Journal **16** (8), e05377, DOI: https://doi.org/10.2903/j.efsa.2018.5377.

Haas, J., M. Zaworra, J. Glaubitz, G. Hertlein, M. Kohler, A. Lagojda, B. Lueke, C. Maus, M.-T. Almanza, T.G.E. Davies, C. Bass, R. Nauen, 2021: A toxicogenomics approach reveals characteristics supporting the honey bee (Apis mellifera L.) safety profile of the butenolide insecticide flupyradifurone. Ecotoxicology and Environmental Safety **217**, 112247, DOI: https://doi.org/10.1016/j.ecoenv.2021.112247.

Jager, T., C. Albert, T.G. Preuss, R. Ashauer, 2011: General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology. Environmental Science & Technology **45** (7), 2529-2540, DOI: 10.1021/es103092a.

Zaworra, M., H. Koehler, J. Schneider, A. Lagojda, R. Nauen, 2019: Pharmacokinetics of Three Neonicotinoid Insecticides upon Contact Exposure in the Western Honey Bee, Apis mellifera. Chemical research in toxicology **32** (1), 35-37, DOI: 10.1021/acs.chemrestox.8b00315.

4.7. Honeybee and bumblebee exposure to post-flowering applications of an insecticide in apple orchards

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Abstract

Pollinators such as Honeybees and bumblebees may be exposed during their foraging to a range of pesticides that are applied in agricultural fields. Applications during flowering to crops which are highly attractive for pollen and nectar represent a worst-case exposure scenario for bees. However, other exposure scenarios have been proposed such as exposure to weeds present in fields, flowering plants at field margins, adjacent flowering crops and succeeding crops. Risk assessment schemes have proposed tier I dietary exposure estimates based on worst case food consumption rates combined with default exposure levels. These exposures

are expressed as quantity/bee in line with the endpoints from test guideline studies (e.g. OECD 213, 245, 239). A risk assessment can then be conducted by comparing the ratio of the exposure to the study endpoint value to an agreed trigger value or specific protection goal (SPG). One of the drawbacks of this approach is that it assumes 100% of the dietary exposure comes from each scenario. In the case of a flowering bee-attractive crop such as oilseed rape a significant proportion of the foraged pollen and nectar may come from the treated field. In comparison the number of attractive weeds in the same crop either pre- or post-flowering offers a much lower reward as do flowers present in the field margins. The difference in the proportion of food obtained from weeds and flowers in the field margins compared to a mass flowering crop is not accounted for at tier I and the risk assessment is based on a colony receiving 100% of its dietary needs from these sources alone. It seems unlikely that because weeds occur in fields at low densities compared to the crop that colony dietary needs could be met completely by these plants and hence the exposure to the colony at tier I is overestimated. One way to deal with this problem could be to introduce a landscape factor to account for the proportion of diet coming from the weeds or margins at the colony or population level. To try to overcome some of issues surrounding exposure estimates for post-flowering applications we conducted a study to measure the concentration of an insecticide found in pollen and nectar of returning forager bees sited at the edge of five apple orchards which had received two post-flowering applications.

Post-flowering apple orchards were not highly attractive to bees, however when sited at the edge returning foragers carried pollen nectar originating from the treated area. Surveys of vegetation in the orchard and surrounding areas indicated that bees forage on a wide range of plants. The test item and major metabolite were detected in pollen and nectar confirming exposure to the treated field but at low levels. These findings shed light on the the relationship between honey and bumblebees to their environment to estimate landscape factors which could be used to achieve a more realistic exposure assessment for applications made when a crop is not in flower.

4.8. Nutritional stress exacerbates impact of a novel insecticide on solitary bees' behaviour, reproduction and survival

Anina C. Knauer, Cedric Alaux, Matthew J. Allan, Robin R. Dean, Virginie Dievart, Gaétan Glauser, Tomasz Kiljanek, Denis Michez, Janine M. Schwarz, Giovanni Tamburini, Dimitry Wintermantel, Alexandra-Maria Klein and Matthias Albrecht

Abstract

Pesticide exposure and food stress are major threats to bees, but their potential synergistic impacts under field-realistic conditions remain poorly understood and are not considered in current pesticide risk assessments. We conducted a semi-field experiment to examine the single and interactive effects of the novel insecticide flupyradifurone (FPF) and nutritional stress on fitness proxies in the solitary bee *Osmia bicornis*. Individually marked bees were released into flight cages with monocultures of either buckwheat, wild mustard or purple tansy, which were assigned to an insecticide treatment (FPF or control) in a crossed design. Nutritional stress, which was high in bees foraging on buckwheat, intermediate on wild mustard and low on purple tansy, modulated the impact of insecticide exposure. Within the first day after application of FPF, mortality of bees feeding on buckwheat was 29 times higher compared to control treatments, while mortality of FPF exposed and control bees was similar in the other two plant species. Moreover, we found negative synergistic impacts of FPF and nutritional stress on offspring production, flight activity, flight duration, and flower visitation frequency. These results reveal that environmental policies and risk assessment schemes that ignore interactions among anthropogenic stressors will fail to adequately protect bees and the pollination services they provide.

4.9. From lab to field: a solid methodology for Bombus terrestris dalmatinus side effect studies

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Abstract

A solid methodology for trials testing the side effects of PPPs on the large earth bumblebee, *Bombus terrestris dalmatinus*, from the laboratory through to large-scale field studies, will be presented. The initial step is the study under laboratory conditions, through simulating the three possible means of bumblebee exposure to the compound: topical, oral by sugar water, and oral by pollen. In order to achieve higher uniformity, R&D colonies (IPM Impact-Koppert) are used. The products are mainly tested according to the maximum field recommended concentration (MFRC), but a sequential dilution testing scheme may be applied to the oral sugar water application, if triggered. In order to enable controlled exposure under semi-field conditions a tunnel setup is designed, following a customized protocol, again using R&D colonies. The final step, if needed, is the large-scale field studies where colonies are exposed to natural conditions. The assessment parameters for all studies mentioned above include the presence/vitality of the mother queen, colony strength, brood volume, the number of queen-brood cells, and the number of newly-formed queens (gynes). Finally, the treated colonies' developmental and reproductive abilities are evaluated by comparing them to those that are untreated or water-treated. Extrapolation of the results to commercial colonies used for pollination and/or to natural colonies, concerning biodiversity, is provided.

Keywords: *Bombus terrestris dalmatinus*, methodology, laboratory studies, semi-field studies, large-scale studies

5. Session – Monitoring

5.1. Honey bee lifecycle assessment and homing success in field observations with the help of visual bee monitoring technology

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Abstract

New technologies such as automatic bee counters and other monitoring devices/equipment gain insights into open questions. As of now, they were to detect changes in activity and pollen foraging at colony level, but not at the level of individual bees.

A technology to observe survival, and/or flight duration and frequency at colony level are RFID chips. With their help, the homing flight behaviour of chipped bee can be observed to find out if there is an influence of a plant protection product on the orientation of the bees (OECD GD 332).

Combining data about the flight activity and life cycle of individual honey bees with data at colony level from an automatic bee counter could be very insightful for a better understanding of effects and their magnitude.

Being partners in the improvement of a visual bee monitoring technology in the BMEL funded research project OCELI (FKZ 281C307B19), apic.ai and EAS Ecotox designed and performed a proof of concept experiment. In the experiment the apic.ai monitoring systems with computer vision technology were used as an instrument to observe individual bees. Queen markers were attached to bees to identify them individually. This novel approach could enable the inclusion of life cycle, homing success and individual behavior in studies where visual monitoring technology is already in use to assess other behavioral endpoints like activity, pollen collection or share of foragers. Visual markers would be comparable to RFID chips and an RFID reader would not be needed if a visual monitoring system is used.

References

OECD 2021 GUIDANCE DOCUMENT ON HONEY BEE (APIS MELLIFERA L.) HOMING FLIGHT TEST, USING SINGLE ORAL EXPOSURE TO SUBLETHAL DOSES OF TEST CHEMICAL Series on Testing and Assessment, No. 332

Alberto Prado, Fabrice Requier, Didier Crauser, Yves Le Conte, Vincent Bretagnolle and Cédric Alaux (2020) Honeybee lifespan: the critical role of pre-foraging stage Royal Society Open Science Volume 7, Issue 11

5.2. Monitoring of pesticide residues with beehives in different agroecosystems

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Abstract

The starting point of this work was beekeeper´s and farmer´s concern about the pollution of “Laguna del Cisne”, an important lagoon basin in Uruguay, which is going into a productive reconversion towards pesticides use reduction. Based on previous studies of beehives as biomonitors of pesticide residues, a monitoring was designed and jointly developed. Swarms from the region were captured and hives with new material installed. Five beehives were settled in 8 selected environments: a native forest and agroecosystems involving rice crops, soybean, fruit orchards and horticulture. Five seasonal samplings (January 2019 - May 2020) were performed. The botanical richness of pollen and honey samples was determined (Louveaux et al, 1978). A total of 156 samples of bees (40), wax (40), beebread (36) and honey (40) were analyzed. QuEChERS based multiresidue methodologies followed by GC-MS/MS and LC-MS/MS determinations were employed.

From a selection of the most used and toxicologically relevant pesticides, 89, 82, 104 and 103 analytes in bees, wax, beebread and honey respectively were validated according to SANTE/11813/2017 guidelines. LOQs ranged 0.0001-0.100 mg kg^{-1}. From the 44 pesticides and metabolites found 10 were herbicides, 15 fungicides and 19 insecticides. Except 3 samples, concentrations ranged 0.001 - 0.05 mg kg^{-1}. Highest frequencies and number of detections were found in wax and beebread in accordance with our previous monitoring study (2014- 2017). Pesticides profile found in each apiary reflected the land use within its ecosystem. A highlight was the involvement and dialogue between producers and academia in order to advance towards bee protection.

Keywords: pesticide residues, validated methodologies, bees, wax, beebread, honey

Abstracts: Posters

1. Session – Non-Apis bees

1.1. Leafcutter bee *Megachile rotundata* semi-field test design

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Abstract

With the 2014 published draft guidance document for the risk assement of pollinators, solitary bees came into regulatory focus. Before, only honey bees were tested as a surrogate species. A semi-field test design with the red mason solitary bee *Osmia bicornis* L. was ringtested by the ICPPR non-Apis working group in 2016 and 2017. The result of the ringtests was presented and published by the ICPPR non-Apis working group with a recommendation for a semi-field test design in 2021 (Franke et al 2021).

With the knowledge on differences in exposure pathways between the solitary bee *Osmia bicornis* and leafcutter bees (e.g. *Megachile rotundata* F.) (Sgolastra et al 2019), it is expected that the same plant protection products will impact those species differently. In addition, a higher sensitivity of *Megachile* species to selected plant protection products was estimated (Hayward et al 2019).

Since there is no established guidance on solitary bee studies with Megachile so far, the main objective of the test was the methodological development of a standardised Tier II study semi-field design based on the recommended test design for *Osmia*.

In the *Megachile* study design, bees (*Megachile rotundata* F.) were released as emerged adults in tunnels containing a bee attractive flowering alfalfa (*Medicago sativa* L.) in Spain and were exposed during their reproductive period. The semi-field tunnel study included a water treated control and a reference item (dimethoate) sprayed treatment. After the application of dimethoate, the bees collected all relevant nest and food items from the treated crop. This included not only pollen and nectar but also treated leaves for nest building. The evaluated endpoints were the establishment of actively nesting females at the nesting units

(nest occupation), observations of the flight activity in front of the nesting units and the production of brood cells.

The assessed endpoints were evaluated with respect to their potential for the use in the risk assessment of plant protection products. Preliminary results will be presented and recommendations for the adaptation of the semi-field test design to an additional species will be given.

Keywords: Solitary bees, leafcutter bees, Megachile, risk assessment

References

Franke, L., C. Elston, C., Jütte, T., Klein, O., Knäbe, S., Lückmann, J., Roessink, I., Persigehl, M., Cornement, M., Exeler, N., Giffard, H., Hodapp, B., Kimmel, S., Kullmann, B., Schneider, C., Schnurr, A., 2021: Results of 2-year ring testing of a semi-field study design to investigate potential impacts of plant protection products on the solitary bees Osmia bicornis and Osmia cornuta and a proposal for a suitable test design. Environ. Toxicol. Chem., 40 (2021), pp. 236-250.

Hayward, A., Beadle, K., Saurabh Singh, K., Exeler, N., Zaworra, M., Almanza, M.T., Nikolakis, A., Garside, C., Glaubitz, J., Bass, C., Nauen, R., 2019: The leafcutter bee, Megachile rotundata, is more sensitive to N-cyanoamidine neonicotinoid and butenolide insecticides than other managed bees. Nature Ecology & Evolution, vol 3, 1521–1524.

Sgolastra, F., Hinarejos, S., Pitts-Singer, T.L., Boyle,N.K., Joseph, T., Lūckmann, J., Raine, N.E., Singh, R., Williams, N.M., Bosch, J., 2019: Pesticide Exposure Assessment Paradigm for Solitary Bees. Environmental Entomology, Volume 48, Issue 1, February 2019, Pages 22–35.

1.2. A more diverse pollen nutrition matters for developing solitary bees but does not mitigate the negative impact of pesticides

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Abstract

In agricultural landscapes, bees are subjected to diminishing floral resources and exposure to pesticides. Potential interactions of nutritional stress and pesticide exposure on solitary bees are largely unknown. We investigated the development and survival of the solitary bee *Osmia bicornis* provisioned with different pollen nutrition and exposed to pesticides in a full-factorial design in the laboratory. We used three nutrition types characterized by a low pollen diversity and a mixture of these (higher pollen diversity). We investigated two field-realistic concentrations of the insecticides thiacloprid, sulfoxaflor and flupyradifurone, as well as of the fungicides azoxystrobin and tebuconazole. We explored whether a higher pollen diversity is beneficial for *O. bicornis* development and survival, how the pesticides affect various fitness measures and whether pesticide impacts are mitigated by the higher diversity pollen. We found that a more diverse pollen was beneficial for *O. bicornis* development time, cocoon weight, pollen efficacy and pollen consumption. Thiacloprid, sulfoxaflor and flupyradifurone elongated development time. Sulfoxaflor and flupyradifurone lowered cocoon weight and pollen efficacy, and sulfoxaflor reduced survival and pollen consumption. Our results do not support the hypothesis that a more diverse pollen mitigates negative pesticide effects, but highlight the importance of diverse floral resources for bee development and the need for further studies on the interactions of multiple stressors.

Keywords: *Osmia bicornis*, larval development, pollen diversity, nutrition, interactions, detoxification, gene expression

1.3. Nutritional stress exacerbates impact of a novel insecticide on solitary bees' behaviour, reproduction and survival

Knauer, Anina[1]; Alaux, Cedric[2]; Allan, Matthew[3]; Dean, Robin[4]; Dievart, Virginie[2]; Glauser, Gaétan[5]; Kiljanek, Tomasz[6]; Michez, Denis[7]; Schwarz, Janine[1]; Tamburini, Giovanni[8]; Wintermantel, Dimitry[9]; Klein, Alexandra Maria[9]; Albrecht, Matthias[1]

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Abstract

Pesticide exposure and food stress are major threats to bees, but their potential synergistic impacts under field-realistic conditions remain poorly understood and are not considered in current pesticide risk assessments. We conducted a semi-field experiment to examine the single and interactive effects of the novel insecticide flupyradifurone (FPF) and nutritional stress on fitness proxies in the solitary bee Osmia bicornis. Individually marked bees were released into flight cages with monocultures of either buckwheat, wild mustard or purple tansy, which were assigned to an insecticide treatment (FPF or control) in a crossed design. Nutritional stress, which was high in bees foraging on buckwheat, intermediate on wild mustard and low on purple tansy, modulated the impact of insecticide exposure. Within the first day after application of FPF, mortality of bees feeding on buckwheat was 29 times higher compared to control treatments, while mortality of FPF exposed and control bees was similar in the other two plant species. Moreover, we found negative synergistic impacts of FPF and nutritional stress on offspring production, flight activity, flight duration, and flower visitation frequency. These results reveal that environmental policies and risk assessment schemes that ignore interactions among anthropogenic stressors will fail to adequately protect bees and the pollination services they provide.

Keywords: bee health, foraging, nectar, pesticide, pollen, reproduction

1.4. Method development for the acute contact test on the solitary bee Megachiles rotundata. – LD50 toxic reference

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Abstract

New methodologies, for solitary bees, need to be developed to fulfill the EFSA requirements. *Megachile rotundata*, or the alfalfa leaf cutter bee, could be a good candidate for it. Cocoons of the *M. rotundata* are commercially available and adults are used as pollinators.

Females of *M, rotundata* are more exposed to the PPPs (Plant Protection Products) than males. Adult females collect not only pollen and nectar but also pieces of leaves to build their own nest. That's why, the new acute methodologies should be developed with adult females only.

To test the methodology, two consecutive tests were run. Commercial cocoons from Northstar Seed Ltd. Canada were incubated at 33 ± 2 °C and 60 ± 10 % RH in the dark. Once the males started to emerge, cocoons were transferred to the test conditions at 30 ± 2 °C and 70 ± 5 % RH with a light cycle of 16 : 8 h (L : D).

Ten newly emerged, meconium free, adult females were introduced per cage (at 20 °C). Female bees were acclimatised to the test conditions for 24 h, before the application. For food, pollen paste was supplied *ad* libitum.

Application was carried out at 20°C. After the application, bees were evaluated and mortality was recorded after 4, 24, 48, 72 and 96 h.

After 96 h, control mortality was below 10 % (6.7 %) and the LD_{50} values for both test were nearly the same, 0.175 µg a.i. / bee for the first test and 0.174 µg a.i. / bee for the second test. Although the results showed the methodology could be considered valid, as the control mortality was below 10% and the LD_{50} values were the same, this methodology needs to be tested again next year and a step farther with the acute oral test needs to be done.

2. Session – Risk Assessment/ Microbials

2.1. Brood termination rate in honey bees in two consecutive brood cycles: a comparison

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Abstract

The potential impact of pesticides on honey bee brood (*Apis mellifera* L.) is often investigated under higher-tier semi-field, worst-case exposure conditions, according to OECD GD 75, with the brood termination rate (BTR) as one of the key measurement endpoints to be considered. Historical data from such semi-field studies, where brood cells with eggs are marked out and the 7-day exposure period takes place under tunnel conditions, show a high variability in the BTRs within the untreated control groups. In contrast, control BTRs obtained under full-field conditions with free-flying honey bees are substantially lower and less variable.

The current analysis by the ICP-PR Bee Brood Working Group investigated the magnitude and variability in BTRs for a negative control and a reference chemical (i.e., fenoxycarb) at two subsequent brood cycles, the first started under semi-field conditions (i.e., while colonies are confined in the tunnels), while the second started under full-field conditions after completion of the first brood cycle when colonies were in the post-exposure monitoring phase of the study and colonies are no longer confined. In addition, the results obtained for the reference chemical fenoxycarb provide insight on the duration of effects on brood caused by an active substance with known insect growth regulating (IGR) properties. These results were compared and discussed regarding the interpretation of BTRs gathered from such bee studies.

2.2. BEEHAVE and brood termination rate - A modelling study how timing, magnitude and duration of effects determine colony strength

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Abstract

The brood termination rate (BTR) investigated in OOMEN and OECD GD 75 studies for pesticide risk assessments is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult and thus influences colony strength. Colony strength as the number of worker bees per colony affects pollination services, yield of hive products and colony viability. In this context, a honey bee colony is regarded as viable and strong enough for successful overwintering and subsequent development to a vital colony in the following year, if at least 5000 worker bees are recorded prior to hibernation according to the respective EFSA Bee Guidance Document of 2013. The EFSA bee guidance gives levels of forager mortality due to pesticide exposure at which colony strength is assumed to fall below this threshold.

Impacts of pesticides on honey bee brood are commonly investigated under semi-field, worst-case exposure conditions according to OECD GD 75 with the BTR as one key endpoint. Until now it remains unclear how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and thereby the hibernation ability and viability in the following season.

Using the honey bee colony model BEEHAVE, we simulated how BTRs at different times in the year and of different magnitude and duration affected colony strength after two brood cycles and prior overwintering. Our results show how different BTR values influence the colony size, aiding the interpretation of experimentally observed BTRs in terms of consequences for colony strength and viability.

2.3. Conceptual framework for the selection of higher-tier refinement options with focus on honey bee (*Apis mellifera*) brood

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Abstract

The outcome of a screening-level honey bee (*Apis mellifera*) risk assessment using laboratory-based studies of individual larvae may indicate potential risk to honey bee brood that require further refinement involving colony-level studies. At present, different study designs (i.e., OECD Guidance Document 75, acute and chronic Oomen feeding studies, and large colony feeding studies (LCFS)) are available to investigate potential effects on bee brood under more realistic exposure conditions. However, without a decision framework, the choice of the suitbale test design can be challenging.

Therefore, a conceptual framework has been developed by the International Commission for Plant-Pollinator Relationships (ICP-PR) Bee Brood Working Group to inform decisions regarding the currently available refinement option(s). The framework consists of a decision tree for determining whether there is exposure of honey bee brood after the use of a plant protection product based on different exposure scenarios. If the outcome indicates that the exposure of the brood cannot be excluded, refinement options are listed. The possible refinement options (i.e., study designs) are tabularised in a table that includes the strengths and limitations of the study.

2.4. Bumblebee (*Bombus terrestris*) versus honey bee (*Apis mellifera*) acute sensitivity – Final results of a CropLife Europe data evaluation

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Abstract

A data evaluation was conducted by CropLife Europe companies to compare the acute sensitivity of the bumblebee *Bombus terrestris* L. with that of the honey bee *Apis mellifera* L. to plant protection products. For the evaluation, 97 data sets were available for oral toxicity and 108 data set for contact toxicity for both bee species. The data comprised 27 and 29 sets for oral and contact toxicity testing of fungicides, 42 and 41 for oral and contact exposure for herbicides (including one plant growth regulator), and 28 oral and 38 contact data sets for insecticides (including one nematicide), respectively. For data sets with definitive endpoints for honey bees (most insecticides), the sensitivity ratio (SR) was determined by dividing the honey bee LD_{50} by the bumblebee LD_{50} value. Endpoints of data sets with unbound '>' endpoints (most fungicides and herbicides) for honeybees were assigned to toxicity classes.

For data sets with unbound honey bee LD_{50}-values the data evaluation indicated similar or lower sensitivity of bumblebees versus honeybees by contact or oral exposure for all fungicides and herbicides. Likewise, similar or lower contact sensitivity of bumblebees than honey bees was determined for all insecticidal data sets (including the nematicide) with definite honeybee endpoints. For the oral exposure, this was also the case except for 5 active substances. For two insecticide active ingredients the SRs were between 3.3 and 5.1. For two insecticide formulations with the same active ingredient and with unbound LD_{50}-values for honey bees which generated SRs of approximately 95, results of higher tier semi-field

data do not indicate any negative impact on B. terrestris and their colony development under more realistic semi-field conditions.

Overall, the current data supports that, for a wide range of chemistry, the honey bee is a sensitive surrogate test species for bumblebees based on acute toxicity testing of plant protection products. Therefore, routine regulatory testing of the bumblebee (B. *terrestris*) in context of registration of plant protection products and/or using a standard safety of 10 on basis of honey bee endpoints is not justified on basis of available data review.

2.5. Compilation and statistical analysis of pesticide residue levels in pollen and nectar: refined Residue Unit Doses (RUDs) for Tier 1 dietary bee risk assessment in North America

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Abstract

Current Tier 1 bee risk assessment in North America (US EPA, 2014) relies on an exposure estimation and risk assessment model called BeeREX. This model uses a Residue Unit Dose (RUD) approach to estimate residues in nectar and pollen. The RUD is the parameter expressing the residue concentration of a pesticide in pollen and in nectar for a standardized application rate of 1 kg/ha or 1 lb/A. For foliar spray applications, the current approach involves the use of the tall grass residue value from the T-REX model (v.1.5) as a surrogate for pesticide concentrations in nectar and pollen. For soil treatments, the Tier I method involves the use of the Briggs' soil-plant uptake model, which is designed to estimate pesticide concentrations in plant shoots, and these are used as a surrogate for concentrations in pollen and nectar. For seed treatments, the Tier I exposure method is based on 1 mg a.i./kg concentration as an upper-bound for pesticides in nectar and pollen. In

comparison, the European Union (EU) Tier 1 risk assessment uses a database of nectar and pollen residue data (Kyriakopoulou *et al*., 2017). The US EPA has received in recent years residue studies from several applicants that can be used to adequately describe the distribution of pesticide residues that occur in pollen and nectar relative to application rate, method of application, and crop. By combining the US EPA and EFSA nectar and pollen databases a statistically refined estimation of RUD values can be calculated. The calculated nectar and pollen RUD values will then inform the BeeREX model with dietary exposure data relevant to the bee risk assessment.

Keywords: residues in pollen and nectar, Tier 1 exposure estimates, refined RUD values, BeeREX.

References

Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K. M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G., Glossioti, M., 2017: Collection and analysis of pesticide residue data for pollen and nectar. EFSA Journal, 2017;14(10).

USEPA, PMRA, CDPR, 2014: Guidance for Assessing Pesticide Risks to Bees. Office of Pesticide Programs United States Environmental Protection Agency, Health Canada Pest Management Regulatory Agency (PMRA), California Department of Pesticide Regulation (CDPR). June 19, 2014. Access: 29. July 2022, URL: https://www.epa.gov/sites/default/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

Name of Section/Session – Laboratory/semi-field/field

3. Session – Laboratory/Semi-field/Field

3.1. The lethal and sublethal effects of synthetic miticide tau-fluvalinate (tech.) on adult honeybees

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Abstract

Pyrethroids (e.g., flumethrin and tau-fluvalinate) are frequently related to long half-life inside the hive matrices, which may adversely affect the health of bee colony. In this study we assessed potential harmful lethal and sublethal effects of synthetic miticide tau-fluvalinate (tech.) on winter adult honeybees according to OECD 245 (2017). In vitro reared winter honeybees showed no dose-dependent mortality after the oral 10-days exposure to sucrose solution (50% w/w) spiked with a maximum concentration of 750 µg tau-fluvalinate /kg diet; the No Observed Effect Concentration (NOEC) appears to be higher than or equal 750 µg a.i./kg diet.

The results of tau-fluvalinate testing for the sublethal effects on bee immune system showed up-regulated gene expression for abaecin, lysozyme, and defensin in the test groups (1/1 FLU and/or 1/10 FLU), however the expression of hymenoptaecin gene was reduced.

Keywords: Toxicity, Tau-fluvalinate, Apis mellifera, Exposure, Immune system

Introduction

Tau-fluvalinate is the active ingredient of several registered plant protection products (Apistan®, Klartan®, Mavrik®), which leave residues in hive matrices (wax, propolis, and honey). Moreover, tau-fluvalinate is used in apiculture as miticide, the market offers several authorised veterinary medicinal products. Several studies detected a wide range of agricultural and apicultural pesticides contaminating in-hive matrices, among the most common of which was tau-fluvalinate (Wallner, 1999; Tsigouri et al., 2004; Johnson et al., 2010; Mullin et al., 2010; Lambert et al.,

2013; Martinello et al., 2020). This creates a dangerous environment for honeybees that are chronically exposed to the residues, as well as they contaminate the substances they require for nutrition and energy, food storage and/or brood rearing. The intensive and long-term use of authorised miticides in apiculture has raised the question of safety of these medicinal products to honeybees. Both, direct lethal and the sublethal effects on immune system of tau-fluvalinate were tested in this in vitro study.

Material and methods

Toxicity bioassay

To determine the lethal and sublethal effects of tau-fluvalinate (tech.) to honeybees after continuous 10-days exposure, we performed chronic in vitro study according to OECD 245 (2017). Selected concentration of 750 µg tau-fluvalinate/kg diet was based on the highest value reported by Atienza et al. (1993).

RNA isolation, cDNA synthesis and gene expression analysis (qPCR)

The gene expression of abaecin, defensin-1, hymenoptaecin, lysozyme-2, and reference β-actin was determined in this study. After 10 days of continuous exposure, tested bees were anaesthetised at + 4 ◦C for 30 min and then their intestinal tracts (n = 15/group) were harvested under aseptic conditions. Guts of tested bees were washed with PBS. Following the manufacturer's instructions, the total RNA of guts was isolated by Purezol™ reagent. Then using Nanodrop 8000, the purity and quantity of isolated total RNA was determined at 260/280 nm. QuantiTect Reverse Transcription Kit was used for gDNA removal and cDNA synthesis. These cDNA samples were used as a template for quantitative PCR. Real-time PCR was performed in an iCycler CFX96 in 10 µL reaction volume containing iQ™ SYBR® Green Supermix, 0.5 µM of forward and reverse primers and 40 ng of cDNA template. β-actin was used as a reference gene for internal control. Each assay included a No template control without a cDNA template and all the reactions were performed in triplicates. The experimental protocol consisted of the initial denaturation at 95 ◦C for 5 min, followed by amplification including 40 cycles of 4 steps: denaturation at 95 ◦C for 30 s, annealing at 59 ◦C for 30 s, extension at 72 ◦C for 30 s, and final extension at 72 ◦C for 15 min followed by melting curve analysis to confirm amplification of a specific product. The 2-ΔΔCT method was used in calculation of relative expression. The sequence of primers for gene expression and other details are listed in Sabová et al. (2022).

Statistical analysis

Obtained toxicity data were analysed using ToxRat Professional® software (ToxRat Solutions GmbH). Data of gene expression were statistically analysed using the GraphPad Prism 3.00 software (GraphPad Software) by oneway analysis of variance (ANOVA) followed by post-hoc Tukey's Multiple Comparison Test.

Results

According to OECD 245 (2017) this bioassay is valid, because mortality observed in the control group and the solvent control group was < 15% and the mortality in the higher reference control group was 100% at the end of the experiment. No dose-dependent mortality was observed in in vitro reared honeybees in any of the test groups. The NOEC was determined to be ≥ 750 µg tau-fluvalinate/kg diet (Bonferroni-Holms corrected, one-sided, P ≤ 0.05).

Table 1 Cumulative mortality of honey bees during the exposure period of 10 days

Test item	Treatment nominal [µg a.i./kg diet]	Cumulative mortality (%)									
		D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10
Control	n.a.	0	0	0	3.6	5.5	5.5	5.5	5.5	5.5	5.5
Solvent control	n.a.	0	0	0	0	0	0	7.3	7.3	9.1	9.1
Test item tau-fluvalinate (tech.)	750	0	0	0	0	0	0	3.6	5.5	5.5	5.5
	75	0	0	0	0	0	0	3.6	3.6	3.6	5.5
Reference item	500	0	0	0	0	14.5	21.8	29.1	36.4	40.0	40.0
	1000	0	0	0	0	16.4	30.9	76.4	100	100	100

The gene expression of abaecin was almost at the same level in groups fed with 1/1 tau-fluvalinate, dimethoate and in solvent control group compared to the untreated control (Fig. 1). However, abaecin gene expression in the group exposed to 1/10 tau-fluvalinate was significantly up-regulated as compared to other tested groups (P < 0.001). In the second antimicrobial compound (lysozyme), we can see

statistically increased gene expression in both tau-fluvalinate groups (1/1 FLU as well as 1/10 FLU) compared to the control. In the dimethoate group, expression of the gene encoding lysozyme had the same trend as with abaecin. A significant up-regulation of gene expression of defensin was recorded only in 1/1 FLU, while in other groups the expression was reduced compared to the untreated control group. The last one of the genes studied was hymenoptaecin, which appears to be the most sensitive antimicrobial peptide. Gene expression in all the experimental groups was significantly lower compared to the untreated control.

Despite no direct lethal effect of tau-fluvalinate was found, we can conclude that repeated low-dose treatments with synthetic acaricide tau-fluvalinate affects bee immunity by modifying the transcription of genes encoding antimicrobial peptides which are considered as the first line of host immune defence against different pathogens.

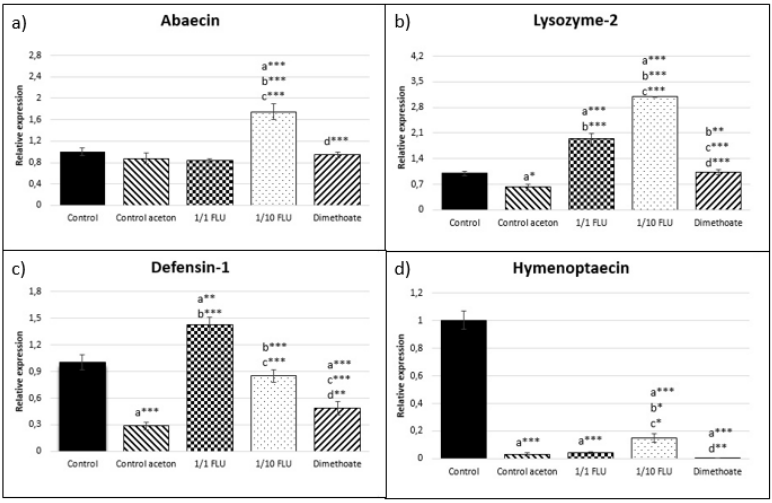

Figure 2 The effect of tau-fluvalinate on the gene expression of bee immunologically important molecules: a) Abaecin, b) Lysozyme-2, c) Defensin-1, d) Hymenoptaecin. a – significantly different from Control; b – significantly different from Control aceton; c – significantly different from 1/1 Fluvalinate; d – significantly different from 1/10 Fluvalinate; *P < 0.05; ** P < 0.01; *** P < 0.001.

Conclusion

Despite no direct lethal effect of tau-fluvalinate was found, we can conclude that repeated low-dose treatments with synthetic acaricide tau-fluvalinate affects bee

immunity by modifying the transcription of genes encoding antimicrobial peptides which are considered as the first line of host immune defence against different pathogens.

Acknowledgements

This research was supported by the National Reference Laboratory for Pesticides of University of Veterinary Medicine and Pharmacy (UVLF) in Kosice, Slovakia.

References

Atienza, J., Jiménez, J., Bernal, J., Martín, M., 1993: Supercritical fluid extraction of fluvalinate residues in honey. Determination by high-performance liquid chromatography. J. Chromatogr. A. 655, 95–99. https://doi.org/10.1016/0021-9673(93)87016-F

Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010: Pesticides and honey bee toxicity - USA. Apidologie 41, p. 312–331. https://doi.org/10.1051/apido/2010018

Lambert, O., Piroux, M., Puyo, S., Thorin, C., L'Hostis, M., Wiest, L., Bulet´e, A., Delbac, F., Pouliquenet, H., 2013: Widespread Occurrence of Chemical Residues in Beehive Matrices from Apiaries Located in Different Landscapes of Western France. PLoS ONE 8 (6), e67007. https://doi.org/10.1371/journal.pone.0067007

Martinello, M., Manzinello, C., Borin, A., Avram, L.E., Dainese, N., Giuliato, I., Gallina, A., Mutinelli, F., 2020: A Survey from 2015 to 2019 to Investigate the Occurrence of Pesticide Residues in Dead Honeybees and Other Matrices Related to Honeybee Mortality Incidents in Italy. Diversity 12 (1), 15. https://doi.org/10.3390/d12010015

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., Vanengelsdorp, D., Pettis, J.S., 2010: High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One 19; 5 (3), e9754. https://doi.org/10.1371/journal.pone.0009754

Sabová, L., Cingeľová Maruščáková, I., Koleničová, S., Mudroňová, D., Holečková, B., Sabo, R., Sobeková, A., Majchrák, T., Ratvaj, M., 2022: The adverse effects of synthetic acaricide tau-fluvalinate (tech.) on winter adult honey bees, Environmental Toxicology and Pharmacology 92, ISSN 1382-6689, https://doi.org/10.1016/j.etap.2022.103861

Tsigouri, A.D., Menkissoglu-Spiroudi, U., Thrasyvoulou, A., Diamantidis, G., 2004: Fluvalinate residues in honey and beeswax after different colony treatments. Bull. Environ. Contam. Toxicol. 72 (5), p. 975–982. https://doi.org/10.1007/s00128-004-0339-7

Wallner, K., 1999: Varroacides and their residues in bee products. Apidologie 30, p. 235–248

3.2. Comparison of Dead Bee Traps for Honey Bees

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Abstract

Dead bee traps are a widely used tool for evaluating honey bee mortality in ecotoxicological (semi-) field studies. Many models exist, all having their specific advantages and faults, which in turn influences the acquired mortality data. We here compared two trap types for their efficiency by adding stained dead bees into four hives over several days. One trap was a flat rectangular mesh box at the floor in front of the hive (Underbasket trap); bees drop dead bodies while flying over the trap. The other was a square mesh box fixed on the hive enclosing also the hive entrance (Todd trap), and bees have to drop dead bodies in order to exit the trap. Traps were switched between bee hives once. For both trap types dead bee recovery was 60%. Bee hives as well as days varied substantially in dead bee recovery, regardless the trap type.

Keywords: dead bee trap, stain, honey bee

Introduction

Dead bee traps are a widely used tool for evaluating in-hive honey bee mortality in ecotoxicological (semi-) field studies. Bees clean their hives by carrying dead bodies while flying out and drop them outside the hives. Many trap models exist, all having their specific advantages and faults, which in turn influences the acquired mortality data. Closed traps (e.g. Todd) covering the entrance can increase stress for honey bees, while open traps (e.g. Underbasket) might not reliably capture mortality if bees fly beside the traps. The underbasket trap is often used in

southern Europe, the US and Brazil where bees can be very agrressive and working with a Todd trap attached to the hive is inconvenient for the bees and the researcher. Furthermore, an underbasket trap can be very usefull when for instance a bee counter or a pollen trap is attached to the hive.

Material and methods

We used four bee hives with two trap models (Fig. 1) in spring 2022.

Todd traps were directly attached to the hive covering the hive entrance with measurements 40 x 40 x 16 cm covered by a mesh with 1 x 1 cm grid size.

Underbasket traps were placed in front of the hive with measuremnts 100 x 50 x 16 covered by a mesh with 1 x 1 cm grid size.

Dead bees were stained with a neon yellow powder.

Figure 1 Two types of dead bee traps, Underbasket trap (A) and Todd trap (B).

Dead bee recovery was measured by following procedure:

100 yellow stained dead bees were added to each hive every morning for 3 days

Stained dead bees were counted in the traps 1, 3, 6, 24 hours after adding. After 24 hrs bottom drawers of the hives were also checked and emptied.

Traps were switched between hives, and we gave the bees several days to acustomize

We repeated the steps above

Results

For both trap types dead bee recovery was on average around 60% (Todd: 60.5±18.0, Underbasket: 58.6±13.2). Bee hives as well as days varied substantially in dead bee recovery, regardless the trap type. Since there was no difference between the efficiency of underbasket traps and the Todd traps either can be used in studies.

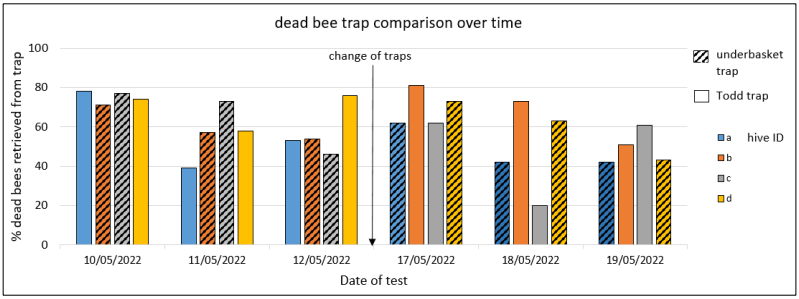

Figure 2 Recovery of stained dead bees in the dead bee trap summed up over 24 hours.

References

Atkins, E. L., F. E. Todd, L. D. Anderson, 1970: Honey bee field research aided by Todd dead bee hive entrance trap. California Agriculture 24(10): 12-13.

Dietemann, V., J. D. Ellis, P.Neumann (Eds.), 2013a: The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research (pp. 636). International Bee Research Association.

Porinni, C., A. G. Sabatini, S. Girotti, S. Ghini, P. Medrzycki, F. Grillenzoni, L .Bortolotti, E. Gattavecchia, G. Celli, 2003: Honey bees and bee products as monitors of the environmental contamination. Apiacta 38: 63-70.

3.3. GLP requirements for using visual bee monitoring technology in ecotoxicological studies

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Abstract

New technologies can help enhance the risk assessment of plant protection products prior to market approval. They allow the integration of the continuous data on sublethal effects such as activity and pollen foraging rather than snapshot data collected at points in time by human observers. They also allow for the collection of data on the life of individual bees.

In order to allow the use of such new technologies in trials under the requirements of the OECD Series on principles of good laboratory practice and compliance monitoring (2016), there are a number of challenges to solve. We are presenting the key questions which arise when including visual bee monitoring technology in GLP studies and the solutions we have developed in order to ensure compliance. Among the critical challenges are:

- Raw data storage
- Performance validation in the field
- Responsibility assignment for device monitor during the study
- Data handling for the analysis by the test facility
- Distinction of Installation Qualification and Operation Qualification

Keywords: visual bee monitoring, new technologies, ecotoxicology, good laboratory practice, sublethal effects, validation

3.4. Chronic larval and adult honey bee laboratory testing: which dietary additive should be considered when a test substance is not solubilized in acetone?

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Abstract

Chronic toxicity tests on adult and larval honey bees (*Apis mellifera*) can require the use of dietary additives (solvents, emulsifiers, adjuvants and viscosifier agents) when the active ingredient of plant protection products cannot be dissolved or does not remain stable and homogeneous within the test diets. Acetone is the widely used and accepted solvent allowed for in the international regulatory guidelines, but it can be ineffective in keeping certain compounds in solution and can cause toxicity to adults and larvae at certain levels. Here we evaluate six dietary additives including five solvents (ethanol, isopropanol, n-propanol, propylene glycol and triethylene glycol) and a viscosifier agent (xanthan gum) at five concentrations as alternative additives in the adult and larval diets. The safe levels for bees were determined for each of the additives used in the 10-day chronic adult and 22-day chronic larval tests. Ethanol and isopropanol were the least toxic dietary additives for both endpoints in the 10-day chronic adult study and in the emergence endpoint in the 22-day chronic larval study and therefore can be used at higher concentrations to achieve solubility of a test substance while xanthan can only be used safely and effectively at lower concentrations. The optimal agent selected for a study will vary based upon the physical and chemical properties of the test substances, yet our study provides empirical data to support the use of alternatives to acetone to generate robust honey bee toxicity data for adults and larvae.

4. Session - Monitoring

4.1. Evaluation of bee counters - introduction of a new protocol for measuring the accuracy of daily losses.

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Abstract

Automated bee counters have evolved and become more diverse over the last hundred years. To date, however, there is no method for standardized validation of counting accuracy and thus no reliable data on daily bee losses or background mortality in bee colonies. Such data, however, are urgently needed by regulatory agencies to establish future guidelines for pesticide risk assessment. In this work, we combined existing approaches into a new protocol for validating bee counters. In a case study with a visual artificial-intelligence-based monitoring system, we demonstrated that the protocol is sufficiently practical to determine the measurement accuracy of a commercial counting system. Measurement accuracy was modeled by the difficulty of specific measurement conditions. The daily loss, i.e., the difference between incoming and outgoing bees, can be used to assess colony health and environmental impact, and to draw conclusions about the effect of pesticides on bee colonies. The protocol developed makes innovations in this field measurable and creates a basis for benchmarking different types of bee counting systems. We discuss how it can be used to advance the sector in the future.

Keywords: Robbers test, Automated bee counting device, Regulatory risk assessment methodology, Harmonized validation protocol, Precision beekeeping, visual bee monitoring

5. Session – Microbials

5.1. Assessing the impact of microbial plant protection product mixtures on honeybee workers

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Abstract

The importance of microbial plant protection products (PPPs) in agriculture is steadily increasing, especially since they are considered to substitute chemical PPPs. Tank mixes are often common practice by farmers to reduce costs and increase the effectivity by controlling a broader spectrum of pests. However, there is no available information on the possible interactions between microbial PPPs and bee´s responses after exposure to such combinations. We studied several tank mixes of microbial PPPs depending on application of the products on the same crops. Five products with different microorganisms as active ingredients and their combination were tested, including *Bacillus thuringiensis* ssp. *aizawai* (strain: ABTS-1857), B. *thuringiensis* ssp. *kurstaki* (strain: EG 2348), B. *amyloliquefacien* (strain: QST 713), *Beauveria bassiana* (strain: ATCC 74040) and *Cydia pomonella* granulosis virus (GV0005). Caged winter honey bees were placed in an incubator at 26°C and 65% humidity and exposed orally either acute or chronic (over 10 d) to the maximum recommended application rate of solo-product or mixture of two products. Mortality and food uptake amount was recorded daily over 15 d. Our results show that mixture of products containing B. *thuringiensis* ssp. *aizawai* and B. *amyloliquefacien* caused higher mortality rate compared to the solo products, whereas the effects in other mixtures are mostly related to the solo products which have the strongest effects. On the other hand, mixtures containing C. *pomonella* granulosis virus and/ or B. *thuringiensis* ssp. *kurstaki* did not affect the bee's survival compared to the other microbial PPPs. In conclusion, further stud-

ies are necessary to assess the effects of such mixtures as the effects of tank mixtures of two or more PPPs on honey bees, as these are not routinely assessed in the risk assessment of plant protection products.

Keywords: *Bacillus thuringiensis*, *Apis mellifera*, tank mixture, microbial plant protection product

5.2. Bacillus thuringiensis ssp. aizawai – Observations on honey bees and distribution in colony matrices under field conditions

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Abstract

Microbial pest control products are commonly applied worldwide as alternatives to avoid potential adverse effects of chemical plant protection products. Here, we aimed to evaluate the biosafety of a commercial product containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) using four different approaches: 1) laboratory chronic exposure to evaluate the survival of adult and larval bee, 2) in-hive feeding under field conditions to examine the effect of B. t. on brood development and the core gut microbiome of adult bees, 3) semi-field colony-feeding to determine contamination levels of B. t. spores in various matrices, and 4) a field trial with spray application in a bee-attractive crop to estimate potential environmental accumulation and exposure of honey bee colonies.

Adult bee and larval survival were negatively affected after chronic exposure depending on the tested concentrations; however, pollen feeding to adults promote survival of treated bees and delay the effects. Under colony conditions, treated colonies showed a higher brood termination rate and a significantly lower normalized abundance of the core gut microbiome in worker bees. B. t. spores

were detectable in all matrices at different concentrations, decreasing over time under semi-field conditions. High spore levels were present in honey sacs and pollen pellets immediately after application. No spore reduction was seen in stored matrices like nectar and bee bread.

In conclusion, the pest control product containing *B. t.* strain ABTS-1857 showed a negative effect on exposed bees under laboratory as well as field conditions, for instance on colony development and caused dysbiosis of the gut microbiome. However, further field-realistic exposure studies in bee attractive crops are needed to evaluate the potential risk of such products on honey bees.

Keywords: *Bacillus thuringiensis*, microbiome, microbial pest control

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