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Preface

From the first meeting of International Commission for Plant-Pollinator Relationships (ICP-PR) Bee Protection Group in Wageningen, Netherlands, in 1980 the group has continue to evolve over the last 35 years and is recognized as established expert forum for addressing the potential risks of pesticides to bees. The initiative to form the Bee Protection Group was in response to the need of regulatory authorities for expert advice to support achieving better regulations for protecting honey bees from potential harmful effects of pesticides. As of 2022, the Bee Protection Group has organized fifteen international symposia. The 15th International Symposium of the ICP-PR Bee Proteciton Group in York, UK, provided participants with a renewed opportunity to meet in person and learn how the science of assessing risks of pesticides to honey bees (*Apis mellifera*) and non-*Apis* bees has continued to evolve in response to both academic and regulatory testing needs. However, the symposium underscored that while the Corona Virus (COVID) pandemic limited the extent of social interactions, critical research continued. At the start of the symposium there were presentations from the European Commission, the European Food Safety Authortiy (EFSA) and the Organization for Economic Cooperation and Development (OECD) which reiterated the interest of regulatory authorities in having suitable tools for assessing both bee exposure to and effects from plant protection product toward providing increasingly realistic estimates for both solitary and social bees. Consistent with the mission of the ICP-PR Bee Protection Group, participants reflected a commitment toward developing test methods which have undergone ring-testing to ensure that study protocols provide consistent, reproducible and reliable data with which to inform decision making across multiple sectors (*i.e.*, government, academia and industry). Many of these methods have been and continue to be advanced to the OECD for consideration as internationally recognized test guidelines and guidance documents.

Consistent with preceding symposia, the conference in York provided an opportunity for workgroups to report on their activities. As is clear from the multiple platform and poster presentations from the Non-*Apis* Bee, Honey Bee Brood, Laboratory/Semi-Field/Field Testing, Monitoring, Microbial Testing, and the Risk Assessment workgroups, researchers have invested a considerable amount of effort toward method development/refinement. The titles and abstracts presented in the following sections collectively represent a significant accomplishment given the challenges faced by our global society as the result of the pandemic.

Similar to preceding years, declines in honey bee health and in the numbers of some species of non-*Apis* bees have been associated with multiple factors (*i.e.*, pests, pathogen, pesticides, loss of habitat, managment practices and climate change). It remains challanging to identify specific combinations of these factors which consistently account for the variability associated with declines. The vagaries of climate change and emerging pests/pathogens and novel pesticides have continued to challenge our understanding of how these multiple factors interact and how to mitigate them. Awareness of these declines though have fueled concerns/demands that more needs to be done. With respect to evaluating the risks of plant protection products to bees, various schemes are continuing to evolve internationally and reflect differences in the number and scope of laboratory- and field-based data needed to inform the decision-making process. These differences though raise uncertainties regarding the extent to which standardized guidance documents and test guidelines should be developed in response. Responses though must be calibated against limited resources and evidence-based understanding as to the extent that additional modifications/data are needed.

Regulatory authorties generally rely on a tiered testing process which transitions from lower-tier laboratory-based studies of individual bees to higher-tier semi- and full-field studies of social and/or solitary species under increasingly realistic conditions. The lower-tier studies and assessment process as well as trigger values therein are intended to be conservative and serve as a means of effectively screening out products which are not considered likely to represent a threat to non-target organims (*e.g.*, bees). This process though relies on assumptions of surrogacy with the understanding that it is neither realistic nor feasible to test all species that may come in contact with the product. Having an effective screen (*i.e.*, testing regime) enables regulatory authorities to focus resources on those products which represent significant uncertainties with respect to their potential effects in situations where exposure is likely and cannot be readily mitigated, as well as avoids unnecessary testing. While there is considerable discussion and expectations regarding the nature/rigor of the various tiers of evaluation, there is a reasonable expectation that the various processes rely on a common understanding of the underlying science and the level of detail which is needed to inform decisions.

As with each of the past symposia, the 15th International Symposium of the ICP-PR Bee Protection Group helped to further advance the science underlying assessing exposure and effects of plant protection products on bees. The Symposium organizers and participants had an opportunity to learn the interests/needs of various regulatory authorities and the opportunities/challanges associated with testing novel products across multiple species of bees. There is an increasing number of opportunities to leverage existing data toward the development of predictive tools/models to reduce reliance on resource-intensive whole animal tests and to develop enhanced methods for monitoring the effectiveness of mitigation practices. There are also opportunities for further harmonizing assessment approaches since the science should not be defined by political boundardies as it is clear that the problems which the science is attempting to address are global.

The Steering Committee would like to thank all of the participants for their continued commitment to advancing testing and assessment methods and in particular for their willingness to share and effectively communicate their research. The Steering Committee would like to particuarly thank FERA Science for their willingness to host the 15th International Symposium of the ICP-PR Bee Protection Group.

Disclaimer: Any views/opinions expressed in any of the papers/abstracts/posters do not necessarily reflect the constituency of the ICP-PR Bee Protection Group, the Bee Protection Group Steering Committee, not the various organizations with whom participants are affiliated.

Mission and Role of the ICPPR Bee Protection Group

The International Commission for Plant-Pollinator Relationships (ICP-PR) Bee Protection Group is a non-profit organisation of researchers in a broad range of disciplines from within and outside Europe who voluntarily share their common interest of improving tools for assessing and understanding bee protection within the context of modern, sustainable agriculture. The information provided by the experts within the Bee Protection Group is intended to serve as a reasonable foundation with which to base regulatory decision-making efforts.

The ICP-PR Bee Protection Group serves as a forum for addressing challenges and uncertainties associated with protecting and enhancing the health of honey bees (*Apis mellifera*) and non-*Apis* bees and to provide a means of coordinating international research efforts within academia, government, and industry to develop suitable testing and evaluation methods for assessing exposure and effects of factors impacting bee health. The ICP-PR provides a means of ensuring that testing methods are fit-for-purpose in terms of providing consistent, reproducible and reliable data to

inform decision making. The underlying methods developed through the collaborative efforts of researchers within the ICP-PR have served as a foundation for informing formal regulatory test guidelines and guidance documents of the Organization for Economic Cooperation and Development (OECD) and have contributed to global harmonization of testing and assessment methods. The composition of the ICP-PR Bee Protection Group provides a means of effectively ring-testing testing methodologies to ensure that they are compliant with international good laboratory practice standards prior to their consideration and testing at the OECD level.

The ICP-PR Bee Protection Group consists of multiple subgroups (*i.e.*, Brood Testing, non-*Apis* Bee Testing, Semi- and Full-field Testing, Microbial Testing, Monitoring, and Risk Assessment/Management) which meet independently to advance testing and assessment methods.

About the 15th International Symposium of the Bee Protection Group in York

Although in the past, the symposia of the ICPPR Bee Protection Group have been organised principally every three years, following the 13th International Symposium in 2017 in Valencia, Spain, a decision was made to place the symposia on a 2-yr cycle. This decision was based on the understanding that a more frequent meeting would better assist the developmement of methodologies to address testing needs and regulatory requirements. Although, the symposium in Valencia was difficult to match, the 14th International Symposium in Bern, Switzerland, at the Agroscope Swiss Bee Research Center proved to be a resounding success. Who would have thought though, that with a short period of time, the world would be thrust into a pandemic as the result of COVID. Although Fera Science Ltd. had generoulsly agreed to host the 15th International Symposium at their Biotech Campus in York, UK, although a lot of effort had already been undertaken for an event in 2021, the notion of a 2-yr cycle had to be abandoned due to uncertainties regarding possible travel restrictions resulting from the virus. While the Steering Committee considered the option of having a vitual meeting to keep to the 2-yr cycle, the importance of direct interactions outweighed the electronic option and the symposium was delayed a year. FERA Science staff members, principally Selwyn Wilkins and Claire Boston-Smithson, worked tirelessly to organize the symposium in York for autumn 2022, even though there were major uncertainties at the time of planning whether the pandemic would settle into remission. Despite challenges, the symposium held on 18 - 21 October 2022, included about 129 participants from 12 European, 2 South American and 2 North American countries; **Figure 1** depicts a group shot of participants on the grounds of Castle Howard Estate.

Figure 1 *Participants in the 15th International Symposium of the ICP-PR Bee Protection Group on the Grounds of Castle Howard Estate and Gardens, York UK.*

The syposium started with welcoming remarks from Dr. Jens Pistorius and was opened by Andrew Swift and David Phillips of Fera Science. The first platform session included presentations by Leon van der Wal of the Organization for Economic Cooperation and Development (OECD), followed by William Garthwaite of the Food and Agriculture Organization (FAO) of the United Nations, and by Sofie Hoefkens of the European Commission. These presentations on possible collaborations with international organizations were then followed by a series of presentations regarding the updated guidance on bees by the European Food Safety Authority (EFSA).

The symposium included three and a half days and a total of 23 scientific oral presentations of 20 minutes each along with 16 poster presentations. Plenary sessions consisted of presentations on Non-Apis bees, Risk Assessment, Lab/Semi-Field/Field studies, and Mirobial Testing. In the afternood of Day 2, participants were treated to a tour of Castle Howard Estate and Gardens followed by a medieval banquet at the Merchant Adverturer´s Hall.

The opportunities to learn about research progress made during the "*time of COVID*"and to share some long-needed scientific and personal exchange helped make this an outstanding symposium.

Country	**Participants**	**Country**	**Participants**
Austria	2	Netherlands	4
Belgium	5	Norway	1
Brazil	8	Slovakia	1
Canada	3	Spain	8
France	9	Switzerland	6
Germany	45	United Kingdom	25
Ireland	1	Uruguay	1
Italy	3	USA	3
		Total	129

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EFSA Bee Guidance

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

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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. 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 respecting the protection goal as agreed by the risk managers. A series of presentations explained the most important changes compared to the 2013 version and the main characteristics of the reviewed guidance document.

Keywords: EFSA, Pesticides, Risk assessment

Introduction

In 2013, the European Food Safety Authority (EFSA) issued its bee guidance document to be considered for the pesticide regulation (Regulation (EC) No 1107/2009). However, due to some concerns from stakeholders and MSs, this guidance has never been fully implemented. Therefore, in 2019, the European Commission tasked EFSA to review its 2013 Guidance Document.

The specific protection goal (SPG) – which forms the basis of the risk assessment – was rediscussed by the risk managers. Risk managers agreed in all aspects of the SPG for honey bees, but the acceptable magnitude of effects for bumble bees and solitary bees is still subject for further discussions. The EFSA guidance presented here, fully respects all the agreed elements of the SPG as set by the risk managers.

Material and methods

The review was performed by a working group that consists of experts from academia, regulatory experts from EU member states and EFSA staff specialised in different professional disciplines. Experts from other sectors were invited as hearing experts for specific issues. A large number of studies from the open literature as well as studies from regulatory dossiers have been considered.

Results

In the review process, which is still ongoing, apart from the risk related to dust drift, nearly all aspects of the guidance have been reviewed. The most important exposure routes, such as the contact (e.g. direct overspray) and the dietary (via pollen and nectar consumption) are covered.

Oral Presentation

Already at Tier 1, a number of scenarios and risk cases (acute and chronic risk to adults, risk to larvae) are considered for the three bee groups (honey bees, bumble bees and solitary bees). The mathematical models to describe those exposure routes have been revised and reparametrized. Revision of the key parameters was supported by literature reviews. Guidance has been developed for appropriate refinement options for many of the parameters (Tier 2 risk assessment).

The characterization of the hazard was intensively reviewed. Unlike any other previous guidance, the Tier 1 hazard is no longer represented by a single endpoint. In contrast, the full dose-response curve is considered. Since standardized laboratory test methods are not available for all risk cases for bumble bees and solitary bees, the method to extrapolate toxicity information from honey bees to other bee species was also reviewed and further developed.

The method for risk characterization was significantly revised. In the proposed method for Tier 1 and Tier 2, the estimated level of exposure is combined with the full dose-response to calculate the predicted level of effect for each of the risk cases. Then, in a later step, the predicted level of effect from each risk case and from both exposure routes (oral and contact) are combined, resulting in an overall predicted effect for the colony or population (effect on colony strength or population abundance, i.e. decrease in the number of individuals). For honey bees, this predicted effect level can directly be compared to the agreed SPG, since the SPG also relates to the effect on the colony. Similar predictions can be made for bumble bee colonies or solitary bee populations. However, since the acceptable magnitude of effects is not yet defined for those bees, the acceptability of the predicted effect cannot be defined by the risk assessors.

Specific aspects were reviewed. The new document includes comprehensive guidance for chemicals prone to time-reinforced toxicity, for sublethal effects, for predicting the toxicity of mixtures (i.e. PPP with two or more active substances) and guidance for metabolites.

As in all risk assessment schemes, the lower tier methods include a number of worst-case assumptions and worst-case parameter values. Therefore, the risk might be further addressed with higher tier studies. This will be particularly relevant for bumble bees and solitary bees, because in lack of a comprehensive SPG (i.e. without the decision on the acceptable magnitude of effects), it is expected that definitive conclusions from lower tiers will less frequently be available than for honey bees. The preferred option is to conduct field tests, but for certain conditions, semi-field or colony feeder tests might be considered as surrogate test methods. In any case, the power of the test is a crucial issue, since alignment with the SPG can only be guaranteed if sufficient power is demonstrated. Guidance on the statistical analysis and recommendations for the test design of the higher tier tests are given in the document.

An overview of the number and general content of the comments received during the consultation process on the draft version of the guidance document was given in the presentation.

References

EUROPEAN FOOD SAFETY AUTHORITY, 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, 268 pp., doi:10.2903/j.efsa.2013.3295

REGULATION (EC) No 1107/2009 Of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

Oral Presentation

Section 1. - Non-Apis bees

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

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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 instance for the genus *Osmia*, are currently inadequate and face numerous obstalces. A major concerning being inadequate feeding methods. Thus, unlike the acute oral test systems for honey bees (OECD Guideline 213) and for bumblebees (OECD Guideline 247), there is still a lack of a guidance document for solitary bees. Here, we propose a novel testing system for an acute oral toxicity test using the model organism *Osmia bicornis*. To both improve feeding success and ensure that bees ingest the desired amounts of sucrose solution within a short period of time (e.g., within 4 hours), we tested a novel cage design and feeding device and subjected bees to a training period prior to testing to increase feeding success. Compared to Nicot cages, the use of our novel transparent cages that had an increased volume and pipette tips as feeding devices greatly improved acute oral dosing and reduced evaporation of the test substance. Furthermore, control mortality in the control group was low (11.8%), monitoring of bee bahavoiur and handling was simplified which reduced stress on bees as well as decreased labor. Ultimately, our novel method appears a promising approach for testing acute oral toxicity in solitary bees, yet additional studies are required to confirm our findings.

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

Introduction

Solid laboratory-based acute oral toxicity test guidelines are urgently required to evaluate of the risks of plant protection products and their active ingredients on solitary bee species, e.g. on the genus *Osmia*. Despite several attemps to develop and ring test such a testing system, the finalization of a robust guidline has yet to be established. In contrast to the acute oral test systems used for honey bees (OECD guideline 213) and for bumble bees (OECD guideline 247), which have both successfully been implemented, the protocols developed for solitary bees are currently inadequate and face numerous obstacles. For instance, the low and highly variable consumption success rates as well as the insufficient consumption of the tested substances reflects to major concerns. In order to improve both the feeding success as well as to ensure that bees consumed desired volumes within a short period

(e.g. up to 4h for acute dosing) of sucrose solution, we tested a novel cage design and feeding device, as well as subjected the bees to a training phase prior to the test to increase feeding performance.

Material and methods

Female and male *Osmia bicornis* cocoons were kept together in a flight cage [150 ♀ & 300 ♂) for three days at RT and indirect natural light, in order to enable hatching and mating. Bees in the flight cage were provided with sucrose solution (30 % w/v) *ad libitum* in a 5 mL disposal syringe. After three days, single females were then transferred into individual cages (round transparent plastic cages (bella plast 100 cm^3) and offered sucrose solution *ad libitum* in a pipet tip 250 µL (Rainin RT-L250WS wide orifice tips) and kept for 48 h. This was considered as "training phase", and thereafter the pipet tip was removed and the bees starved for 18 h. Only bees that have clearly consumed a certain amount of sucrose solution during the training phase (i.e., trained feeders) were used for the subsequent acute oral feeding test. Four treatment groups with each 16 to 17 test bees were established, in which each bee was offered 25 µl in a pipet tip of: 1.) sucrose solution (30 % w/v) (control group), 2.) 0.15 µg dimethoate/bee, 3.) 0.45 µg dimethoate/bee or 4.) 1.35 µg dimethoate/bee in sucrose solution (30 % w/v). The weight of the pipet tips was determined before and after a four-hour exposure phase to assess the ingested amount of feeding solution, hence to calculate the exact intake (dose) of Dimethoate per bee. Additionally, the feeding success was assessed visually after one, two, three and four hours. Calculations for the oral toxicity test were based on ingested doses and the oral LD_{50} and their 95% confidence limits for dimethoate was calculated by Probit analysis.

In parallel we compared the evaporation loss of the Nicot® cups (currently suggested feeding method by other laboratories) and the pipet tip feeding device used here in our experiment (Fig. 3). Therefore, twice ten of each feeder were filled with sucrose solution (30 % w/v) and kept under the same laboratory conditions as the caged test bees. To account for the evaporation, the weight of each feeder was assessd before and after 4 hours.

Results

Our results showed an equal feeding success rate (complete ingestion) of 75 to 88 % of the control group sucrose solution (30 % w/v) and the two lower dimethoate concentrations after 2 hours (Fig. 1). In the group with the highest dose of dimethoate (1.35 µg dimethoate/bee), only 25% of the bees fully consumed the 25 µl of spiked sucrose solution within two hour (Fig. 1). There was no change in feeding success after 2 hours of exposure compared to 3 hours in all treatment groups. Survival rate after 96h oral exposure phase was 88.2%, 75%, 6.2%, and 6.2% in the control group and the treatment groups with 0.15, 0.45, and 1.35 µg dimethoate/bee, respectively (Fig.2).

A statistically significant dose /response was found ($p<0.05$). Base on the calculation the LD_{50} -24h of *O. bicornis* was determined to be 0.140 µg dimethoate/bee (Table 1), which, is within the recommended LD_{50}-24h range given in the OECD 213 guideline for honey bees (oral LD_{50}-24h range 0.10-0.35 µg a.i./bee).

The evaporation observed in nicotine cups after 4h was significantly higher with a mean evaporation of 6.5 % compared to 1.9 % in the pipette tips tested (Fig. 3).

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Table 1 *Calculation of LD_{50} dose of Dimethoate by Probit analysis in an acute oral feeding test for O. bicornis.*

ToxRatPro Probit-Analysis:		
LD_{50}	**Dimethoate (µg/bee)**	Lower/upper 95%-cl
24h	0.140	(0.001-0.501)
48h	**0.114**	(0.002-0.338)
72h	0.084	(0.002-0.229)
96h	0.084	(0.002-0.229)

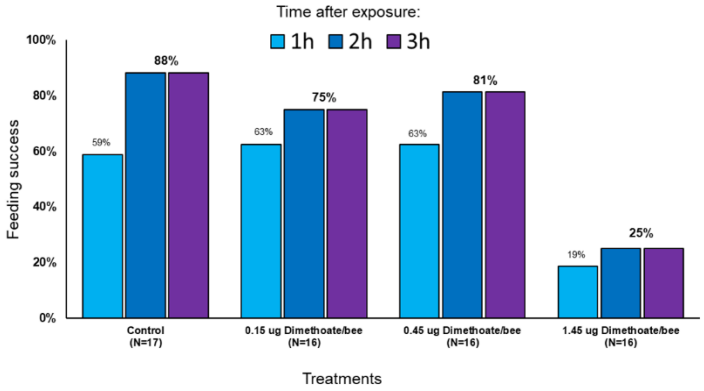

Figure 1 *Feeding success (complet consumption) in treatment groups after 1, 2 and 3 hours.*

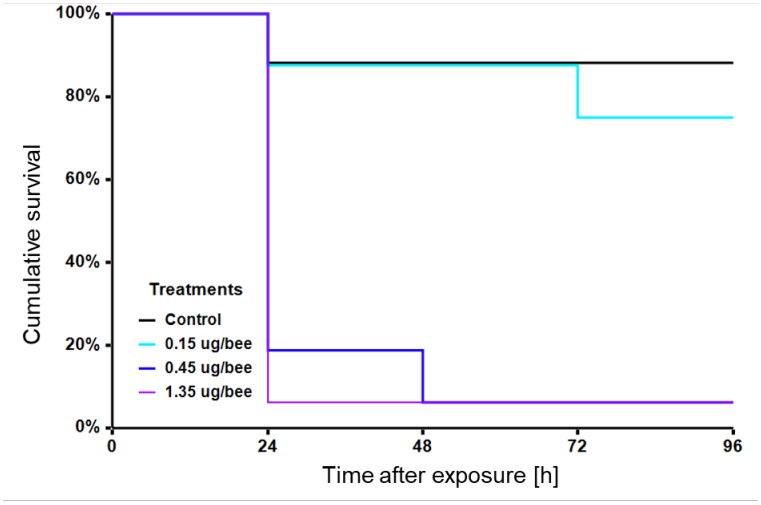

Figure 2 *Cumulative survival (%) 0 - 96 h*

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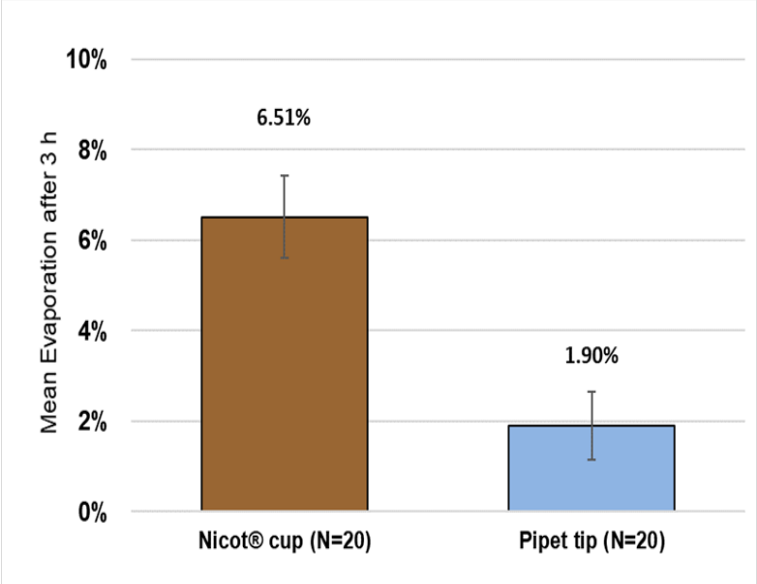

Figure 3 *Evaporation of feeding solution after 4 hours in two diffent feeders: Nicot cups and in pipet tips.*

Discussion

With regard to our preliminary pre-tests and final test, a "training phase" of the bees' feeding system (pipette tip) and selection of bees (successful feeders), as well as a starvation phase prior to the definitive test solution, seems to be the key to ensure high and consistent feeding success rates in our acute oral test. A similar pattern was observed in Knautz et al. (2022), where *O. bicornis* bees were also trained prior to their use in an acute oral test.

In contranst to Nicot cages, the use of transparent cages with larger volumes and pipette tips as a feeder greatly simplified handling and monitoring of the bees. Furthermore, the reduced evaporation of the surcosre solution, which can affect dosage, was several orders of magnitude lower, thus improving the accurarcy (Figure 4).

The preliminary data and results are highly promising, yet further assessments are required to verify our findings. Nevertheless, we would highly encourage and recommend for current and future oral acute toxicity ring tests unsing solitary bee species to consider and apply our new method.

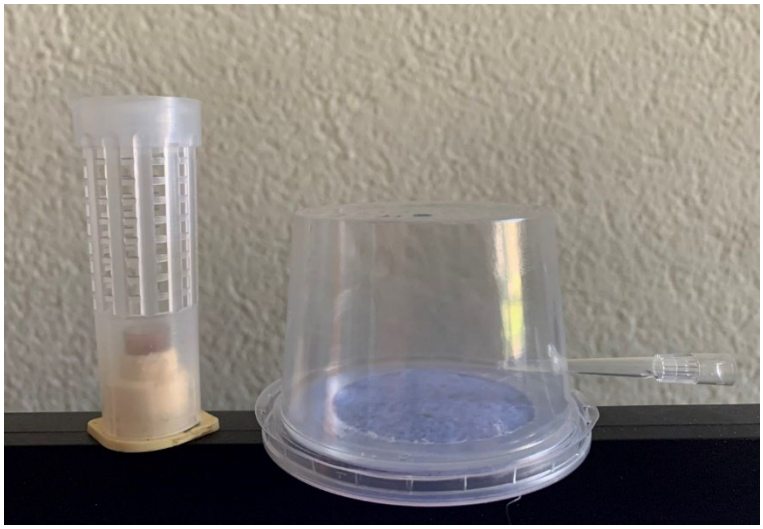

Figure 4 *Left: Assembled Nicot cage with feeder cup, right: clear bell plast cage with pipet tip feeder*

Oral Presentation

References

KNAUTZ, T. , C. BERG, A. BARME, T. SEKINE, 2022: How to feed the Red Mason bee *Osmia bicornis* – Establishing a new feeding technique for the acute oral Bioassay with solitary bees, Poster at SETAC Europe 32nd annual meeting, Copenhagen, 15-19 May 2022.

OECD TEST NO. 213: Honeybees, Acute Oral Toxicity Test, DOI: 10.1787/9789264070165-en.

OECD TEST NO. 247: Bumblebee, Acute Oral Toxicity Test, DOI: 10.1787/9789264284128-en.

DAVIES, R. G. (1971): COMPUTER PROGRAMMING IN QUANTITATIVE BIOLOGY, ACADEMIC PRESS, LONDON.

FINNEY, D. J. (1971): PROBIT ANALYSIS, 3RD EDITION, CAMBRIDGE UNIVERSITY PRESS, LONDON

Oral Presentation

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

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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

Oral Presentation

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

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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

Oral Presentation

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

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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.

Oral Presentation

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

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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

Oral Presentation

Section 2. - Risk Assesment

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

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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.

Oral Presentation

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

Oral Presentation

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.

Oral Presentation

Bee-longing together – Application of BEEHAVEecotox 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).

Oral Presentation

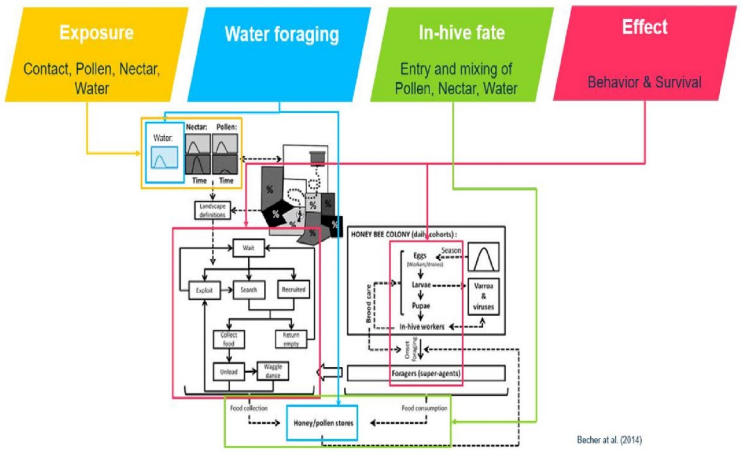

Figure 1 *Flow-chart of BEEHAVE (Becher et al., 2014) and the BEEHAVEecotox additions. Black: original model. Orange: Landscape exposure module. Blue: water foraging module. Green: in-hive exposure module. Red: effect module for survival of different cohorts*

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.

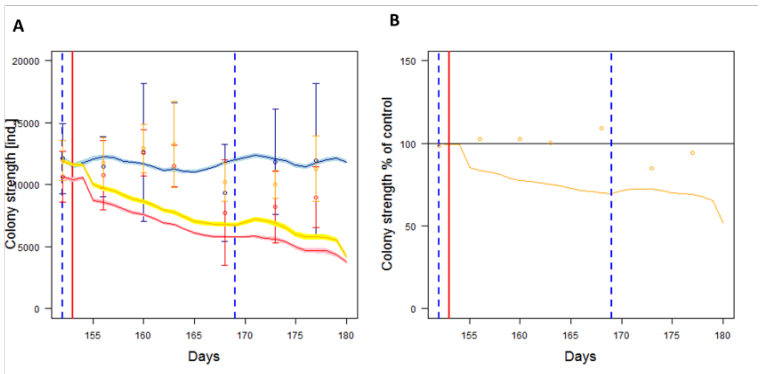

Figure 2 A*: Measured (dots as an average with SD of three hives) and simulated (lines as an average with 95% CI) colony strength in absolute numbers over time for control (blue), a toxic reference (red) and an insecticide (orange) for semi-field study. B: Relative impact of the insecticide on the colony 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.

Oral Presentation

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.

Oral Presentation

Section 3. - Microbials

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*

Oral Presentation

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

Oral Presentation

Section 4. - Laboratory/Semi-field/Field

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

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

Abstract

Repro-toxicity tests reveals a new approach in the assessments of effects to honeybees with the particularity to present a mid-term evaluation and an undirect exposure of individual honeybees. This article follows a previous one since the improvement has not progressed during the pandemic.

The French Methodological Bee Group is committed to provide guidance and protocols to assessors about local or international methodologies. In this working group, 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 reference 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 (in 2016, 2017 and 2021 respectively). The behavior of forager honeybees under tunnels as well as the measurement of HPGs (Hypopharyngeal glands) are still under French 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. As the repro-toxicity concept could cover the fertility, the fecundity, and the prolificacy of the queen in the colony, it has been selected at this stage to consider the drone fertility only.

Drone fertility methodology.

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

Oral Presentation

There were 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 queen less 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) for 20 days similarly to LD_{50} exposure.

About 1000 nurse bees are necessary for the care of 150 young drones and the nurse have to be replaced after on week.

Discussion

Despite the methodology is not finalized yet we already have wondering discussions about the data collection and interpretation of the results. It is unusual to set up a protocol with undirect exposure of the individuals. In this repro-toxicity test the nurse honeybees are exposed to contaminants for feeding the young drones whereas the potential effects are later assessed on the sexually mature drones.

In this way the aim of the protocol remains close to the OECD 75 (Brood impact evaluation) where the forager bees are exposed on flowering crops under semi-field conditions to provide potential effects on the brood development in the beehive.

On the other hand, technically counts of spermatozoids from the mature drones are still under inclusion in the protocol and should be assessed under electronic microscope.

Currently the protocol is not yet finalized but the collection of mature drones is efficient, and the validity criteria are still discussed. A guidance document is 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 influences the sexual maturation of honeybee drones.

Oral Presentation

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.

Oral Presentation

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 flight activity. Furthermore, we describe our setup and show results from 'robbers tests' performed in 2021 and 2022 and find that the ratio of incoming and exiting bees is accurate within (1 ± 0.05) for 31 out of 34 tests (in 2021) and for 15 out of 17 tests (in 2022). 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 (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). Automatic hive monitoring systems, especially bee counters, can provide 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 devices 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 test' 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.

Oral Presentation

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 tests', 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 sensors) 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/May 2021 and in May/June 2022, using a similar set-up as introduced by Struye (1999). For the experiments in 2021 we set up five 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 experiment, bees enter the food source, 'rob' food, and bring it back to their hive. After each test and for each tent dead bees that remained in the box with the food were counted along with the 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 the five hives into two tents (leading to one tent with two and one tent with three hives) 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 has

[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/)

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been analyzed. We derived the accuracy of a counter as the corrected ratio between exiting and incoming bees, namely for a counter at the food sources as

$$\text{accuracy}_{food} = \frac{count_{food,out} + bees_{food}}{count_{food,in}} = 1 + \frac{(count_{food,out} - count_{food,in}) + bees_{food}}{count_{food,in}}$$

and for the counters at the hives as

$$accuracy_{hive} = \frac{count_{hive,in} + bees_{food} + bees_{tent}}{count_{hive,out}} = 1 + \frac{(count_{hive,in} - count_{hive,out}) + bees_{food} + bees_{tent}}{count_{hive,out}}$$

where $count_{food/hive,\ out/in}$ refers to the count of exiting/returning bees at the respective counter and $bees_{food/tent}$ refers the dead bees counted manually at the food source or at the tent floor after each day.

In 2022 we performed 'robbers tests' in three different tents with natural grass floor. Each tent was equipped with one hive and one food source and bee counter were only installed at the entrances to the food sources. The summer honey used in 2021 had stuck to the bees as they had to walk over it (see Fig. 2), leading to accumulated honey on the light barriers that can reduce their reliability. To avoid this, we we used honeycombs as a food source in 2022.

Figure 1 *Setup of the 'robbers tests' in 2021. Left: covering the floor with plastic tarpaulins, which enabeled counting of bees on the tent floor. 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).*

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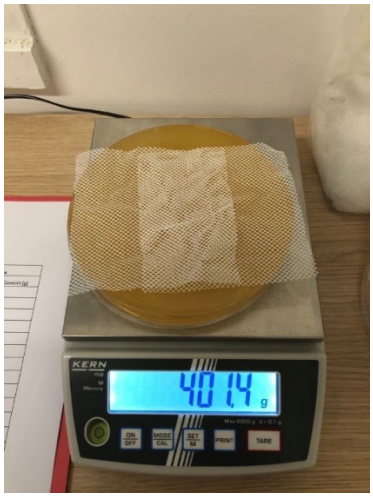

Figure 2 *Bowl with honey used as a food source in the 'robbers tests' during the 2021 season. We used summer honey, which is very bee attractive. As the honey sticked to the bees and the light barriers, we switched to using honeycombs for the experiments in 2022.*

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. 7).

Results and discussion

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 smaller. 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 (r-squared=0.624; p-value of the model<0.001; intercept=208.819 with p-value <0.001; slope=0.397 with p-value<0.001). 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.

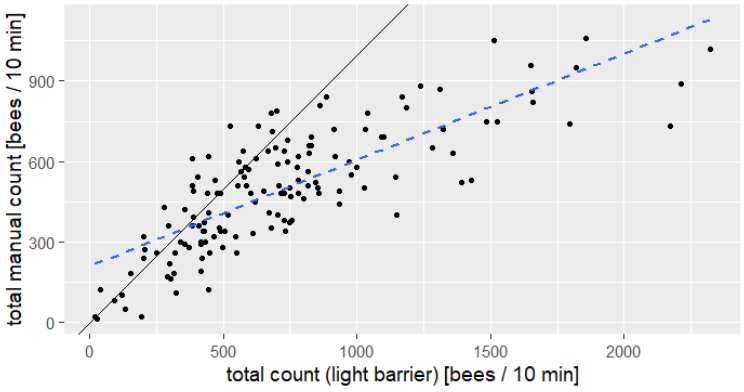

Figure 3 *Data from a trial in April 2020. Total ("in+out") count from the light barriers compared to manual counts at the hive entry, scaled to correspond to 10 minutes of accumulated data. To guide the eye, the black line is the y=x diagonal, while the blue, dashed line is a simple linear fit to the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

Robbers test

We performed series of 'robbers tests' in 2021 and 2022. Fig. 4 shows the data collected during one test in 2021, 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.

In 2021, 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. 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 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 2021 '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 36444 (at the food sources), and between 9708.5 and 99612 (at the hives). For the correction factors (manually counted 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 a 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 or better. The dashed line in Fig. 5 is a linear Ordinary Least Squares (OLS) fit to the data, which returns a slope that is close to zero and not significant, indicating that, in the range of flight

activities we could test with our setup, the accuracy did not depend on the flight activity (r-squared=0.000; p-value of the model=0.986; intercept=0.985 with p-value <0.001; slope=-6.12e-09 with p-value=0.986).

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 dirty, which can reduce their precision. During a repetition of the tests in 2022 we exchanged the bowl with honey against a complete honeycomb, which reduced the dirt significantly. The bees will still mark the entry to the food source with wax, which could limit the functionality of the counter. We note that during a trial with a bee-attractive, flowering crop, there is no entry to a food source and therefore, this kind of dirt would not be an issue.

In 2022 we conducted a new series of tests with only new light barriers in three tents. Each light barrier was tested once, i.e., each dot in the overview (Fig. 6) corresponds to a different counter. We did not exclude any data in 2022 that had been recorded after training the bees and finalizing the technical set-up, and we only installed counters at the food sources. The largest correction factor in 2022 was 80 dead bees (0.25% of the total flight activity) in the food source in tent number two, for the same day and tent a total flight activity of 31593.5 bees and an accuracy of 1.05 was recorded. All 2022 experiments are summarized in Fig. 6, overall, 15 out of 17 tests have an accuracy of 1 ± 0.05 or better.

Interestingly, the accuracy in Fig. 6 also seems to depend on the tent in which experiment was conducted. Results from tent number one are very stable with accuracies very close to one, while the two outliers come from tent number two and the experiments from tent number two tend to count more exiting than entering bees. In tent number three the bees were not flying reliably (hence the flight activity is close to zero for two experiments) and fewer tests were conducted in this tent. Our hypothesis to explain the increased number of exiting bees in tent number two is that the box around the food source was not perfectly sealed and the bees found a hole to squeeze in without going through the counter. We have observed that, if such a hole exists, the bees tend to use it in only one direction, which would explain the systematic shift of the accuracy. A linear OLS fit shows a slightly declining slope with increased flight activity, however the fit of the slope is driven by the two outliers and not statistically significant (r-squared=0.047; p-value of the model=0.401; intercept=1.036 with p-value<0.001; slope=-5.34e-07 with p-value=0.401).

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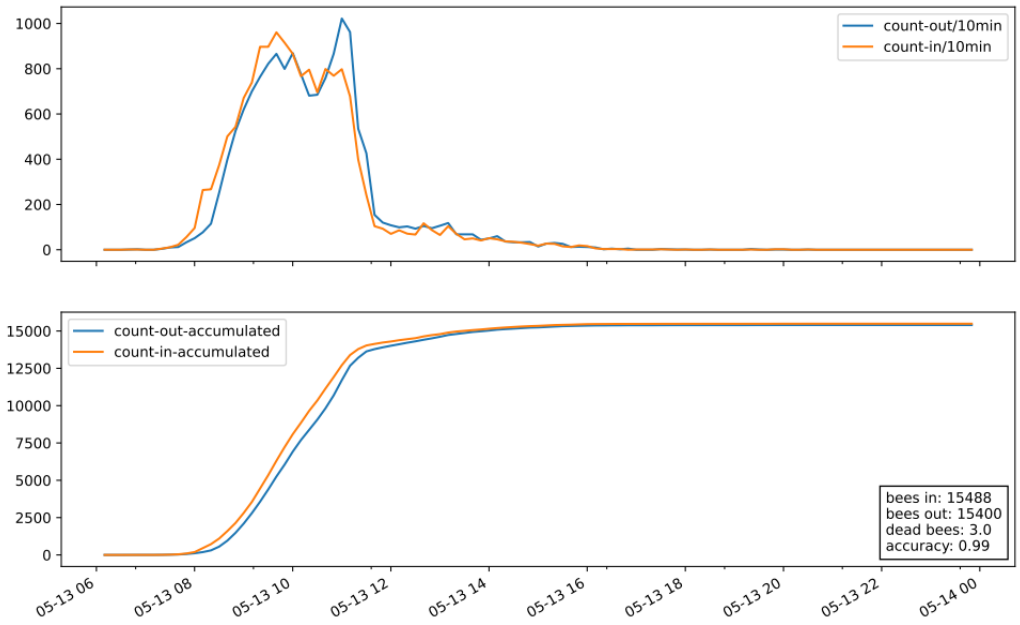

Figure 4 *Robbers test for one counter, the count is from the light barrier at the food source. The upper panel shows the bee count per 10 minutes for incoming bees (orange) and leaving bees (blue). The lower panel shows the aggregated counts.*

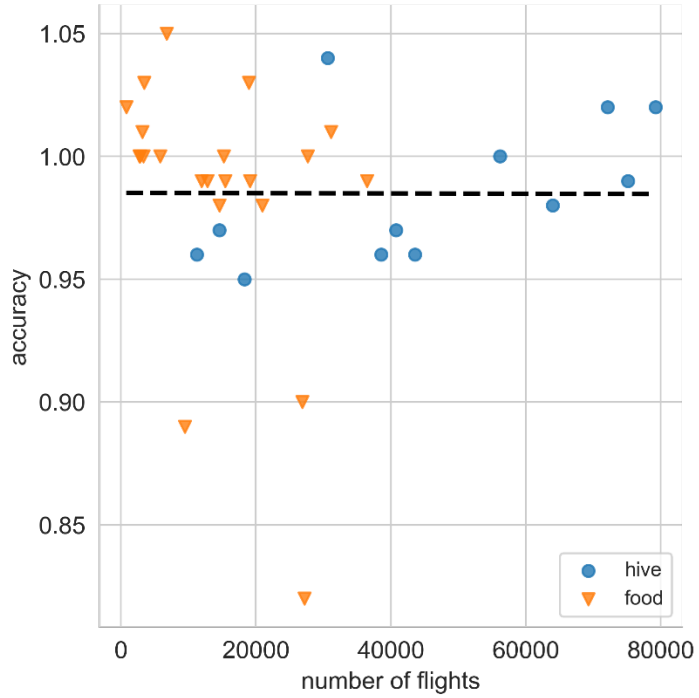

Figure 5 *Overview of the results of our 2021 'robbers tests': the accuracy of each experiment, plotted against the total number of flights ($(count_{in} + count_{out})/2$). Four old light barriers that repeatedly showed large errors have been excluded. Blue or orange color (different markers) indicates whether the count has been taken at a hive or at a food source. As expected, the activity at the hives is higher. The dashed line is a linear OLS fit to the data, which indicates that the accuracy does not depend on the flight activity (see text for details).*

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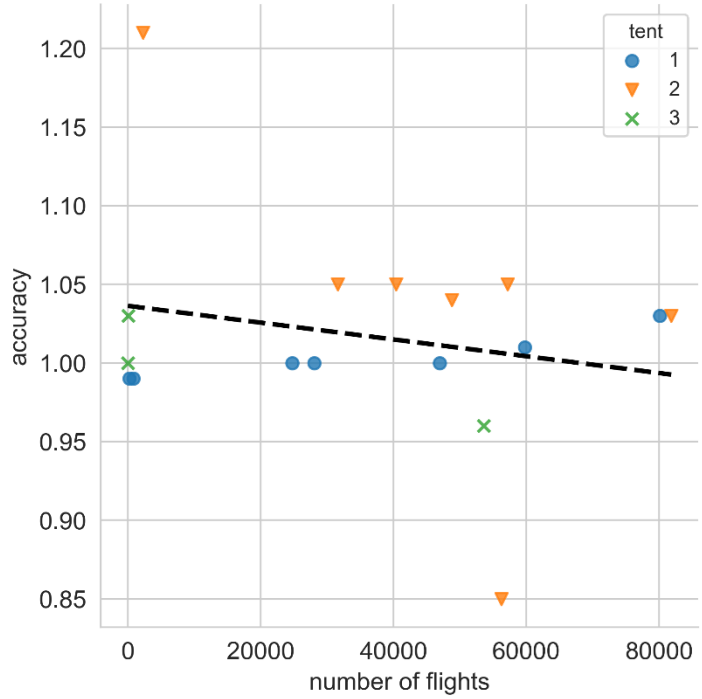

Figure 6 *Overview of the results of our 2022 'robbers tests': the accuracy of the different tests, plotted against the total number of flights ($(count_{in} + count_{out})/2$). In 2022 all measurements have been performed at the food sources and each measurement corresponds to a different light barrier. Different colors (markers) indicate in which tent the experiment has been performed. The dashed line shows a linear OLS fit to the data (see text for details).*

Comparison to count data provided by apic.ai

Fig. 7 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. Only one hive has been plotted for better readibility, which is the same hive as the hive represented by the black line in the upper panel. The grey lines are the 95% confidence interval.

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. For the second hive analyzed by apic.ai (not shown in the second panel) the difference is larger.

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 difference of a factor of two or more in bee activity is not common. As both hives monitored by apic.ai show a higher bee activity compared to the light barriers, we assume that the video-based method tends to return higher counts.

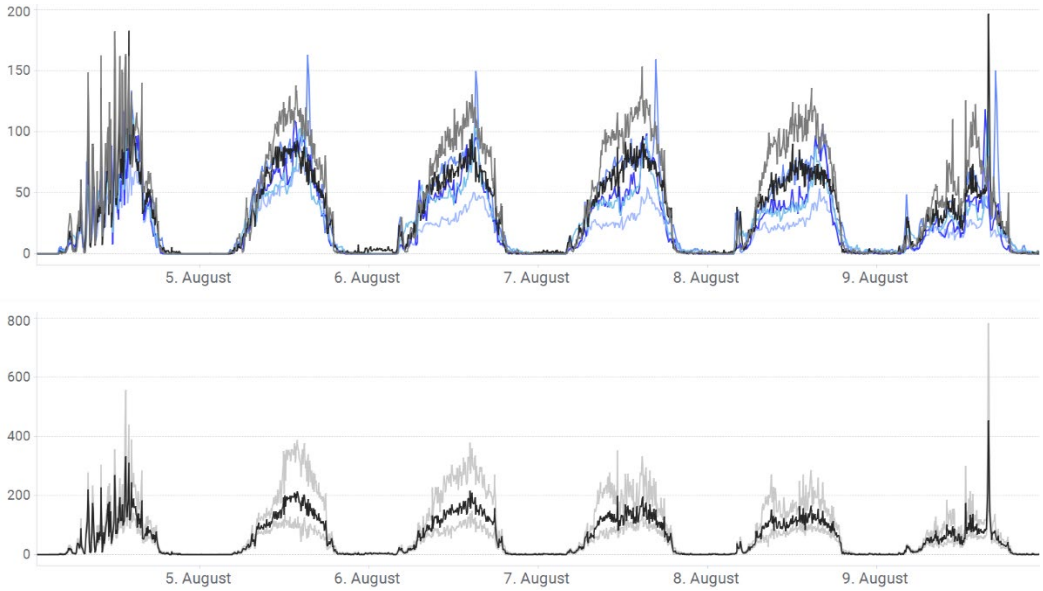

Figure 7 *Count of incoming bees from six hives recorded during a trial in summer 2020. Six days have been selected for readability. The upper panel shows the data from four hives that have been monitored with light barriers (lines in different shades of blue) and raw data from two hives analyzed by 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 interval.*

Conclusion

We have conducted different experiments to assess the accuracy of state-of-the-art light barrier-based bee counters. In 2021 and 2022 we ran series' of 'robbers tests' with the result that an accuracy of 1 ± 0.05 is realistic for the tested counters. Some outliers with larger errors remain (3 out of 34 tests (9%) in 2021 and 2 out of 17 tests (12%) in 2022). It is desirable to understand how these can be removed in the future. In the 2022 series we see a dependency of the accuracy on the tent in which the test was conducted and speculate that the systematically increased numbers of exiting bees in tent number two might be due to a not perfectly sealed box around the food source. This supports the hypothesis that further technical refinements could improve the accuracy. Furthermore, we excluded repeatedly failing light barriers in 2021, but could use the complete data set in 2022, showing that we could improve the set-up. The honey used as a food source in 2021 caused a lot of dirt on the light barriers, which has been improved in 2022 by using honeycombs and would not occur in a trial with a flowering crop. Using new devices or cleaning and checking the light barriers carefully before each trial will increase the quality of the results. We do not see a decrease in accuracy with higher flight activity, however, we note that the 'robbers tests' can only measure the error on the ratio between incoming and exiting bees. Systematic errors affecting the count of exiting and returning bees in the same direction cannot be detected. A first comparison to video-based counts suggests that, at high flight activities, the light-barriers might miss more bees than the '5% or better' suggested by the 'robbers tests'.

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Comparing the light-barrier with the video-based counters, we conclude that the choice of the technology will depend on the specific research goal. The light barriers are easy to handle, and the returned data can be analyzed without the need for complex algorithms. Consequently, it is feasible to scale the set-up to observe larger numbers of hives. On the downside quantifying the error on the absolute counts is currently not possible. The more complicated, video-based counters can quantify these errors and have the potential to further increase their accuracy via algorithm improvements.

Due to the errors discussed above and the large number of bee flight per day, directly assessing the bee mortality (as specified in the EPPO guideline No. 1/170 (4)) with the counters is currently not feasible. Still, bee counters can add value to bee trials as they provide the continuous flight profile over time and can help us to understand environmental influences on the trial. Furthermore, knowledge of the flight activity of the individual hives in a trial can be used to detect outliers and improve the trial design.

Acknowledgements

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References

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION, Pp 1/170 (4): Side-effects on honeybees, Bulletin OEPP/EPPO Bulletin 40 (2010), pp. 313-319

A. E. LUNDIE, The flight activities of the honeybee, United States 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 (2021), pp. 73-89

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

Oral Presentation

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.

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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 an 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.

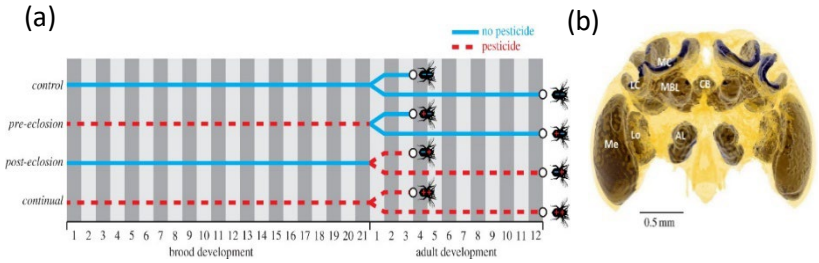

Figure 1. *Panel a) Graphic showing the developmental and exposure periods of individuals inside colonies for the four colony treatments (control, pre-eclosion, post-eclosion and continual) and the eight cohorts of workers tested. 'Brood development' represents the larval and pupal stages of workers, 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.

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(a)

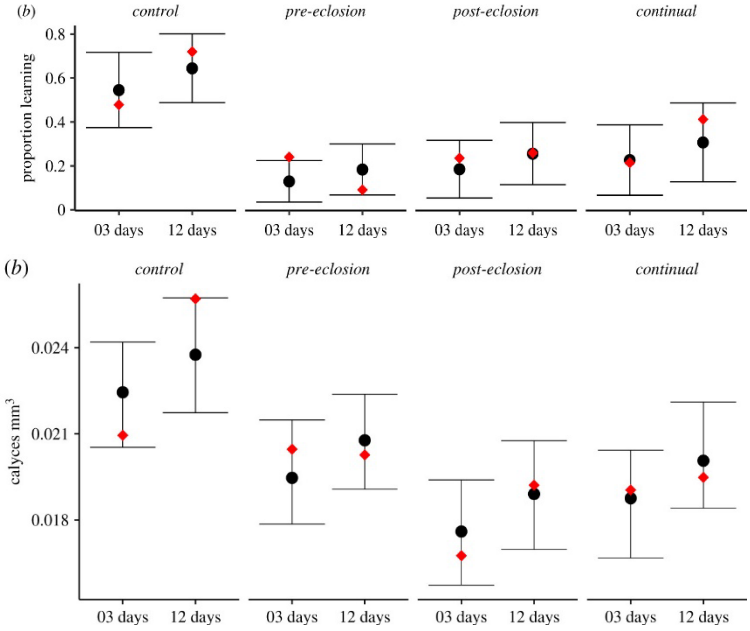

Figure 2 *Panel a) Proportion of learners between treatments. Sample sizes of 3- and 12-day worker cohorts was: control = 23 and 25, pre-eclosion = 25 and 33; post-eclosion = 17 and 27; continual = 14 and 17. Panel b) Relative volumes of bumblebee worker mushroom body (a) calyces, Sample sizes of 3- 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.

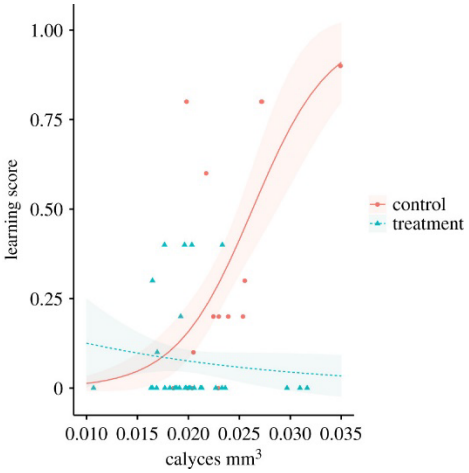

Figure 3. *Relative mushroom body calycal volume plotted against the respective worker's learning score. Workers from all three pesticide treatments were pooled (blue triangles, n= 29; pre-eclosion = 11, post-eclosion= 12, continual= 6) and compared against controlworkers (red circles, n= 15), with fitted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

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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.

Oral Presentation

Observation of Repellence Effects on Honey Bees regarding their Activity and Pollen Collection Behaviour under Semi-Field Conditions with an automated bee counter

Beobachtung von Repellent-Effekten auf die Aktivität und das Pollensammelverhalten auf Honigbienen unter Halbfreilandbedingungen mit Hilfe einer visuellen Bienenmonitoring Technologie

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Abstract

New technologies such as bee counters and other monitoring devices/equipment offer the possibility to answer questions that can help us understand the life history of bees better.

Including continuous information on honey bees' behaviour during semi-field studies would help to increase the understanding of their results. For this purpose, 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 in two cohorts of individually marked bees. A study was conducted to determine the repellence effect on honey bees under semi-field conditions using automated bee counter in parallel with classic assessments comprising 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, among which each treatment group contained 4 hives and the other six colonies served as a control group. For the exposure, 40 m tunnels of Phacelia were used. The hives stayed 17 days in the tunnels, until the end of flowering. Observation continued for a period of 14 days at another monitoring site, where bees could forage freely.

The aim for the study was to use two different active ingredients to see which influence they have on the activity and pollen foraging. A further aim for the study is 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 systems model BEEHAVE.

Keywords: bees semi-field study, automatic counter, repellence, artificial intelligence, visual, monitoring

Zusammenfassung

Neue Technologien wie automatische Bienenzähler eröffnen die Möglichkeit den Einfluss von Pflanzenschutzmittel auf Bienen besser zu verstehen.

Die Einbeziehung kontinuierlicher Informationen über Honigbienen während Halbfreilandstudien könnte helfen, das Verständnis für Effekte innerhalb des Versuches besser zu verstehen. Zu diesem Zweck sind apic.ai und EAS Ecotox Partner bei der Verbesserung einer visuellen

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Bienenüberwachungstechnologie im Forschungsprojekt OCELI (FKZ 281C307B19). Innerhalb dieses Projektes wurde eine Halbfreilandstudie durchgeführt. Das apic.ai-Monitoringsystem mit Computer Vision Technologie wurde eingesetzt, um die Aktivität und den Polleneintrag auf Volksebene und in zwei Kohorten von einzeln markierten Bienen zu erfassen. Dafür wurden Individuen mit Königinnenmarkern versehen. Die erste Kohorte waren Sammlerinnen und die zweite Kohorte frisch geschlüpfte Bienen.

Ziel der Studie war es, den Einfluss von zwei verwendeten Wirkstoffen auf die Aktivität und die Pollensuche der Bienen sichtbar zu machen. Ergänzend wurden klassische Erhebungen, wie Kolonieschätzungen, Gewichtsermittlung der Bienenstöcke, Flug und tägliche Mortalität, durchgeführt. Die Studie fand im Juli/August 2022 in Deutschland statt. Für die Exposition wurden 40-m-Tunnel mit *Phacelia* verwendet. Die Applikation erfolgte während des Bienenfluges. Die Bienenstöcke blieben bis zum Ende der Blüte im Tunnel (17 Tage).

Die Beobachtung wurde für weitere 14 Tage im Feld mit freiem Zugang zu Nahrung fortgesetzt.

Ein weiteres Ziel der Studie ist es, Informationen beizusteuern, die zur Validierung des Modells BEEHAVE verwendet werden können.

Stichwörter: Lebenszyklus, Bienen, Zählgerät, Künstliche Intelligenz, Monitoring

Introduction

The honey bee is an important beneficial insect due to its pollination activity in fruit, berry and seed growing. Honey bee colonies involved in (migratory) beekeeping for the purpose of providing pollination services are a significant and valuable component of a productive agricultural sector. Additionally, they contribute to the preservation of a multitude of flowering plants because of their high constancy in pollination activity. However, conventional agricultural practices require the application of plant protection products (PPP) to keep the yield at a constant level. Therefore, it´s necessary to assess the effect of PPP on the health of bees. The first testing requirements were introduced more than 30 years ago. They are constantly improved and developed further.

At the moment, there are fast advances in the technology available for bee monitoring. There is a strong desire to enhance the knowledge creation on bee health and to get a better understanding of how available resources of the surrounding landscape contribute to bee health. New technologies such as automated bee counters and other monitoring equipment carry a vast potential to gain insights into these questions and improve the risk assessment of PPP. They make it possible to move from sample-assessments of individual time points to continuous observations.

The objective of the study was to determine the repellence effect of *Lambda*-Cyhalothrin (Pyrethroid) and the effect of a low dosage of Thiamethoxam (Neonicotinoid) on the honey bees (*Apis mellifera* L.). For this purpose, classic testing as well as automated bee monitoring were used for studying effects on activity, pollen collection behaviour and life history traits under semi-field conditions in *P. tanacetifolia* in Germany.

Material and methods

The study was conducted using experimental hives in the south of Germany. There was a control treatment applied with water, a treatment with Thiomethoxam (applied at a rate of 5 g a.i./ha) and a treatment with *Lambda*-cyhalothrin (applied at a rate of 10 g a.i./ha). Each treatment was replicated four times, the control group consisted of six hives. Healthy and queen-right bee colonies were used.

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The hives contained one body with 10 frames and 8,000 – 10,000 honey bees, at least two brood combs with eggs, larvae or capped cells and at least 1 comb containing honey and/ or pollen.

Colonies were free of symptoms of nosemosis, varroosis or other bee diseases. Honey bee colonies were checked to ensure the presence of all brood stages and of an egg-laying queen.

The crop used was *Phacelia tanacetifolia*. The crop was covered with a tunnel. Each tunnel covered an area of 200 m² (5 x 40 m), with approximately 182.52 m^2 of crop and an area of 17.28 m^2 covered with linen sheets. The colonies were moved into the tunnels 3 days before application.

Foliar application with calibrated boom sprayer took place according to local agricultural practice during bee flight. After exposure, the honey bee colonies remained in the tunnels for two weeks before they were relocated to another monitoring location in the field (in the evening after daily honey bee-flight on 14DAA) following a last assessment of mortality, foraging activity and behaviour. Control C and the test item treatment groups T1 (Neonicotinoid-treated) & T2 (Pyrethroid-treated) colonies were placed at the same monitoring site.

The second monitoring site was an area which provided sufficient food sources for the bees to forage on (e.g. wild flowers) but had no intensive agriculture and no flowering main crops in the near surroundings, which might have been attractive to honey bees.

The number of dead bees within the crop area (on the linen sheets) and in the dead bee traps at the entrance of the hives in the tunnels was assessed daily (once almost at the same time of the day before noon) with the exception on the application day where multiple assessments at 2, hour, 4 and 6 hours after application and in the evening after bee flight were carried out. During the assessment period, dead bees (including pupae and larvae) were counted on the linen sheets, in the dead-bee trap and on the bottom drawer inside each hive and afterwards removed. Dead honey bees were differentiated into adult worker bees, pupae and larvae in the raw data and were summarized (one value per replicate and assessment). Dead male bees and male brood were also recorded in the raw data, but were excluded from the evaluation of mortality.

The flight intensity of honey bees was assessed in the crop during the tunnel phase. At each assessment, the number of bees that have been both foraging on flowers and flying over the crop were counted. Three random flight observation areas of 1m2 were chosen in each tunnel and the number of foraging bees were counted for a duration period of 10-15 seconds per assessment. Flight assessments were performed once a day except on the day of application and the following day where 6 assessments (shortly before application, twice within the 1st hour, 2-hour, 4-hour and 6-hour after application) and three assessments (in the morning, noon and afternoon) were performed, respectively.

Colony assessments were carried out five times during the study (before the start of the study, 8, 15, 21 and 28 days after the application) according to the Imdorf & Greig (1999) and Imdorf et al. (1987).

apic.ai hive monitoring systems were placed in front of the hives. They started counting on the day before application and continued until the last assessment. The monitoring systems generated data from the movements and features of the bees, which they detect visually. The technology consists of both hardware and software components. The systems are powered by solar panels with an included backup battery to insure operability for several days in times without sun or in case of bad weather.

When entering or leaving, all bees are moving through an illuminated area where they are captured by a camera. Between sunrise and sunset white light is used to illuminate the yield of view through

which the bees enter and leave, during the night-time the systems switch to red light in order to not interfere with the natural diurnal rhythm of the colonies.

The monitoring systems continuously analyse image data from the camera field of view. This is done in an automated process using neural networks. The systems can distinguish bees entering or leaving through the direction of their movement. Bees moving from the inside to the outside or vice versa are counted as leaving or entering bees. Bees turning around within the camera's field of view and going back the direction they came from aren't counted.

During the trial, the camera stream from the monitoring devices was analysed continuously in real time. The analysis results were stored on external storage devices. After the end of the study, the raw data was collected for further analysis and storage.

Data was generated on:

Bee activity

Activity describes the number of bees entering and leaving. For the activity, the end results are the number of bees entering and leaving in changeable time intervals, such as per 5 minutes or per day.

Pollen collection

Pollen foraging behaviour is measured by detecting foragers which carry pollen into the hive. Both the total number of pollen foragers and the share of pollen foragers among all bees entering the hive are recorded.

Simultaneous to the storage of the raw data, the measured data on activity and pollen collection was sent to apic.ai via the internet for the purpose of monitoring that the devices functioned and operated successfully.

Results

Weather Conditions

The weather conditions during the study period are shown in Figure 1. The temperature ranged from 8.4 °C to 37.5 °C with rainfall observed on 4 days throughout the study period.

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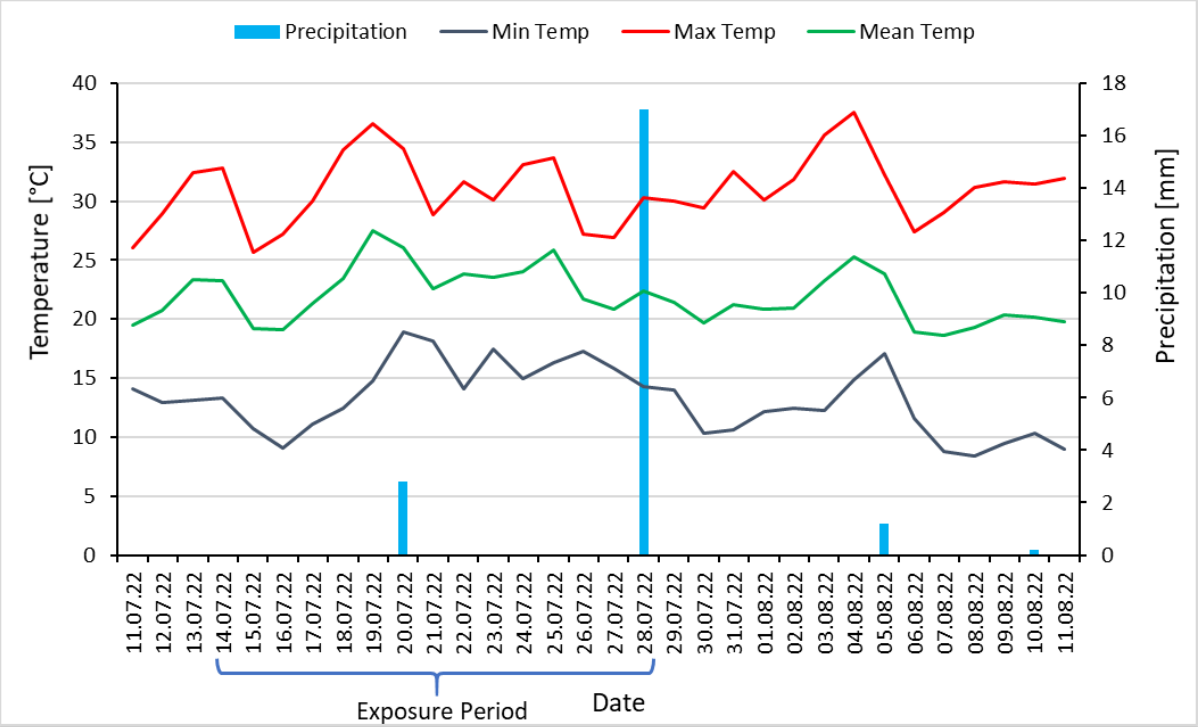

Figure 1 *. minimum, maximum and average temperature and daily rainfall during the study period. Application was done on 14 July 2022 and the colonies where in the tunnels until 28 July 2022 before being relocated to the monitoring site.*

Mortality

The mortality of adult worker honey bees during the tunnel phase is presented in Figure 2. Statistically significantly high mortality was observed for both Neonicotinoid and Pyrethroid treated hives compared to the control on the day of application (Dunnett's t-Test, $p \leq 0.05$). Majority of the observed adult worker bee mortality was in-hive mortalities on the day of application (dead bee trap and bottom drawer). A statistically significantly high mortality was also observed in Pyrethroid treated group the following day after the application and in Neonicotinoid treated group 13 days after the application (Dunnett's t-Test, $p \leq 0.05$). However, the high mortality observed on 13 days after application in Neonicotinoid group does not seem to be test item related. The incremental increase of mortality towards the end of the tunnel phase within all treatments is due to the tunnel effect and maturing of the crop.

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The mortality of adult worker bees after relocation to the monitoring site was much lower than in the tunnel and comparable among all treatment groups. No statistically significant differences were observed on any assessment day (Figure 3).

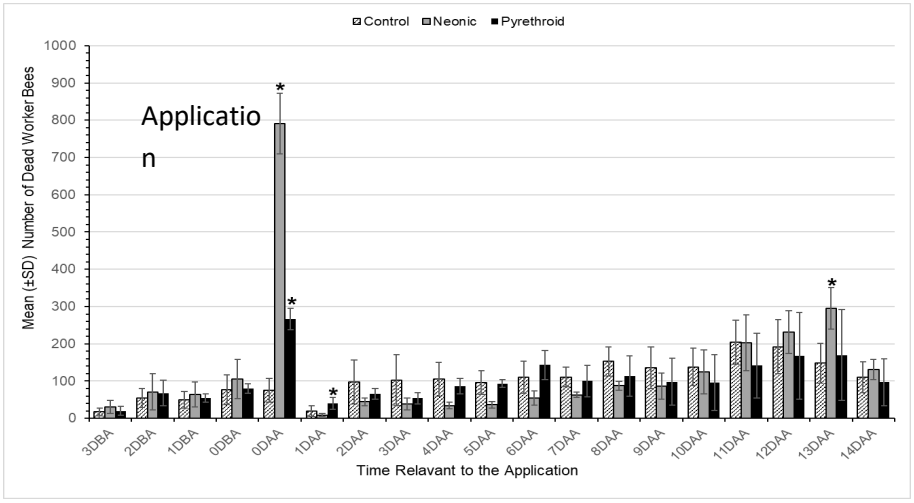

***Figure 2**. Mortality of adult worker honey bees during the tunnel phase. DBA/DAA represents days before/after the application.*
**statistically significantly different than the control group (Dunnett's t-Test, $p \leq 0.05$).*

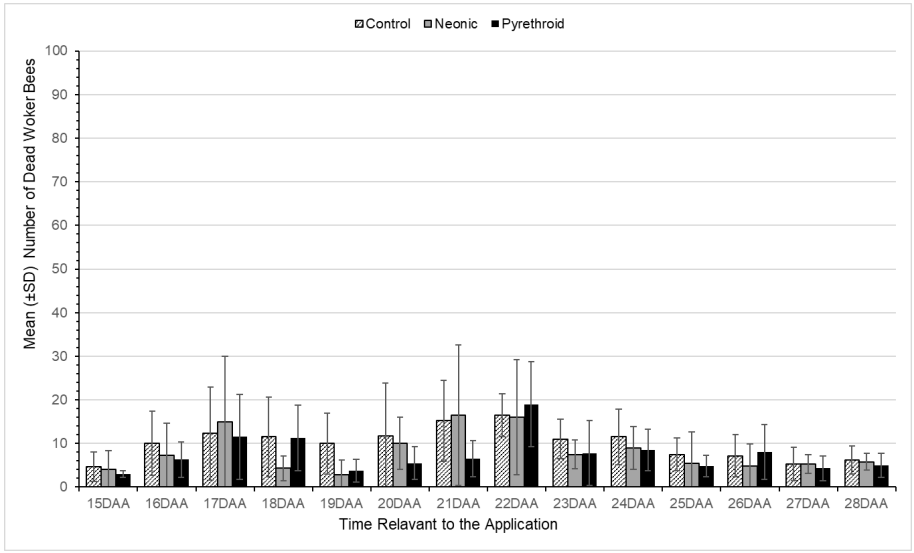

***Figure 3**. Mortality of adult worker honey bees at the monitoring site. DBA/DAA represents days before/after the application. No statistically significant differences were observed.*

Figure 4 depicts the activity of the honeybees entering and exiting the hives on the day of application, recorded and averaged by the apic.ai system over a time interval of 5 minutes. A clear and strong repellence effect of the Pyrethroid treatment group can be observed in Figure 4. The data indicates that honey bees returned to the hives immediately after the application in the Pyrethroid treated groups and their activities stopped completely for a duration of about 1 hour after application. It returned to regular level after about three hours. The repellence effect observed for the Pyrethroid group can explain the lower mortality compared to Neonicotinoid treated group. No effect on the activity of honey bees was observed in any of the treatment groups on the following day (Figure 5).

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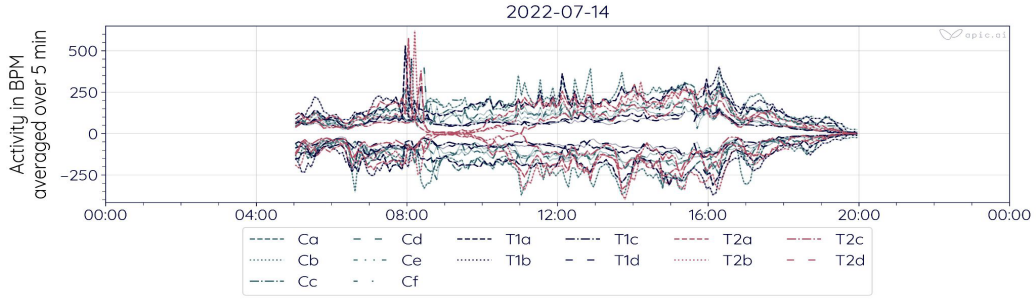

***Figure 4**. Activity in bees per minute on the day of application, averaged over 5 minutes between 5 am - 8 pm UTC, respectively 7 am - 20 pm local time. Treatment/ water application was around 8 am UCT/ 10 am local time. T1 was exposed to the Neonic, T2 was exposed to the Pyrethroid, C is control.*

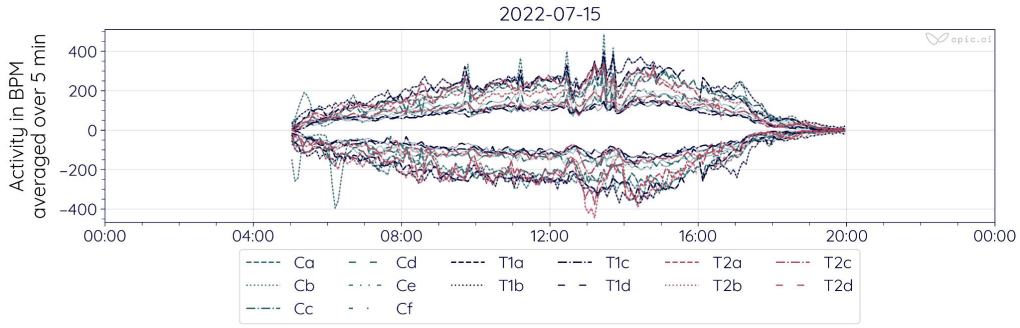

***Figure5**. Activity in bees per minute on the day after application, averaged over 5 minutes between 5 am - 8 pm UTC, respectively 7 am - 20 pm local time. T1 was exposed to the Neonic, T2 was exposed to the Pyrethroid, C is control.*

Pollen Collection

Figure 6 shows the pollen collection behaviour recorded by apic.ai on the day of application and the following day. As it can be seen on the day of application (14.07.2022), there is a slight reduction in pollen foraging for the Neonicotinoid group whereas in Pyrethroid treatment group, the pollen foraging ceases almost completely but starts again higher than in control and Neonicotinoid treatment after about three hours. The higher pollen foraging activity on the next day, indicates a compensation of pollen collection (Figure 7).

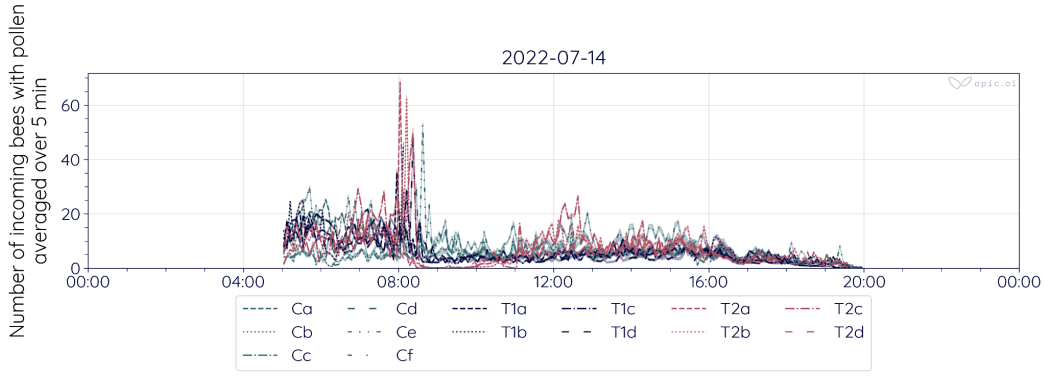

***Figure 6**: Bees entering the hive with pollen per minute on the day of application, averaged over 5 minutes between 5 am - 8 pm UTC, respectively 7 am - 20 pm local time. Treatment/ water application was around 8 am UCT/ 10 am local time. T1 was exposed to the Neonic, T2 was exposed to the Pyrethroid, C is control.*

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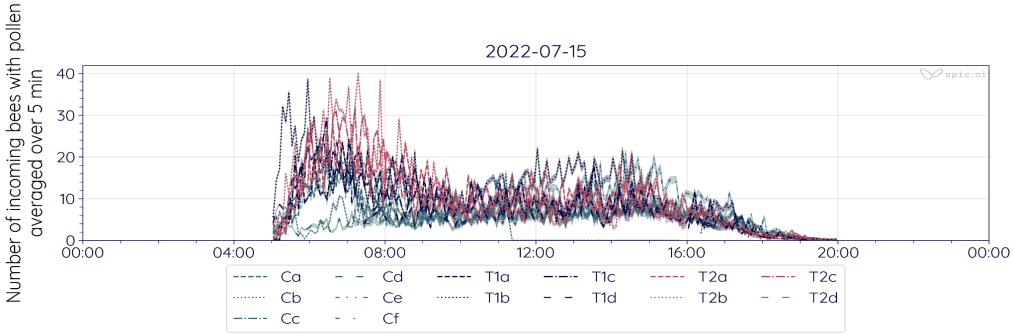

***Figure 7**: Bees entering the hive with pollen per minute on the day after application, averaged over 5 minutes between 5 am - 8 pm UTC, respectively 7 am - 20 pm local time. T1 was exposed to the Neonic, T2 was exposed to the Pyrethroid, C is control.*

Pollen foraging seemed to increase throughout the tunnel phase for all treatment groups, as can be seen in figure 8. The Neonicotinoid treatment group seemed to have the highest pollen collection activity among treatments. The share of pollen foragers increased towards the end of the tunnel phase for all treatment groups, while the general activity declined.

Figure 8: Total number of bees entering the hive with pollen per day and group. The lines indicate the variability within the groups. Bars are displayed if at least 95% of data in the time window from 7 am to 10 pm local time is available from at least three hives per group.

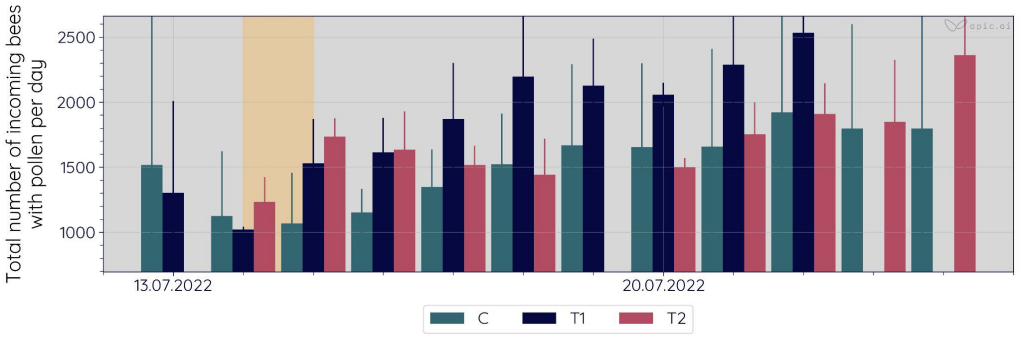

***Figure 8**: Total number of bees entering the hive with pollen per day and group. The lines indicate the variability within the groups. Bars are displayed if at least 95% of data in the time window from 7 am to 10 pm local time is available from at least three hives per group. T1 was exposed to the Neonic, T2 was exposed to the Pyrethroid, C is control.*

Conclusion

A significant increase in mortality of honeybees was observed in both Neonicotinoid and Pyrethroid treatment groups on the day of application, which could be verified by AI monitoring based technology. The mortality was higher in the Neonicotinoid group. A reduced foraging activity was noticed for both Neonicotinoid and Pyrethroid groups through both manual and automated bee monitoring observations. The automated assessment could confirm a clear reduction of activity (in/out bees) for one-two hours after exposure to the Pyrethroid treatment and a return to a regular activity level after three hours. The same was not recorded for the more toxic Neonicotinoid treatment.

Pollen foraging was also reduced in both treatment groups on the day of application, however on the following day, increased pollen collection activity was observed, which could be for compensation.

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No effect was observed on colony weight, colony strength and brood cells. Overall, no long-term effect of Neonicotinoid and Pyrethroid treatments was observed on the mortality and flight activity of the honey bees after the very pronounced acute effects ceased.

The automated bee monitoring system proved to be an effective tool for providing valuable insights in colony behaviour and activity determination. A repellence effect was clearly visible in the data. The continuous data collection by bee monitoring systems can provide a more realistic picture of the colony conditions and behaviour. The collected data will be used for further training of the BEEHAVE model.

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.; 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.

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Oral Presentation

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).

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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.

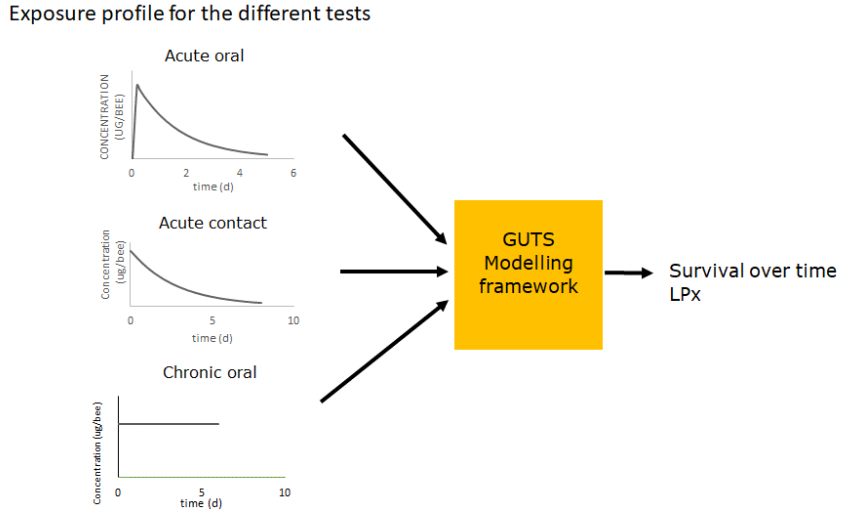

Figure 1 *Overview of the BeeGUTS modelling framework.*

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.

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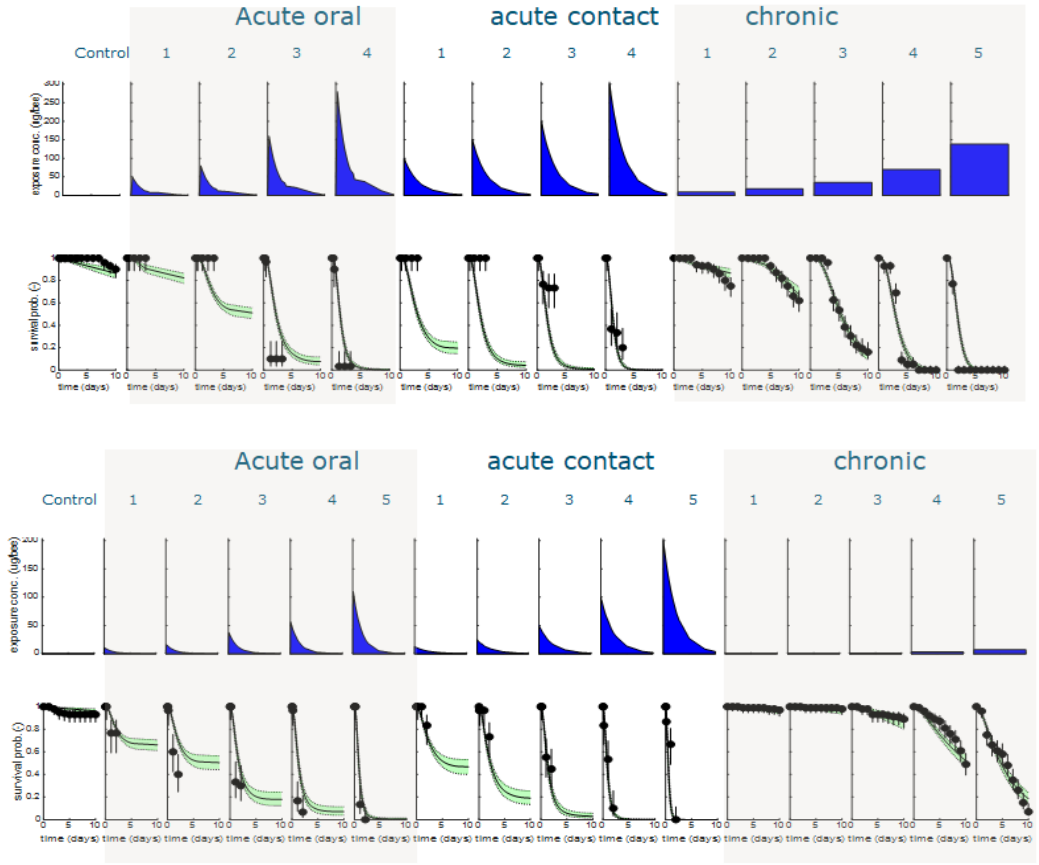

Figure 2 *Results for dimethoate and thiacloprid, showing the the different test results can be integrated within one framework. The top panels show the time -dependent exposure concentration and the lower panels show the measured (dots) and modelled (line, with green 95% conf int) survival over time.*

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.

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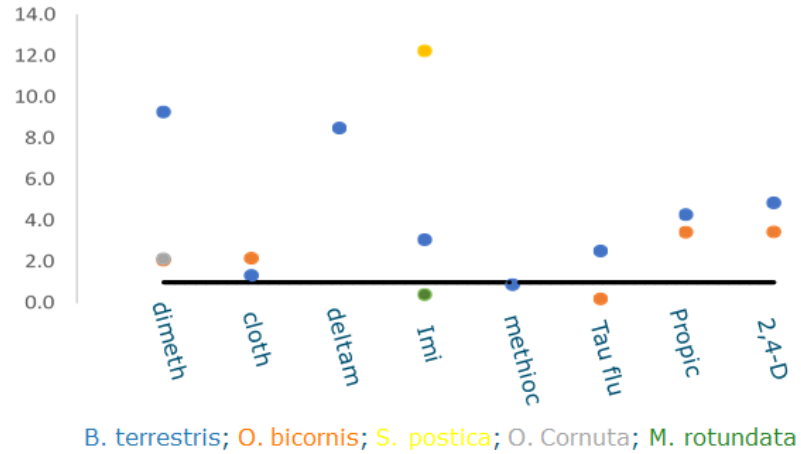

Figure 3 *Comparison of bee sensitivity with the sensitivity of the honey bee set to a value of 1. Any dots below the line indicate a higher sensitive than honey bees and all dots above the line indicate a lower sensitivity than honey bees.*

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

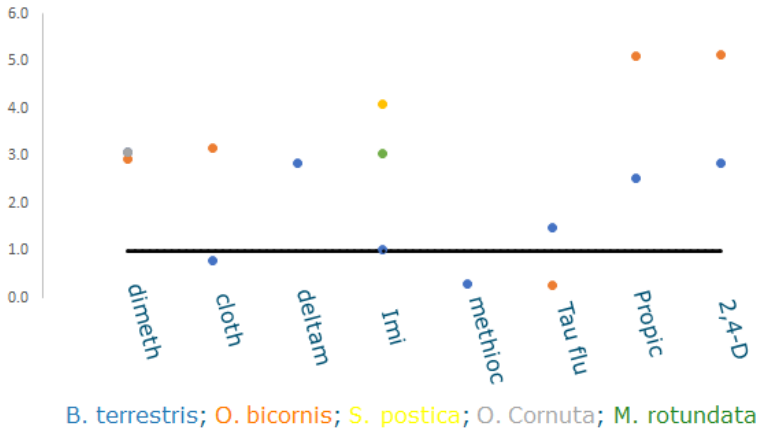

Figure 4 *Comparison of bee sensitivity on a weight basis with the sensitivity of the honey bee set to a value of 1. Any dots below the line indicate a higher sensitive than honey bees and all dots above the line indicate a lower sensitivity than honey bees.*

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.

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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 Toxicokinetic/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.

Oral Presentation

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.

Oral Presentation

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

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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.

Oral Presentation

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 to large-scale field studies is presented with a stepwise approach. The study designs may serve various purposes, such as practical help regarding commercial pollination, safeguarding biodiversity or for risk assessments in the registration process under GLP. In order to achieve high uniformity between bumblebee colonies, specially standardized R&D colonies (IPM Impact-Koppert) are used and adapted to the different test designs. The initial step is the study under laboratory conditions, through simulating the three possible means of bumblebee exposure to the compound: acute contact, oral by sugar water and/or by pollen. 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 exposure, if triggered. In the next step, simulating a more field-realistic but still controlled exposure scenario, R&D colonies can be tested under semi-field conditions in a tunnel set-up. The test design can be customised according to the specific requirements. The final step, if needed, is to monitor bumblebees in a field study where colonies are exposed to the product in a common agricultural landscape. In all study types, the following assessment parameters are recorded at regular intervals during the experimental phase: the presence/vitality of the mother queen, colony strength, colony weight, brood volume, the number of queen-brood cells, and the number of newly-formed queens (gynes). In a final assessment of the colonies, the reproduction rate of the control and treated colonies expressed as number of newly formed queens and, if possible, drones and the colony strength as the number of worker bees is compared at the end of the experiment.

In conclusion, an extrapolation of study results to commercial colonies used in practice for pollination and/or to natural colonies, concerning biodiversity, will be provided.

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

Introduction

The worldwide acceptance of Integrated Pest Management (IPM) and its increased uptake in many cultivations, mainly in the European Union, brought several new, less toxic Plant Protection Products (PPP) onto the market, with reduction of the hazardous pesticides use and their replacement by biopesticides or microbials. One of the main reasons for this change was the protection of the beneficial and pollinator organisms exposed to the PPP in indoor and outdoor cultivations and nature (Source: https://environment.ec.europa.eu/topics/nature-and-biodiversity/pollinators_en). Bumblebees' high foraging capacity and ability to pollinate in glasshouses, wildflowers, and field cultivations are worth mentioning. Therefore many techniques and methodologies have been

introduced to evaluate the PPPs' effects regarding acute or long-term toxicity. However, the methodologies vary mainly regarding the number and age of exposed bumblebees, the size of the colonies, the presence or absence of queens, the time of exposure etc. We summarize a graduate methodology that has been applied and evolved since 1994 (Sterk et al. 1996). The first bumblebee studies took place in the lab, investigating the direct toxicity of pesticides with a small colony consisting of a low number of workers (microcolonies) or the behavioural effects with double microcolonies. In recent years, R&D colonies of commercial size have been introduced and mainly used for lab, semi-field and field studies. The effects of PPPs under practical and applied conditions have been studied under semi-field and field trials, while the biodiversity and further fate of the free-living colonies have been studied in large-scale field trials.

Material and methods

The sub-species that is used in the present methodology is *Bombus terrestris dalmatinus* (Hymenoptera Apidae). The presented methodology has been followed in the studies of IPM Impact (Belgium) in collaboration with tier3 solutions (Germany) concerning the semi-field and field studies.

Laboratory studies

- Microcolonies: a group of bumblebees housed in a small-sized container, where a queen is absent. The queenless environment stimulates one worker to become dominant and begin laying unfertilized eggs, producing males (drones). These studies evaluate the chronic exposure effect of PPPs under defined laboratory conditions on adults and brood through its development (Klinger et al. 2019). In our studies, an initially standardized low number (10 to 15) of newly emerged workers (callows) is introduced in a small container made of transparent plastic on top, allowing observation. The colonies are purchased by Koppert (the Netherlands) or Biobest (Belgium). They are placed in complete darkness (0:24 L:D) at 26-28^{o}C, and the parameters of adult mortality, longevity, reproduction, and larval development are recorded weekly.
- Double-colonies: these studies are designed to evaluate microcolonies' foraging behavior (Mommaerts et al. 2010b). The double colony consists of a microcolony nest compartment connected to a 20 cm long tube with an empty nest containing food (pollen and sugar water). The orientation capacity and sugar water consumption are recorded in addition to the parameters of the standard microcolonies studies. Temperature and light conditions remain as with the microcolonies studies.
- R&D colonies: the question of the reproductive success of the colonies and the survival both of the newly formed queens and the successive generation led to the use of full-size colonies where a queen is present. However, the commercially available colonies are highly variable since workers and queens of different ages are initially randomly collected to form such a colony. This problem is solved by introducing R&D colonies designed by IPM Impact (Belgium) and Koppert (the Netherlands). These colonies consist of a mother queen from the same hibernation batch and the same number of callows. The trials are conducted under continuous darkness (0:24 L:D) or 16:8 (L:D) and a temperature of 26-28^{o}C.

In all lab studies, the exposure of the bumblebees to the PPPs is achieved through three ways of application: a) contact exposure, where the PPPs are applied onto the bumblebees with a pipette, or through spraying the colonies from above with a Birchmayer 1L hand sprayer, b) oral exposure through contaminated sugar water, which is prepared as a spraying solution and c) oral exposure through pollen, where a pollen ball, which is formed until saturation of pollen and PPP solution, is

given ad libidum to each colony. The contact exposed colonies are sprayed once (or more than once if applied in practice), while the control is sprayed with tap water. The colonies treated with spiked pollen and sugar water are exposed for four weeks, while for the post-exposure time, the sugar water is replaced with untreated sugar water, and water-sprayed pollen balls are provided to each colony, respectively.

The PPP's maximum field recommended concentration (MFRC) is applied in most studies. If required, a sequential dilution testing scheme is followed, where a decimal dilution of the MFRC is applied to the bumblebee colonies until no or low toxicity is recorded.

These studies focus on the most critical biological endpoints: acute lethal effects, colony development and reproductive success by the formation of newborn queens (gynes), as these will hibernate and start a new colony the next spring. Therefore, in a weekly assessment the presence/vitality of the mother queen, the number of adults and gynes, the weight of the colony and the volume of the brood are recorded. When the colonies reach their end, a final assessment is conducted: the number of queens (mother queen, gynes, and queen cells), the colony's strength (workers, drones, and non-identified gender bumblebees), the colony's weight and the brood volume are recorded. Concerning the brood volume, IPM Impact and Koppert have introduced a new classification system, where the colony development can be categorized according to the size/volume of the brood (Sterk et al. 2019a,b) (Table 1).

Tab. 1. *Size (cm^3) of a bumblebee colony's brood and description*

Code	Size (cm^3)	Description
A	30 cm^3	Basic colony in center of hive
B	235 cm^3	Expanding colony in center of hive
C	382 cm^3	Colony expanding, but not yet reaching the borders of the hive
D	655 cm^3	Colony expanding, and touching at least one side of the hive
E	1763 cm^3	Colony touching more than one side of the hive and growing in height
F	3489 cm^3	Colony covering the whole bottom of the hive and strongly expanding in height
G	4477 cm^3	Colony filling about half the hive
H	5373 cm^3	Colony almost filling the whole hive
I	6034 cm^3	Colony filling the whole hive. No space left for further expansion

Since a classification system for evaluating the PPPs on the pollinators is unavailable, the International Organization of Biological Control (IOBC) classification system for laboratory side effects is used to characterize the PPPs' lethal and sublethal effects on the bumblebees (Tab. 2).

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Tab. 2. *. Range (%) of effect and evaluation categories for laboratory side-effects studies, according to the IOBC*

IOBC Class	Range % effect (mortality, reproduction)	Evaluation category
1	<30	Harmless
2	30-79	Slightly harmful
3	80-98	Moderately harmful
4	>98	Harmful

Finally, conclusions for the PPPs' acute toxicity on queens and adults, and their effect on brood and reproduction are drawn based on the results and the class. Further recommendations for applying the tested PPP with priority on the bumblebee's safety are pointed out. For a harmless product, no actions are needed, while in cases of toxicity, further actions are proposed, e.g., by closing the hive's exit for 1-2 days after spraying. Simultaneous application when bumblebees are present may be prohibited in cases of high toxicity. Finally, if needed, further investigations, such as a sequential dilution testing scheme, may be designed.

Semi-field studies

In the semi-field studies, bumblebee colonies are placed in tunnels to investigate PPPs' side effects under a more field-realistic worst-case scenario. In 2021, IPM Impact and tier3 solutions conducted a semi-field study to investigate the side effects of a microbial product. For this purpose, bumblebee colonies were exposed to the microbial product in tunnels for about 30 days. In total, twelve tunnels were used: five tunnels treated with the microbial, five untreated control tunnels and two tunnels treated with dimethoate as a toxic reference. The 30-day flowering period required for this test was ensured by successively flowering Phacelia strips within the tunnel, and drip irrigation. To reduce the impact of the assessments on the colonies, newly developed R&D plastic hives (IPM Impact and Koppert) were used. During the experimental phase the assessment parameters, being the presence/vitality of the mother queen, colony strength, colony weight, brood volume, the number of queen-brood cells, and the number of newly-formed queens (gynes), were recorded in 3-4 days intervals. In a final assessment of the colonies at the end of the experiment, the reproduction rate of the colonies, expressed as the number of newly formed queens and the final colony strength (number of worker bees), was assessed. Additional to the endpoints mentioned in the draft of the revised EFSA Bee Guidance (2022) for bumblebee studies, colony weight (expressing the colony growth during trial) and reproductive success at the end of the study, the brood volume was successfully recorded. The low-impact assessment methods in combination with the new R&D hives reduced the impact on the test colonies and allowed a natural development. Both the synchronous development and the fact that all control colonies and treatment colonies produced new queens at the end of the colony development underline the necessity of using such strongly standardised colonies for use in eco-toxicological semi-field studies.

Field studies

The paradigms of two large-scale field studies are presented here. The first one was conducted in 2014, where the effects of clothianidin-dressed oilseed rape on *B. terrestris* colonies were monitored (Sterk et. al. 2016). In this study, three R&D colonies (IPM Impact and Koppert) were placed inside each Natupol Tripol box, which is designed for outdoor pollination. The Tripol hives were settled in

the reference and test sites which comprised an area of 65 Km^2 in Northern Germany, where no other crops attractive to pollinators were present. The second large-scale field study was a colony feeder study located in Bokrijk, Belgium. This 550-ha provincial estate site was chosen due to its unspoiled natural vegetation with no agricultural or horticultural cultivations in its periphery. Ten sites in various ecosystems were selected at a distance of at least 300m, and eight R&D colonies were placed in each site.

In both field studies, the following parameters were recorded in each colony assessment: presence/vitality of mother queen, colony strength, number of gynes and queen-brood, and colony weight. Further observations of the colony health, abnormal behaviour, and malformations of bumblebees were made. The weather conditions were also recorded. In the case of the monitoring study, chemical analysis and consumption of the pollen were documented, while in the colony feeder study, the sugar water consumption was noted. By the final assessment, the number of adults (workers, drones, and non-identified gender individuals) and queens (gynes, queen-brood) were recorded. Additionally, the number of eggs, larvae and pupae were counted for the colony feeder study.

Through the semi-field and field studies several conclusions may be drawn, especially regarding the short and long term effects of the applied PPPs in the bumblebee colonies and populations, as expressed in the colonies' development, reproduction rate, total gynes' production, health and turn over point.

Conclusion

B. terrestris dalamtinus is one of the most important pollinators in global size and is constantly exposed to PPPs in closed environments, such as greenhouses or open field sites. The presented solid study methodology draws conclusions about the compatibility between PPPs and bumblebees, as the colony's development reflects its pollination ability just as the number of gynes and males reflects the outcome for biodiversity. The results of the studies are published in a database (https://www.ipmimpact.com/database), where a realistic approach to applied PPPs' side effects on bumblebees in the frame of applied agriculture and biodiversity is presented.

References

EUROPEAN FOOD SAFETY AUTHORITY (EFSA). 2022. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). DOI: 10.2903j.efsa.201Y.xxxx10.2903j.efsa.201Y.xxxx

KLINGER, E., A.A. CAMP, J.P. STRANGE, D. COX-FOSTER, D.M. LEHMANN. 2019. *Bombus* (Hymenoptera: Apidae) Microcolonies as a tool for biological understanding and pesticide risk assessment. Environmental Entomology, 48(6), 1249-1259. DOI: 10.1093eenvz117.

MOMMAERTS, V., S. REYNDERS, J. BOULET, L. BESARD, G. STERK, G. SMAGGLE. 2010b. Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behaviour. Ecotoxicology, 19(1), 207-215. DOI: 10.1007/s10 646-009-0406-2.

STERK, G., K. BOLKMANS, J. EYAL. 1996. A new microbial insecticide for the control of the greenhouse whitefly, PreFeRal, (*Paecilomyces fumosoroseus* (WIZE) Brown and Smith, strain Apopka 97). British Crop Protection Conference - Pest and diseases 1996, 461-466.

STERK, G., J. HANEGRAAF, P. KOLOKYTHA. 2019a. Effects of chemical and biological Plant Protection Products on R& colonies of the Buff-Tailed Bumblebee *Bombus terrestris.* Hazards of pesticides to

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bees - 14th International Symposium of the ICP-PR Bee Protection Group, October 23-25 2019, Bern, (Switzerland), 48-53.

STERK, G., J. HANEGRAAF, P. KOLOKYTHA. 2019b. Effects of *Bacillus thuringiensis* subsp. *aizawai* GC91 (Agree WG) on R&D colonies of the Buff-Tailed Bumblebee *Bombus terrestris.* Hazards of pesticides to bees - 14th International Symposium of the ICP-PR Bee Protection Group, October 23-25 2019, Bern, (Switzerland), 53-57.

STERK, G., B. PETERS, Z. GAO U. ZUMKIER. 2016. Large-scale monitoring of effects of the clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*Bombus terrestris*). Ecotoxicology 25: 1666-1678. DOI: 10.1007s10646-016-1730-y.

https://environment.ec.europa.eu/topics/nature-and-biodiversity/pollinators_en (Revision of the EU Pollinators Initiative - A new deal for pollinators {SWD(2023) 18 final})

Oral Presentation

Section 5. - Monitoring

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

Bewertung des Lebenszyklus von Honigbienen und des Heimkehrerfolgs bei Feldbeobachtungen mit Hilfe visueller Monitoringtechnologie

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Abstract

There is a strong desire to enhance the knowledge creation on bee health and to get a better understanding of how available feed resources contribute to it. New technologies such as automatic carry a vast potential to gain insights into these questions. As of now, they were used to detect changes in activity and pollen foraging at colony level, but have not yet been applied to generate data at the level of individual bees.

One new technology to observe survival, flight duration and frequency at colony level are radio-frequency identification (RFID) chips. With their help, the homing flight behaviour of bees equipped with sensors can be observed to find out if there is an influence of a plant protection product on the orientation of the bees, thus their ability to return to their hive (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.

The companies apic.ai and EAS Ecotox are partners in the improvement of a visual bee monitoring technology in the research project OCELI (FKZ 281C307B19). Automated visual identification of individual bees could enable the inclusion of life cycle changes, homing success, flight duration and frequency as well as individual behaviour in studies where visual monitoring technology is already in use to assess other behavioural endpoints like activity, pollen collection or share of pollen foragers. As part of the project, a proof-of-concept experiment was performed in 2021, where the apic.ai monitoring systems with computer vision technology were used to observe individual foraging bees leaving and entering the hive. Queen markers (opalite plates) with different numbers and colours were attached to bees to identify them individually. For the study, bees from two colonies were marked twice at the interval of one week with a different colour. At each marking, both freshly hatched bees young and forager bees were marked. The marking was successful. Over Time there was no difference between a more experienced marking team than a less experienced team.

In a follow up tunnel trial in 2022 in Germany, marking with opalite plates was conducted to determine whether chemicals have an influence on foraging start and survival of individual foragers. apic.ai monitoring systems with computer vision technology were used to observe the activity and foraging of pollen at colony level and at the level of individuals through the marked bees. The first cohort marked were experienced foragers and the second cohort in hive bees. An algorithm saved multiple images of every bee entering and leaving the hive. These images were subsequently

analyzed for markers using a neural network. Picture were checked by a person to identify the colour and the number on the plate. Thus, data could be collected on:

- the first time the bee was seen leaving the hive
- every time the bee was detected to enter and leave
- every time the bee was detected bringing pollen into the hive
- the last time the bee was seen leaving the hive

Survival curves and changes in foraging and recruiting behaviour were studied using this data. The first results will be presented here to display and discuss the benefits of additional insights at the level of individual bees and the potential of the data to enhance simulation models such as BEEHAVE.

Keywords: semi-field study, honey bees, automatic counter, monitoring, re-ID

Zusammenfassung

Es besteht ein großes Interesse daran, das Wissen über den Gesundheitszustand von Bienen zu erweitern und besser zu verstehen, wie die verfügbaren Nahrungsressourcen ihn beeinflussen. Neue Technologien wie automatische Bienenzähler bieten ein enormes Potenzial, Erkenntnisse über diese Fragen zu gewinnen. Sie werden bereits dafür eingesetzt, Veränderungen der Aktivität und des Verhaltens bei der Nahrungssuche auf der Ebene des Bienenvolks zu messen. Bisher wurden sie jedoch noch nicht eingesetzt, um Daten auf der Ebene einzelner Bienen zu erheben.

Eine neue Technologie zur Untersuchung des Überlebens, der Flugdauer und -frequenz auf Volksebene sind RFID-Chips (Radio Frequency Identification). Mit ihrer Hilfe können individuelle Bienen, die mit Sensoren ausgestattet sind, mit Scannern registriert werden. So lässt sich herausfinden, ob ein Pflanzenschutzmittel das Orientierungsvermögen der Bienen beeinflusst und damit ihre Fähigkeit, zu ihrem Stock zurückzukehren (OECD GD 332).

Die Kombination von Daten über die Flugaktivität und Beginn und Ende von Sammelflügen einzelner Honigbienen mit den von einem automatischen Monitoringsystem gelieferten Daten auf Volksebene könnte sehr aufschlussreich sein, um ein besseres Verständnis von Effekten und deren Ausmaß zu erhalten.

Die Unternehmen apic.ai und EAS Ecotox sind im Forschungsprojekt OCELI (FKZ 281C307B19) Partner bei der Verbesserung einer visuellen Bienenmonitoringtechnologie, welche die Erhebung von Daten auf Volksebene und Individualebene verbinden möchte. Die automatisierte visuelle Identifizierung einzelner Bienen könnte es ermöglichen, dem Beginn von Sammelflügen, den Heimkehrerfolg, die Flugdauer und -häufigkeit sowie das individuelle Verhalten in Studien einzubeziehen, in denen die visuelle Überwachungstechnologie bereits zur Bewertung anderer Verhaltensendpunkte wie Aktivität, Pollensammlung oder Anteil der Sammlerinnen eingesetzt wird. Im Rahmen eines Projekts wurde 2021 ein Proof-of-Concept-Experiment durchgeführt, bei dem die apic.ai Monitoring-Systeme mit Computer Vision Technologie zur Beobachtung einzelner Bienen getestet wurden. An den Bienen wurden zur individuellen Identifikation Königinnenmarker (Opalith-Plättchen) mit unterschiedlichen Nummern und Farben angebracht. Für die Studie wurden Bienen aus zwei Völkern an zwei Tagen im Abstand von einer Woche mit unterschiedlichen Farben markiert. Bei jeder Markierung wurden sowohl Jungbienen als auch erfahrene Sammlerinnen markiert. Die Markierung war erfolgreich, wobei es über eine längere Zeit keinen Unterschied zwischen den Teams gab. Der Ansatz der Re-Identifizierung (Re-ID) wurde auch parallel zu klassischen Beobachtungen in einem nachfolgenden Tunnelversuch im Jahr 2022 in Deutschland angewandt, um festzustellen, ob ein Einfluss von zwei Insektiziden auf Kohorten von Einzelbienen gemessen werden kann. apic.ai-Monitoringsysteme mit

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Computer-Vision-Technologie wurden eingesetzt, um die Aktivität und das Sammelverhalten auf Volksebene und für einzeln markierte Individuen zu beobachten. Bei der ersten markierten Kohorte handelte es sich um aktive Sammlerinnen, bei der zweiten Kohorte um im Stock lebende Jungbienen. Ein Algorithmus speicherte mehrere Bilder von jeder Biene beim Einflug und Ausflug des Bienenstocks. Die Bilder wurden anschließend mithilfe eines neuronalen Netzes auf Marker untersucht. Wurde ein Marker gefunden, wurde das Bild einer Person gezeigt, die die Farbe und die Nummer auf dem Plättchen identifizierte. So konnten folgende Daten gesammelt werden:

- das erste Mal, dass die Biene beim Ausflug des Bienenstocks beobachtet wurde
- jedes Mal, wenn die Biene beim Einflug und Ausflug des Stocks beobachtet wurde
- jedes Mal, wenn die Biene beim Einbringen von Pollen in den Bienenstock beobachtet wurde
- das letzte Mal, als die Biene beim Ausflug des Bienenstocks gesehen wurde

Anhand dieser Daten konnten Überlebenskurven für aktive Sammlerinnen und neu rekrutierte Sammlerinnen dargestellt werden. Die ersten Ergebnisse werden hier vorgestellt, um die Vorteile zusätzlicher Erkenntnisse auf der Ebene der einzelnen Bienen und dass Potenzial der Daten zur Verbesserung von Simulationsmodellen, wie BEEHAVE aufzuzeigen.

Stichwörter: Halbfreilandstudie, Honigbienen, Zählgerät, Monitoring, Markierung, Re-ID

Introduction

The honey bee is considered an important beneficial insect with a crucial role in pollination activity in fruit, berry and seed growing. Honey bee colonies involved in (migratory) beekeeping for the purpose of providing pollination services are a significant and valuable component of a productive agricultural sector. Therefore, it is important to have a better understanding of the lifecycle of honey bees and their pollen collection and handling time. New automated technologies have been developed with the possibility of detecting and following honey bees foraging. Visual bee monitoring systems could be used to include the continuous observation of cohorts of individual bees in bee field-testing. Since the apic.ai bee monitoring system is based on video, it can be used to track individual bees if they were marked uniquely. The technology could be used to include the continuous observation of individual bees in bee field-testing, including foraging time or times bees have their first foraging flight as well as information about the foraging duration and type. In order to determine the efficacy of the automated bee counters and their future potentials, two studies run in succession. The first was a pre-test study (proof of concept experiment) that took place in 2021 followed by a semi-field study in 2022.

The objective of the pre-test study in 2021 was to determine the possibility of tracking individual bees after marking, to assess the presence of winter bees in the next spring and to compare the marking success of experienced and inexperienced teams.

The partial aim of the following semi-field study in summer 2022 was to contribute ground truth data on the flight duration and frequency as well as the age of first foraging after exposure to Neonicotinoid and Pyrethroid treatments. For this purpose, both young in-hive bees and active forager bees were marked using queen markers in all treatment groups.

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Material and methods

Pre-test

The pre-test was conducted in southern Germany, where two hives with access to natural nectar and pollen resources were equipped with monitoring systems. The hives were monitored for a period of 28 days with individual bee detection using imaging processing and neural networks. Two marking sessions took place, with one week in between. The first marking took place 21st September 2021 and the second marking on 28th September 2021. Four different opalite marker colours were used. The first marking was done by experienced and second marking was done by inexperienced bee handlers. Background colour in the table indicates the marker colours used.

Semi-field study

The study was conducted using experimental hives in the south of Germany. There was a control group (treated with water), a treatment group T1 treated with Thiomethoxam (Neonicotinoid, at a rate of 5 g a.i./ha) and a treatment group T2 treated with *Lambda*-cyhalothrin (Pyrethroid, at a rate of 10 g a.i./ha). Each treatment was replicated 4 times, the control group consisted of six replicates. Healthy and queen-right bee colonies were used. The hives were placed in 200 m^2 tunnels with *Phacelia tanacetifolia* as the crop.

40 young bees (taken from the brood frame with grey hairs) were marked in the “a” replicates of each treatment group, and 40 marked forager bees were introduced to the “b” replicates of each treatment group one day before the application. The bees were marked with individually numbered queen markers (opalite plates). For the hives with the individually marked bees, multiple photos of each bee entering and leaving the hives were recorded. The photos were analysed for markers and pollen using neural networks after the end of the trial. Multiple images were assessed for each bee, because markers as well as pollen pellets are sometimes hidden by wings. Additionally, bees can walk with the front towards the camera and a marker can’t be detected under this circumstances.

The resulting data was used to determine handling time/flight duration, age of first foraging and flight as well as foraging frequency. They were also used confirm if individual bees specify on pollen collecting.

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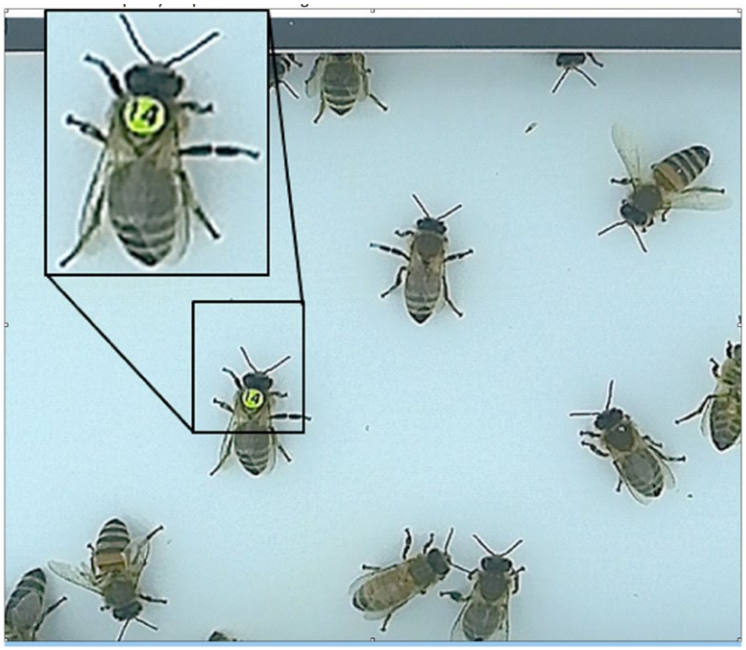

Picture 1 *Marked bee detected by bee counter.*

Results

Pre-test

Table 1 shows the assessment results of the marked bees at various assessments after the marking. Bees were assessed by beekeepers.

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Table 1 *Distribution and number of marked bees. First marking was done by experienced and second by an inexperienced team. Background colours indicate the colours used.*

Time after marking in days	Hive a	Hive b
marking experienced team	57 yooung bees 23 foragers	50 young bees 24 foragers
30	18 yooung bees 0 foragers	24 young bees 0 foragers
182	0 yooung bees 0 foragers	3 young bees 0 foragers
marking inexperienced team	52 young bees 24 foragers	55 young bees 24 foragers
23	22 young bees 0 foragers	19 young bees 0 foragers
175	1 young bees 0 foragers	2 young bees 0 foragers

There was no difference in the results for an experienced and unexperienced team. Of the young bees marked there were still individuals present in the hive about 6 months after marking.

No forager bees survived one month after the marking. Among all the marked bees, only one forager bee (marked green, nr. 83) returned with pollen.

The automated observation of marked bees showed that the young marked bees started to appear 3-4 days after marking. In-hive bees were confirmed to survive for up to six months over winter as few marked bees were still observed in spring 2022. Therefore, it can be concluded that the active forager bees marked at the end of September were not winter bees.Semi-field study

Figure 1 to Figure 3 depict the activity of the marked freshly hatched and forager bees in all treatment groups. Figure 1 shows, in the control group, all the young bees were detected from 17 July 2022 onwards with the exception of one bee that was detected on the day of marking. Marked forager bees were active from the day of marking in the control group and were detected till 19 July 2022, when the first observation interval ended.

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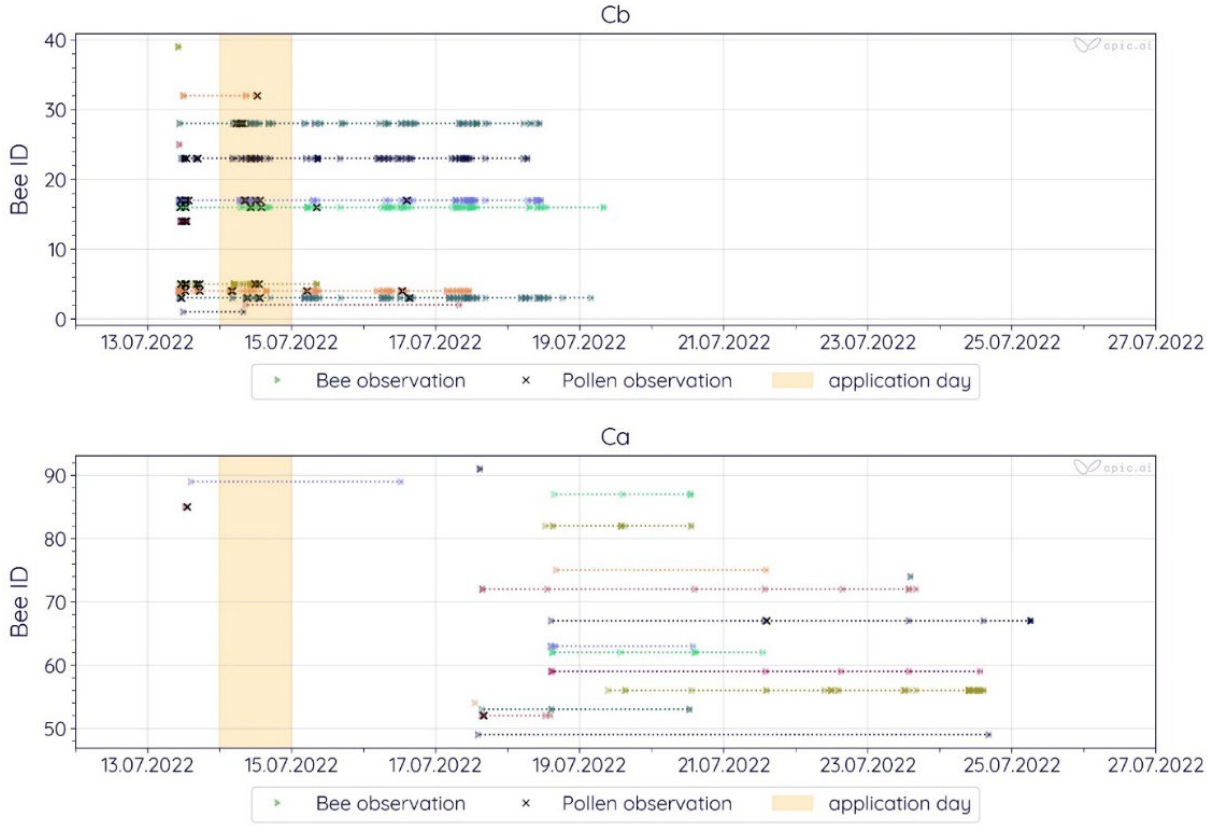

Figure 1 *Foraging flight and pollen sampling in control treatment during tunnel phase (Cb experienced foragers, Ca young bees)*

In the Neonicotinoid treated group, one young bee was observed on 16 July 2022 one time, however, the majority of the young bees' activity started on 20 July 2022 (Figure 2). Marked foragers were detected from the marking day but no detection of marked foragers happened after the application day with exception of one bee that was detected very frequently till 22 July 2022 (Figure 2). It must be noted, that the marking colour in this group (blue), turned out to be not well readable. Therefore, part of the activity in this group was most likely not detected.

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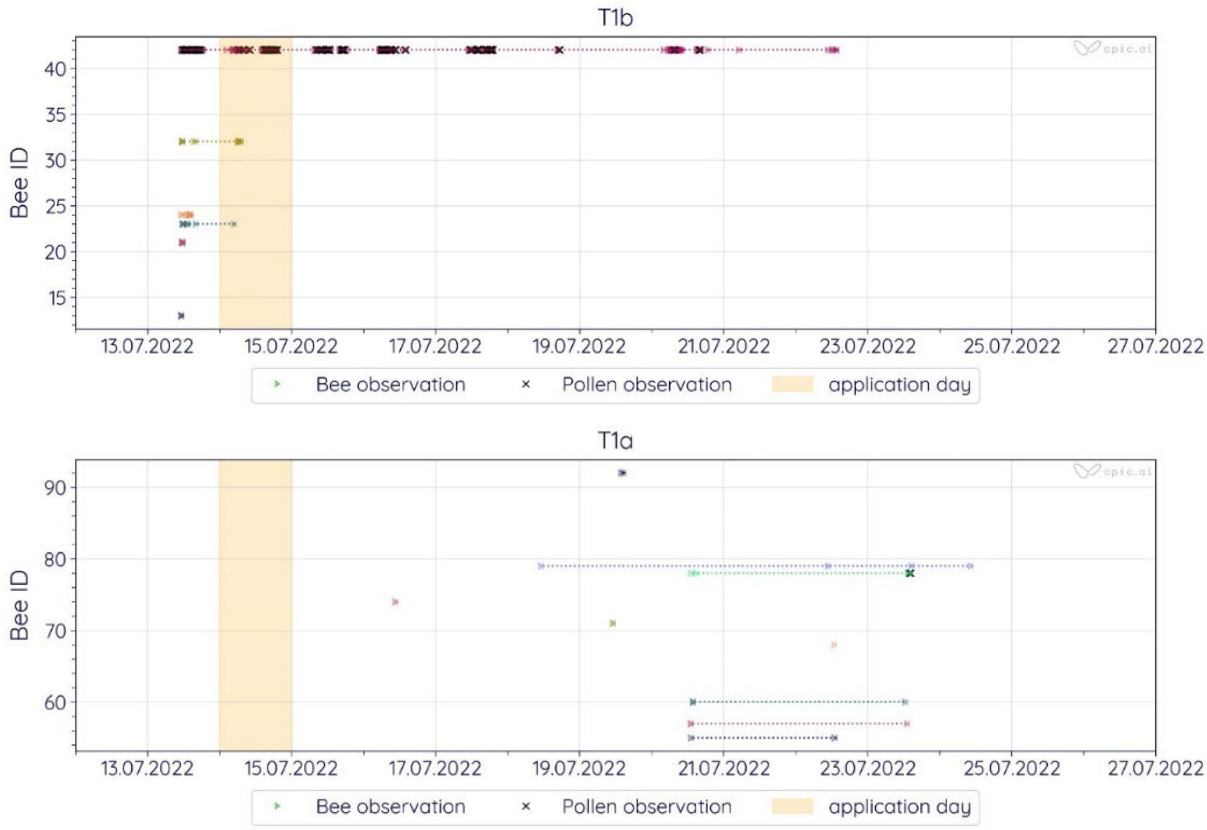

Figure 2 *Foraging flight and pollen sampling in Neonicotinoid treatment during tunnel phase (T1b experienced foragers, T1a young bees)*

In the Pyrethroid treated group young bees were detected from the day of application until the end of the tunnel phase on 26nd July 2022. In the treatment, no marked forager bees were detected from the day of application onwards except for one forager which was detected till 17 July 2022 (Figure 3).

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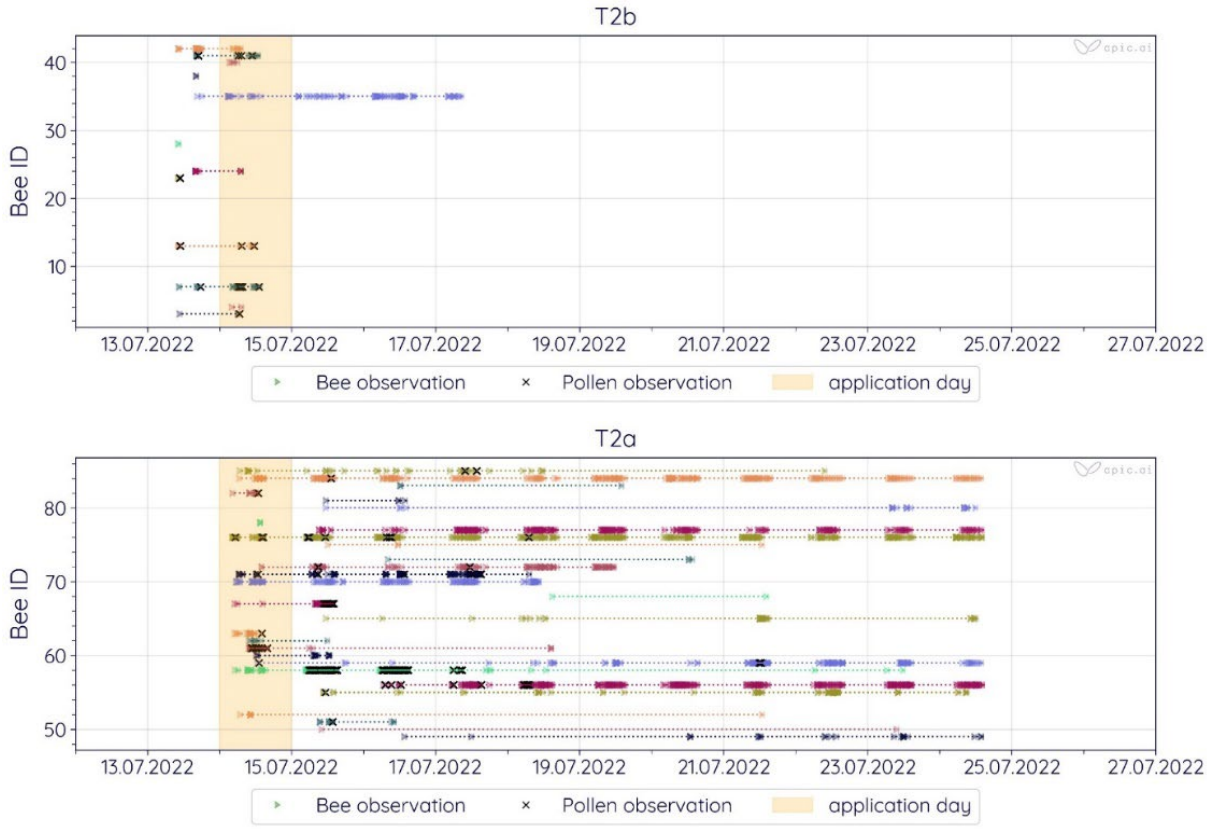

Figure 3 *Foraging flight and pollen sampling in Pyrethroid treatment during tunnel phase (T2b experienced foragers,, T2a young bees)*

Figure 4 shows the flight activity of the marked young bees in the Pyrethroid treated group. Most of the pollen foragers took less than two minutes to return to the hive. The flights of bees that were leaving for other purposes than collecting pollen e.g. collecting nectar, scouting, etc. took longer to be completed.

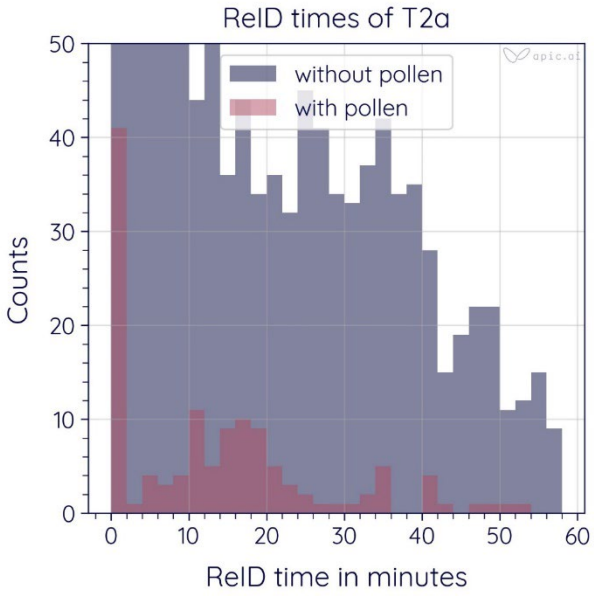

Figure 4 *Distribution of foraging flight duration in the Pyrethroid treatment during tunnel phase (T2a young bees)*

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Comparing the activity among the three treatment groups, it can be seen that experienced forager bees were clearly affected by the application. Only two single bees survived the application in the Neonicotinoid and Pyrethroid treatments respectively. However, in at this stage there is some uncertainty since there were only 5 foragers detected overall in the neonicotinoid treatment, while in the Pyrethroid treatment 13 were found. The forager bees in the control group survived longer to with the last activity recorded 10 days after the marking (Figure 5). As this was the end of the observation interval, they were likely active beyond this point in time. The results show that as expected and visible in the manual mortality assessment, the application of Neonicotinoid and Pyrethroid during the bee fight, had a strong effect on the forager mortality.

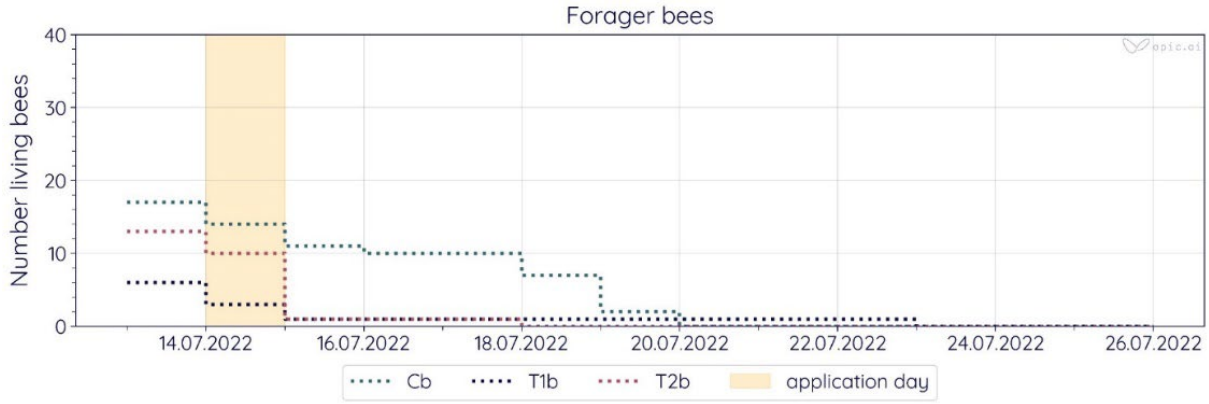

Figure 5 *Survival of experiences foragers for control Cb, neonicotinoid T1b and pyrethroid treatment T2b*

As presented in Figure 6, there was a visible difference in the recruitment of young bees for foraging. Young bees in the Pyrethroid treatment group were recruited directly after the application for foraging. Yet, freshly hatched bees in the neonicotinoid treatment were recruited slower than in the control. All marked freshly hatched bees continued to be detected in all treatment groups till the end of the tunnel phase on 26 July 2022.

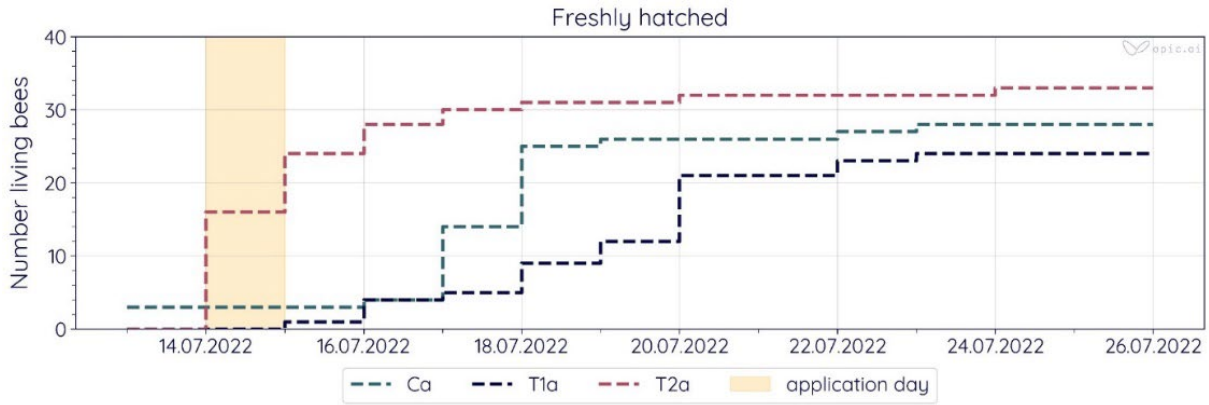

Figure 6 *Recruitment of young bees for foraging for control Ca, neonicotinoid T1a and pyrethroid treatment T2a*

Conclusion

Automated bee monitoring technology proved to be effective in determining survival curves and recruitment of young bees to foraging. The method was successful in comparing treatment groups with each other. In both the pre-test and the semi-field study, the tracking of individual bees,

determining their homing success, flight duration and frequency and pollen foraging behaviour were successful. Since individual bee tracking and pollen foraging is possible, the automated bee counters can provide a better understanding into the lifespan and -cycle of honey bees. Furthermore, they are an alternative for RFID chips. The reduction of forages during application was expected, however why the recruitment of foragers was speeded up in the Pyrethroid treatment is at this stage not clear. Future studies are planned to find out if the observations will be confirmed.

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.; 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].

OECD (2021) Guidance Document on Honey Bee (*Apis Mellifera* L.) Homing Flight Test, Using Single Oral Exposure to Sublethal Doses of Test Chemical, No. 332 (ENV/CBC/MONO(2021)7).

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.Bredemeier, W., 2019: Zukunft der Informationswissenschaften: Hat die Infromationswissenschaft eine Zukunft? Berlin, Simon Verlag für Bibliothekswissen.

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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

Introduction

Honey bees and hive products have been used as environmental biomonitors due to their strong interaction with the ecosystem where they live and specific bioindicator characteristics (PORRINI et al., 2002). During the past decade sample preparation and instrumental analysis has evolved and several methodologies have been adapted to analyze pesticide residues in bees and hive products (HRYNKO et al., 2021). Monitoring campaigns all over the world showed the ubiquitous presence of several pesticide residues in honey, bees, wax, pollen, beebread and honeycombs (MURCIA-MORALES et al., 2022).

The starting point of the presented monitoring study was beekeeper´s and farmer´s concern about the pollution of their ecosystem. This is an important lagoon basin in Canelones, Uruguay (-

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34.751826, -55.832390) called “Laguna del Cisne” shown in figure 1, where a productive reconversion was starting looking for pesticides use reduction, as it is also the tap water source for a population of 150000 inhabitants.

Figure 1 *Laguna del Cisne location*

Based on the previous experience from our group studying beehives as environmental monitors of pesticide residues (NIELL et al., 2017, 2018), a monitoring of agroecosystems was designed and jointly developed.

Material and methods

Monitoring design

Swarms from the region were captured by local beekeepers and hives with new material were installed the following summer. A group of five beehives was settled in each selected environment: a native forest and seven agroecosystems involving rice crops, soybean, fruit orchards and horticulture. Four monitoring units were placed in the lagoon basin and four outside. Five seasonal samplings from January 2019 until May 2020 were performed. A total of 156 samples of bees, wax, pollen (beebread) and honey (40, 40, 36 and 40 samples respectively) were analyzed. Also the botanical richness of pollen and honey samples was determined following Louveaux methods of melissopalynology (LOUVEAUX et al., 1978).

The pesticides multiresidue methodologies consisted of QuEChERScitrate buffered(ANASTASSIADES et al., 2007)
based sample preparations followed by GC-MS/MS and LC-MS/MS analysis previously developed by our group(NIELL et al., 2015; NIELL et al., 2014).Briefly, 2 g of beeswax is extracted with 10 mL of MeCN at 80 °C.Then, the extract is frozen-out, liquid-liquid partitioned with 3 mL of hexane and cleaned up with 25 mg of primary−secondary amine (PSA) and 25 mg of C18 sorbent/mL of extract. Homogenized honey and beebread (5 g) and 2g bees are extracted with water and MeCN. Then the mixture of citrate buffer salts is added. The honey extract is cleaned up with PSA, 25mg/mL, and MgSO4, 150 mg/mL. Beebread has an extra clean up: freeze-out and PSA, 50 mg/mL, C18,50mg/mL while bees extract is freezed-out and GCB is also added. Finally, the extract is acidified with 5% formic acid solution in MeCN (v/v) (10 µL/mL extract) and injected in LC-MS/MS. For GC-MS/MS analysis a concentration factor of 5 is used, the extract is driven to dryness and redissolved in Ethyl Acetate.

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Results

Methods performance

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 fully validated according to SANTE/11813/2017 guidelines. As shown in Table 1, LOQs ranged from 0.0001 to 0.100 mg kg^{-1} depending on the matrix and analyte.

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Table 1 *Limits of quantitation (LOQ) and instrumental analysis for each matrix (bees, honey, wax and beebread).*

	LOQ (mg kg^{-1})				Instrumental analysis
Pesticide	**Honey**	**Beebread**	**Wax**	**Bees**	
Acephate	0.001	0.01	-	-	LC-MS/MS
Acetamiprid	0.001	0.01	0.001	0.001	LC-MS/MS
Acetochlor	-	-	0.005	0.005	GC-MS/MS
Acetochlor	0.001	0.01	-	-	LC-MS/MS
Alachlor	-	-	0.005	0.005	GC-MS/MS
Alachlor	0.005	0.01	-	-	LC-MS/MS
Aldrin	0.005	-	0.005	0.005	GC-MS/MS
Ametryn	0.001	0.005	0.001	0.001	LC-MS/MS
Atrazine	0.001	0.01	0.001	0.001	LC-MS/MS
Atrazine desethyl	0.001	0.005	0.005	0.005	LC-MS/MS
Atrazine desisopropyl	0.005	0.02	0.005	0.005	LC-MS/MS
Azinphos methyl	0.001	0.005	0.001	0.001	LC-MS/MS
Azoxystrobin	0.0001	0.005	0.0001	0.0001	LC-MS/MS
Bifenthrin (sum of isomers)	0.005	-	0.005	0.005	GC-MS/MS
Bifenthrin (sum of isomers)	-	0.005	-	-	LC-MS/MS
Boscalid	-	0.01	-	-	GC-MS/MS
Boscalid	0.001	-	0.001	0.001	LC-MS/MS
Bromopropylate	0.1	0.01	0.1	0.01	GC-MS/MS
Carbaryl	0.001	0.01	0.0001	0.001	LC-MS/MS
Carbendazim	0.0001	0.005	0.001	0.001	LC-MS/MS
Carbofuran	0.001	0.02	0.001	0.005	LC-MS/MS
Chlorantraniliprole	0.001	0.005	0.005	0.005	LC-MS/MS
Chlorothalonil	0.1	-	0.1	0.1	GC-MS/MS
Chlorpyrifos	0.005	0.005	0.005	0.005	GC-MS/MS
Chlorpyrifos Methyl	0.005	0.01	0.005	0.005	GC-MS/MS
Chlorsulfuron	0.001	-	-	-	LC-MS/MS
Chlothianidin	0.0001	0.005	0.001	0.001	LC-MS/MS
Cis-Chlordane	0.005	-	-	-	GC-MS/MS
Clomazone	0.005	0.01	-	0.005	GC-MS/MS
Coumaphos	0.0001	0.01	0.0001	0.01	LC-MS/MS
Cyfluthrin	0.005	0.02	0.1	0.01	GC-MS/MS
Cyhalofop butyl	0.005	0.01	0.005	0.005	GC-MS/MS
Cyhalothrin	0.005	0.01	0.005	0.005	GC-MS/MS
Cymoxanil	-	0.01	-	-	LC-MS/MS
Cypermethrin	0.005	-	0.1	0.1	GC-MS/MS
Cyproconazole	0.001	0.02	-	0.005	LC-MS/MS
Deltamethrin	0.005	0.02	0.1	0.1	GC-MS/MS

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	LOQ (mg kg^{-1})				Instrumental analysis
Pesticide	**Honey**	**Beebread**	**Wax**	**Bees**	
Diazinon	0.001	0.001	0.001	0.001	LC-MS/MS
Diclosulam	0.001	0.005	-	-	LC-MS/MS
Dieldrin	0.005	-	-	-	GC-MS/MS
Difenoconazole	0.005	0.005	0.001	0.001	LC-MS/MS
Diflubenzuron	-	0.005	-	-	LC-MS/MS
Dimethoate	0.001	0.005	0.001	0.0001	LC-MS/MS
Diuron	0.001	-	0.001	0.001	LC-MS/MS
Endosulfan sulphate	0.1	-	0.1	0.1	GC-MS/MS
Epoxiconazole	-	0.01	-	0.005	GC-MS/MS
Epoxiconazole	0.001	-	-	-	LC-MS/MS
Ethion	0.005	-	0.005	0.005	GC-MS/MS
Ethion	-	0.001	-	-	LC-MS/MS
Fenthion	-	0.01	-	-	GC-MS/MS
Fenthion sulfone	-	0.01	-	-	LC-MS/MS
Fenthion sufoxide	-	0.005	-	-	LC-MS/MS
Fenvalerate	-	0.01	-	-	GC-MS/MS
Fipronil	0.001	0.001	0.001	0.001	LC-MS/MS
Fipronil caboxamide	-	0.005	-	-	LC-MS/MS
Fipronil desulfynil	-	0.001	-	-	LC-MS/MS
Fipronil sulfide	0.001	0.001	0.001	0.001	LC-MS/MS
Fipronil sulfone	0.001	0.001	0.001	0.001	LC-MS/MS
Fludioxonil	-	0.001	-	-	LC-MS/MS
Flufenoxuron	-	0.005	-	-	LC-MS/MS
Flumetsulam	0.005	0.02	-	-	LC-MS/MS
Flusilazol	-	0.001	-	-	LC-MS/MS
Fluroxipyr – Meptyl	0.005	-	-	0.005	LC-MS/MS
Fluvalinate (Tau-Fluvalinate)	0.1	0.01	0.01	0.1	GC-MS/MS
Fluvalinate (Tau-Fluvalinate)	-	-	-	-	LC-MS/MS
Haloxifop methyl	0.001	0.005	0.001	0.0001	LC-MS/MS
Heptachlor	-	-	0.005	0.005	GC-MS/MS
Hexythiazox	0.001	0.01	0.001	0.001	LC-MS/MS
Imazalil	0.001	0.005	-	0.05	LC-MS/MS
Imidacloprid	0.001	0.01	0.001	0.01	LC-MS/MS
Iprodione	-	-	0.001	-	LC-MS/MS
Isoxadifen –ethyl	0.005	-	0.005	0.005	GC-MS/MS
Isoxadifen –ethyl	-	0.01	-	-	LC-MS/MS
Kresoxim methyl	0.005	-	0.005	0.005	GC-MS/MS
Linuron	0.001	0.02	0.005	0.001	LC-MS/MS

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	LOQ (mg kg^{-1})				Instrumental analysis
Pesticide	**Honey**	**Beebread**	**Wax**	**Bees**	
Malaoxon	0.001	0.001	0.001	0.001	LC-MS/MS
Malathion	0.005	-	0.005	0.005	GC-MS/MS
Malathion	-	0.001	-	-	LC-MS/MS
Metalaxyl	0.001	0.005	0.001	0.001	LC-MS/MS
Metconazole	-	0.05	-	-	LC-MS/MS
Methidation	0.005	0.01	0.005	0.005	GC-MS/MS
Methiocarb	0.001	0.005	0.005	0.005	LC-MS/MS
Methiocarb sulfone	-	0.001	-	-	LC-MS/MS
Methiocarb sulfoxide	-	0.001	-	-	LC-MS/MS
Methomyl	0.001	0.001	0.001	0.0001	LC-MS/MS
Methoxychlor	0.005	0.01	0.005	0.005	GC-MS/MS
Methoxyfenozide	0.001	0.005	0.001	0.001	LC-MS/MS
Metolachlor	-	0.01	-	-	GC-MS/MS
Metolachlor	0.005	-	0.005	0.005	LC-MS/MS
Metribuzin	0.005	0.01	0.01	0.005	GC-MS/MS
Metsulfuron methyl	-	0.005	-	-	LC-MS/MS
Mirex	0.005	-	0.005	0.005	GC-MS/MS
o,p' DDD	0.005	0.005	0.005	0.005	GC-MS/MS
o,p' DDE	0.005	0.005	0.005	0.005	GC-MS/MS
o,p'-DDT	0.005	0.005	0.005	0.005	GC-MS/MS
Oxyfluorfen	-	-	0.005	0.005	GC-MS/MS
p,p' DDD	0.005	0.005	0.005	0.005	GC-MS/MS
p,p' DDE	0.005	0.02	0.005	0.005	GC-MS/MS
p,p'-DDT	0.005	0.02	0.005	0.005	GC-MS/MS
Parathion	0.01	0.01	0.02	0.01	GC-MS/MS
Parathion methyl	0.01	0.02	0.02	0.1	GC-MS/MS
Penoxsulam	0.001	0.01	-	-	LC-MS/MS
Permethrin	0.005	-	0.005	0.005	GC-MS/MS
Picoxystrobin	0.001	0.005	0.005	0.005	LC-MS/MS
Pirimicarb	-	0.005	-	-	LC-MS/MS
Pirimiphos methyl	0.005	0.01	-	0.01	GC-MS/MS
Profenophos	0.005	0.02	-	-	GC-MS/MS
Propamocarb	0.005	-	-	-	LC-MS/MS
Propanil	0.005	0.005	0.005	0.005	LC-MS/MS
Propiconazole	0.005	0.02	-	-	LC-MS/MS
Propoxur	-	0.01	-	-	LC-MS/MS
Protioconazole	0.001	0.02	-	-	LC-MS/MS
Pyraclostrobin	0.001	0.001	0.001	0.001	LC-MS/MS

Oral Presentation

	LOQ (mg kg^{-1})				Instrumental analysis
Pesticide	**Honey**	**Beebread**	**Wax**	**Bees**	
Pyrazosulfuron	-	0.02	-	-	LC-MS/MS
Pyrimethanil	0.005	0.02	-	0.005	GC-MS/MS
Saflufenacil	-	0.05	-	-	LC-MS/MS
Simazine	0.005	-	0.005	0.005	GC-MS/MS
Simazine	-	0.02	-	-	LC-MS/MS
Tebuconazole	-	0.005	-	-	GC-MS/MS
Tebuconazole	0.001	-	0.0001	0.001	LC-MS/MS
Tebufenozide	-	0.001	-	-	LC-MS/MS
Terbacil	0.001	-	-	0.001	LC-MS/MS
Terbuthylazine	-	0.01	-	-	LC-MS/MS
Tetraconazole	-	0.02	-	-	LC-MS/MS
Thiabendazole	0.001	-	-	-	LC-MS/MS
Thiacloprid	0.001	0.005	0.001	0.001	LC-MS/MS
Thiamethoxam	0.001	0.02	0.001	0.001	LC-MS/MS
Trans- Chlordane	0.005	-	-	-	GC-MS/MS
Trichlorfon	0.005	0.05	-	-	LC-MS/MS
Tricyclazole	0.001	0.005	0.001	0.001	LC-MS/MS
Trifloxystrobin	0.001	0.001	0.001	0.001	LC-MS/MS
Triflumuron	0.001	0.005	-	-	LC-MS/MS
Trifluralin	0.1	-	0.005	0.005	GC-MS/MS
Triticonazole	0.005	0.02	0.005	0.005	GC-MS/MS
Vinclozolin	-	0.02	-	-	GC-MS/MS
α-Endosulfan	0.1	-	0.1	0.1	GC-MS/MS
β-Endosulfan	0.1	-	0.1	0.1	GC-MS/MS
α-HCH	0.005	-	0.005	0.005	GC-MS/MS
β-HCH	0.005	-	-	-	GC-MS/MS
γ-HCH (Lindane)	0.005	-	0.005	0.005	GC-MS/MS
δ-HCH	0.005	-	0.005	0.005	GC-MS/MS
2,4-DB	-	0.02	-	-	LC-MS/MS

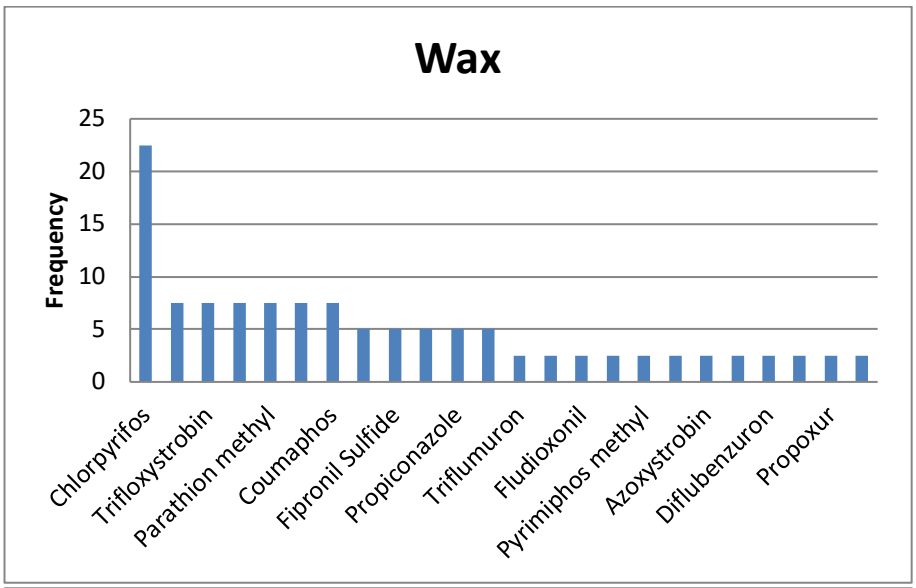
Following the on-going quality control recommendations of SANTE guidelines blanks and a single recovery experiment of all the studied analytes was introduced in each analysis batch. Percentage recoveries of these experiments were used in control charts to determine if samples needed to be re-analyzed.

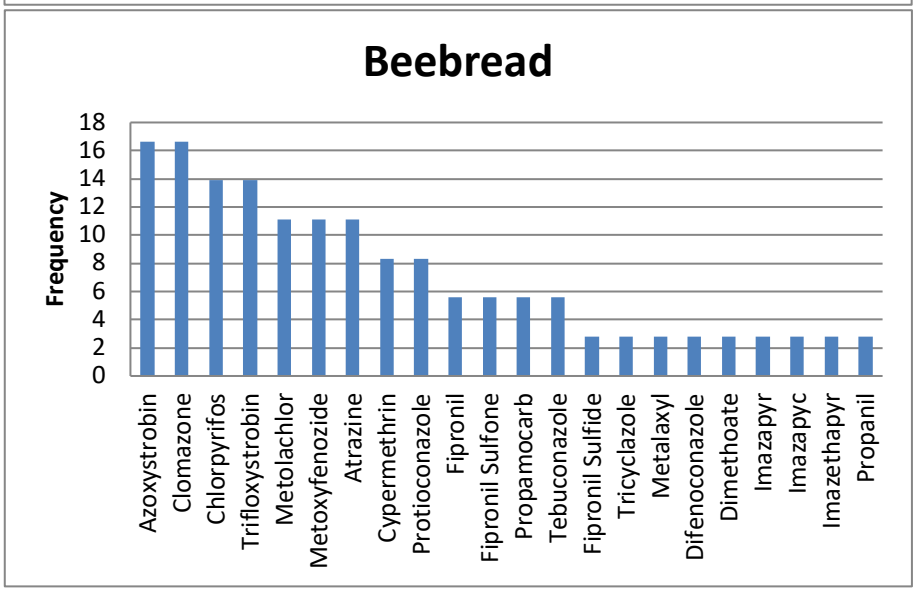
Pesticide residues findings

From the 44 pesticides and metabolites found 10 were herbicides, 15 fungicides and 19 insecticides. Among the most critical for bees, fipronil and its metabolites, chlorpyriphos, bifenthrin, cypermethrin, parathion methyl, acephate, dimethoate, ethion and propoxur were found. Concentrations ranged from 0.001 to 0.05 mg kg^{-1} with the exception of 3 wax samples which had

higher concentrations. As shown in figure 2, the highest frequencies and number of detections were found in beebread followed by beeswax. This is in accordance with our previous monitoring study performed from 2014 until 2017 (NIELL et al., 2018). Pesticide findings were different according to the beehive matrix analyzed. In wax and honey insecticides accounted for 50% of the active principles, while herbicides and fungicides represented the 75% of the compounds detected in beebread. The diversity of insecticides detected in honey was unexpected. Most of the agrochemicals detected in honey had pKows higher than 2 including the herbicide metolachlor, which was the most frequent agrochemical in it.

The pesticides profile of each apiary reflected the pesticide´s use of its surrounding ecosystem. For example clomazone, tricyclazole and propanil are typical agrochemicals from rice productive system. Ethion, pyrimifos methyl and cypermethrin are used to fight ticks. Metolachlor, chlorpyrifos and cypermethrin are used in many productive systems but mainly in soybeans, corn and artificial meadows. The fungicides from the strobilurins and CytP450 inhibitors families found also correspond to the different agroecosystems evaluated. The mixtures of them with some insecticides are of special concern for bee safety.





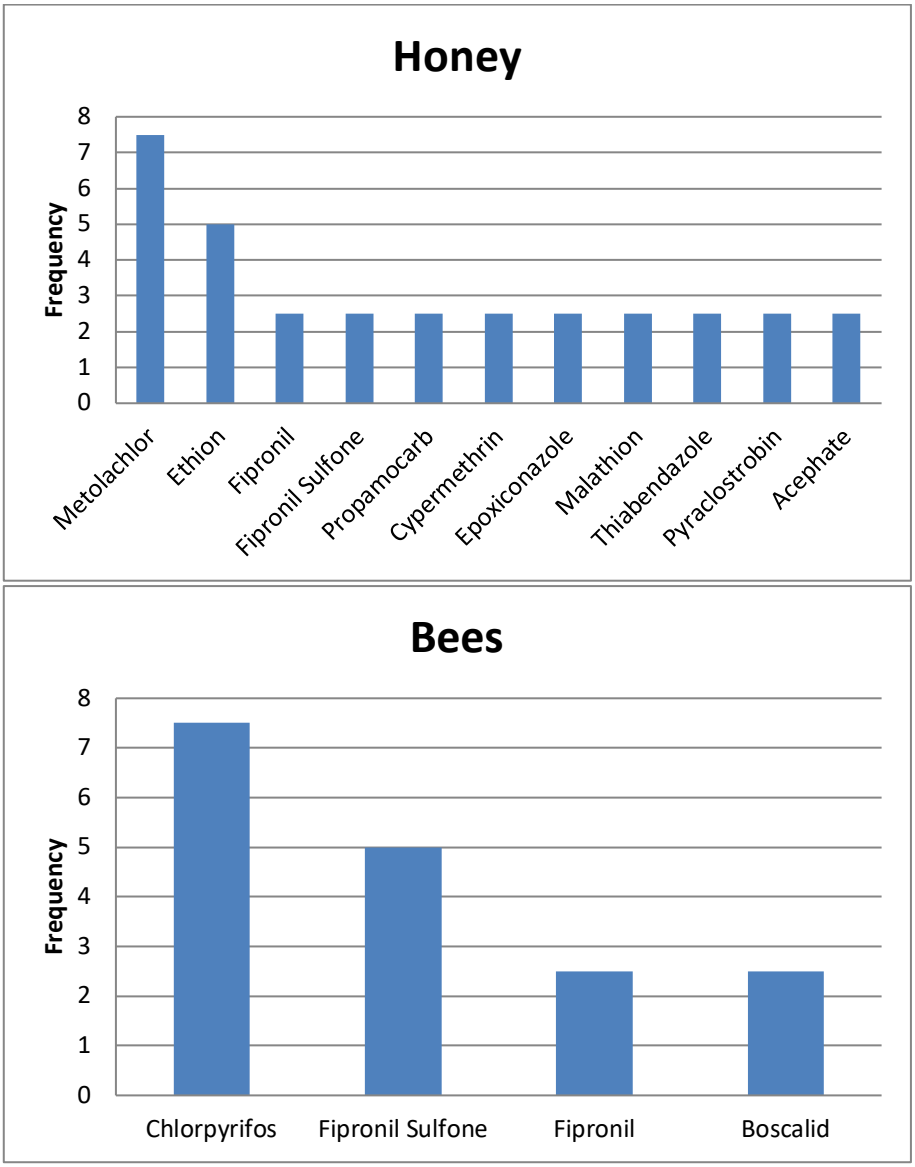


Figure 2 *Frequencies of pesticide residue findings in wax, beebread, honey and bees.*

Melissopalynological findings

The most important botanical resources of the basin that bees used to produce honey were: Eucalyptus sp., Lotus sp. meadows, Acacias (Fabaceae), wildly grown *Brassica napus* and Chircales (Asteraceae). From the crops grown in the analyzed area, the bees used the artificial meadows of *Lotus* sp, *Trifolium pratense* and soybeans, *Glycine max*. The richness of botanical species used by bees in “Laguna del Cisne” was 18 species, while outside the basin it was 28 species.

This monitoring established a useful baseline for the future study of the productive process of the basin. The results show that plant protection products are still intensively employed in the region. Honey bees and hive products were useful biomonitors of the pesticide´s use in the agroecosystems under study. A key point of this work was the involvement and dialogue between producers and academia in order to advance towards effective bee protection.

Oral Presentation

Acknowledgements

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References

ANASTASSIADES, M.; E. SCHERBAUM; B. TAŞDELEN & D. ŠTAJNBAHER, 2007: Recent Developments in QuEChERS Methodology for Pesticide Multiresidue Analysis. Pesticide Chemistry. p. 439–458, DOI: https://doi.org/10.1002/9783527611249.ch46.

HRYNKO, I.; P. KACZYŃSKI & B. ŁOZOWICKA, 2021: A global study of pesticides in bees: QuEChERS as a sample preparation methodology for their analysis – Critical review and perspective. Science of the Total Environment. **792**, DOI: 10.1016/j.scitotenv.2021.148385.

LOUVEAUX, J.; A. MAURIZIO & G. VORWOHL, 1978: Methods of Melissopalynology. Bee World. **59** (4), 139–157, DOI: 10.1080/0005772X.1978.11097714.

MURCIA-MORALES, M.; H. HEINZEN; P. PARRILLA-VÁZQUEZ; M. del M. GÓMEZ-RAMOS & A. R. FERNÁNDEZ-ALBA, 2022: Presence and distribution of pesticides in apicultural products: A critical appraisal. TrAC Trends in Analytical Chemistry. **146**, 116506, DOI: 10.1016/J.TRAC.2021.116506.

NIELL, S; F. JESÚS; C. PÉREZ; Y. MENDOZA; R. DÍAZ; J. FRANCO; V. CESIO & H. HEINZEN, 2015: QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. Journal of Agricultural and Food Chemistry. **63** (18), DOI: 10.1021/acs.jafc.5b00795.

NIELL, Silvina; V. CESIO; J. HEPPERLE; D. DOERK; L. KIRSCH; D. KOLBERG; E. SCHERBAUM; M. ANASTASSIADES & H. HEINZEN, 2014: QuEChERS-Based Method for the Multiresidue Analysis of Pesticides in Beeswax by LC-MS/MS and GC×GC-TOF. Journal of Agricultural and Food Chemistry. **62** (17), 3675–3683, DOI: 10.1021/jf405771t.

NIELL, Silvina; F. JESÚS; R. DÍAZ; Y. MENDOZA; G. NOTTE; E. SANTOS; N. GÉREZ; V. CESIO; H. CANCELA & H. HEINZEN, 2018: Beehives biomonitor pesticides in agroecosystems: Simple chemical and biological indicators evaluation using Support Vector Machines (SVM). Ecological Indicators. **91**, 149–154, DOI: https://doi.org/10.1016/j.ecolind.2018.03.028.

NIELL, Silvina; F. JESÚS; N. PÉREZ; C. PÉREZ; L. PAREJA; S. ABBATE; L. CARRASCO LETELIER; S. DÍAZ; Y. MENDOZA; V. CESIO & H. HEINZEN, 2017: Neonicotinoids transference from the field to the hive by honey bees: Towards a pesticide residues biomonitor. Science of the Total Environment. **581**–**582**, 25–31, DOI: 10.1016/j.scitotenv.2017.01.011.

PORRINI, C.; S. GHINI & S. GIROTTI, 2002: Use of honey bees as bioindicators of environmental pollution in Italy C. PORRINI, S. GHINI, S. GIROTTI, AG SABATINI. In: *Honey Bees*. CRC Press, p. 200–261.

Poster

Section 1 - Non-Apis bees

Leafcutter bee *Megachile rotundata* semi-field test design

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Abstract

With the 2014 published draft guidance document for higher Tier II risk assessment of pollinators, non-Apis bees came into regulatory focus. A semi-field test design with the red mason bee *Osmia bicornis* L. was ringtested by the ICPPR non-Apis working group in 2016 and 2017 and presented and published with a recommendation for a semi-field test design in 2021 (Franke et al 2021). So far, only a few bee species other than honey bees are in the focus for risk assessment studies worldwide, but only little is known about the toxicity of Plant Protection Products (PPPs) for other solitary bee species. Non-Apis bees comprise a wide range of body sizes as well as biological and life history traits which may result in differences in sensitivity and exposure routes in comparison to honey bees. In the EFSA Bee GD it was advised to consider not only honey bees, but also solitary bees in the plant protection product risk assessment.

With the knowledge on differences in exposure pathways and life-history traits between the two managed solitary bee species *Osmia bicornis* and the alfalfa leafcutter bee *Megachile rotundata* F. (Sgolastra et al 2019), it is expected that the same PPPs will impact those species differently. In addition, a higher sensitivity of Megachile species to selected PPPs due to a lower detoxification ability was estimated (Hayward et al 2019). The main objective of the test was the methodological development of a standardised Tier II study semi-field test design based on the recommended concept for *O. bicornis* and to include scenarios for other potential routes of exposure for non-Apis bees towards PPPs to account for these data gaps and uncertainties in a regulatory context and to provide further solutions for the reliability of risk assessment for solitary bees.

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

Introduction

Ring-test studies with solitary bees were conducted by the ICPPR non-Apis working group to develop a general test set-up, based on the draft EFSA guidance document, OEPP/EPPO Guideline No. 170. As test organism the mason bees *O. bicornis* and *O. cornuta* were selected. In a next step, the leafcutter bee *Megachile rotundata* was chosen to test, if the recommended study design would also be feasible for other solitary bee species. *Megachile rotundata* was chosen because it is polylectic and native to Europe and cocoons are available from commercial suppliers. The natural life cycle (Figure 1) can be either bivoltine or univoltine. The flight activity of adults is between June to August. The bees start to emerge from cocoons, in which they stay as dormant overwintering pre pupae. Males are emerging a few days before the females (proterandry). After mating several times, the females start to build nests in pre-existing cavities using leaves as nesting material such as alfalfa. Each female builds up to 30-50 brood cells consisting of a provision of pollen mixed with nectar and a

single egg (Mader et al. 2010). Only the females take care of the brood, meaning that reproductive success mainly depends on the vitality of the females.

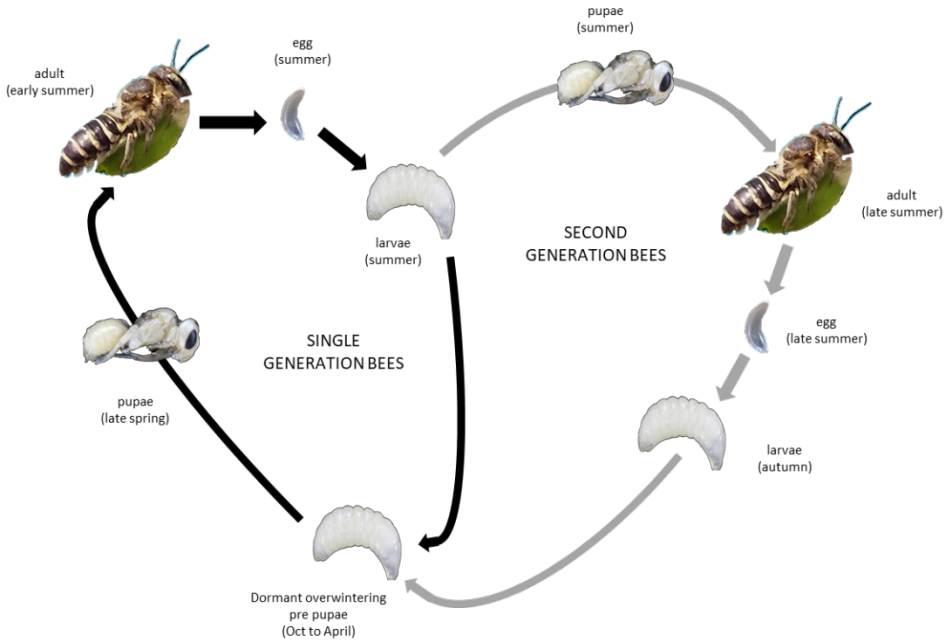

Figure 1 *Life cycle of the alfalfa leafcutter bee* Megachile rotundata

Material and methods

In this study design, *Megachile rotundata* bees 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 4 replicates with a water treated control and 4 replicates with a toxic reference item sprayed treatment (dimethoate with a concentration of 75 g a.i./ha). After the application, 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 construction and building of brood cells. 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 to calculate the reproduction rate.

Nest occupation (nesting activity): was assessed by counting the number of females occupying the cavities inside the nesting units after the daily end of bee flight. In this way, the establishment of females was monitored before the application. After application, the nest occupation was assessed in regular intervals as an indirect measure of mortality until the end of the exposure phase in the tunnels.

Flight activity: was noted shortly before the application to ensure a sufficient exposure of adult bees and directly after the application to assess sublethal effects. To assess flight activity, the number of females entering the nesting cavities in a defined time interval was counted.

Cell production / Reproductive performance (fecundity): was assessed by counting the number of cells built in the nesting cavities after application. This was done by marking on a transparent sheet covering the cavities. The total number of produced cells in the toxic reference item was compared to the control to determine, whether the toxic reference item had an impact on the offspring population size (“cell production per nesting unit”). The reproductive performance (fecundity) of female bees was calculated as “cell production per nesting female”.

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The assessed endpoints were evaluated with respect to their potential for the use in the risk assessment of PPPs.

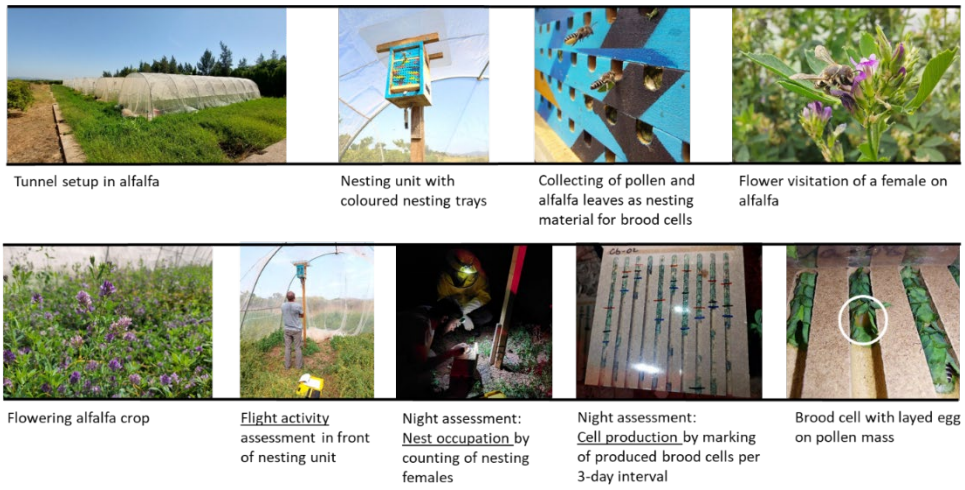

Figure 2 *Set-up and methodological practice*

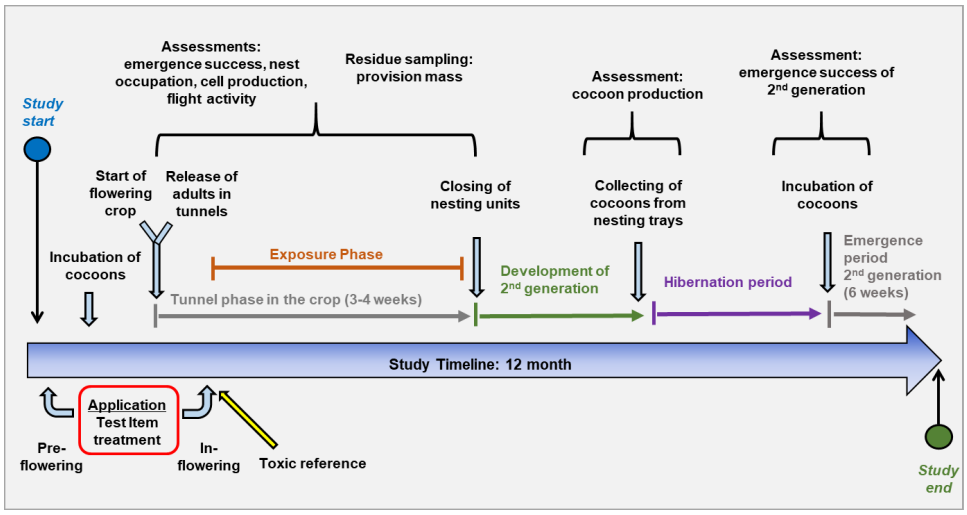

Figure 3 *Time schedule for semi-field trials with solitary bees*

Results

The flight activity was similar in the control and the dimethoate treatment before application and reached maximum values of 15 entering females per minute. After application, flight values decreased strongly for the dimethoate treatment and were statistically significant lower ongoing from 0 days after application (DAA) (Figure 4). Nest occupation values (number of actively nesting females in cavities) were statistically significantly reduced in the dimethoate treatment after application (Figure 5). In the control treatment, nest occupation ranged from 40 to 60 from -2 DAA to 5 DAA and then decreased with lower availability of food and nest provision resources in the tunnel environment. The mean cell production per nesting unit was strongly and statistically significantly reduced in the dimethoate treatment for cohorts of 0-2, 3-5 6-8 and 9-11 DAA (Figure 6) due to a decreasing number of nesting females after the application. If related to the number of nesting females (Figure 7), after an initial statistically significant impact in the first cohort (0-2 DAA), the number of cells produced per nesting female was similar to the control values. 94 % of nesting females died after the application of dimethoate. The surviving 6 % of females was actively nesting again after a 2-day-inactivity-period. Accordingly, the number of cells produced per nesting female

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was also statistically significantly reduced in the first assessment interval after application (0-2 DAA), but not from 3 DAA until the end of the study.

Results for the cocoon production and for the emergence success of the 2nd generation are not available yet, they will be evaluated in next steps.

Table 1 *Flight activity at nesting units.*

Flight activity				
	Control		**Dimethoate (75 g a.i./ha)**	
	Mean	*SD*	**Mean**	*SD*
Mean number exposure phase	**8.2**	*1.1*	**1.5 ***	*0.4*

p ≤ 0.05, two-tailed: pooled t-test

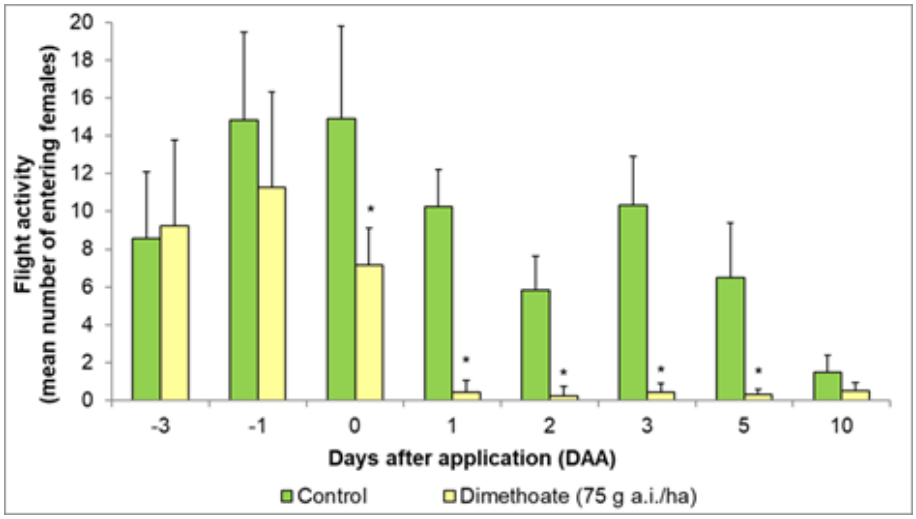

Figure 4 *Flight activity: Mean number of entering females per nesting unit in a time interval of 3x1 min (p ≤ 0.05, two-tailed: pooled t-test, Satterthwaite t-test)*

Table 2 *Nest occupation of females*

Nest occupation				
	Control		**Dimethoate (75 g a.i./ha)**	
	Mean	*SD*	**Mean**	*SD*
Mean number exposure phase	**34.1**	*3.7*	**3.3 ***	*2.6*

p ≤ 0.05, left-sided: pooled test

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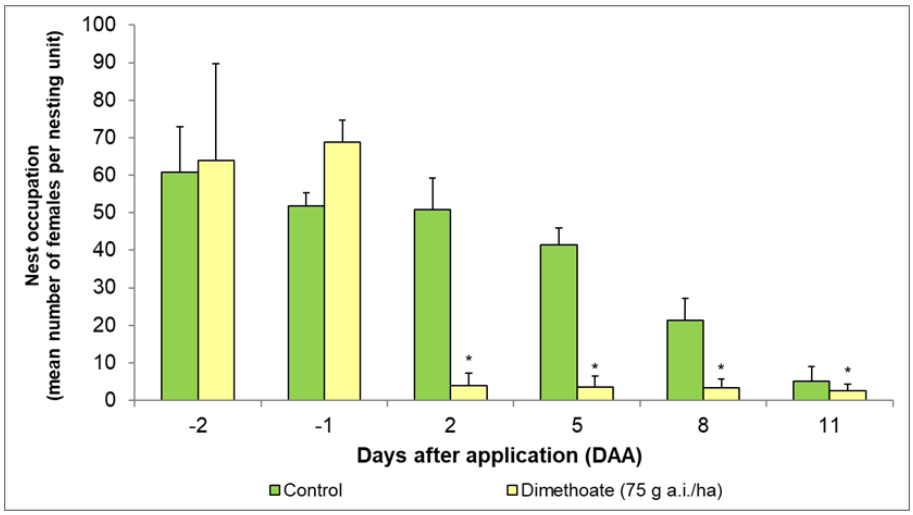

Figure 5 *Nest occupation: Mean number of females per nesting unit (p ≤ 0.05, left-sided: pooled t-test, Mann Whitney exact t-test).*

Table 3 *Cell production per nesting unit: Mean number of produced cells in a time interval of 3 days*

Cell production (numer of produced cells per nesting unit per 3-day-interval)				
Cohorts	**Control**		**Dimethoate (75 g a.i./ha)**	
DAA	**Mean**	*SD*	**Mean**	*SD*
0 to 2	**98.3**	*20.9*	**6.0 ***	*7.1*
3 to 5	**98.3**	*27.8*	**5.8 ***	*6.4*
6 to 8	**33.0**	*11.5*	**1.8 ***	*2.1*
9 to 11	**8.5**	*5.4*	**0.8 ***	*1.0*
Mean of exposure phase	**238.0**	*62.0*	**14.3 ***	*15.8*

(p ≤ 0.05, left-sided: pooled t-test, Satterthwaite t-test)

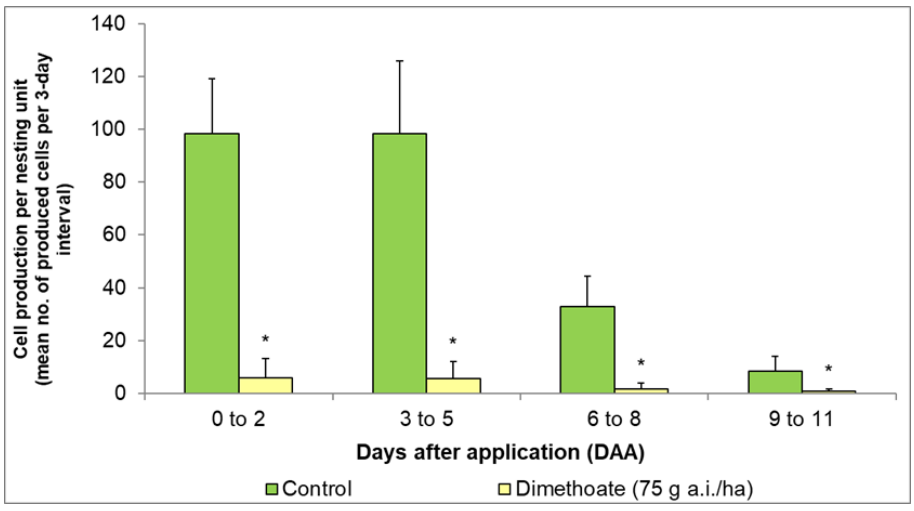

Figure 6 *Cell production per nesting unit: Mean number of produced cells in a time interval of 3 days*

Poster

Table 4 *Cell production per nesting unit: Mean number of produced cells in a time interval of 3 days*

Cell production (numer of produced cells per nesting female per 3-day-interval)				
Cohorts	**Control**		**Dimethoate (75 g a.i./ha)**	
DAA	**Mean**	*SD*	**Mean**	*SD*
0 to 2	**1.9**	*0.4*	**0.1 ***	*0.1*
3 to 5	**1.9**	*0.3*	**1.3**	*0.5*
6 to 8	**0.8**	*0.2*	**0.5**	*0.5*
9 to 11	**0.4**	*0.2*	**0.2**	*0.2*
Number per max. nesting female	**4.3**	*1.1*	**0.3 ***	*0.2*

($p \leq 0.05$, left-sided: pooled t-test)

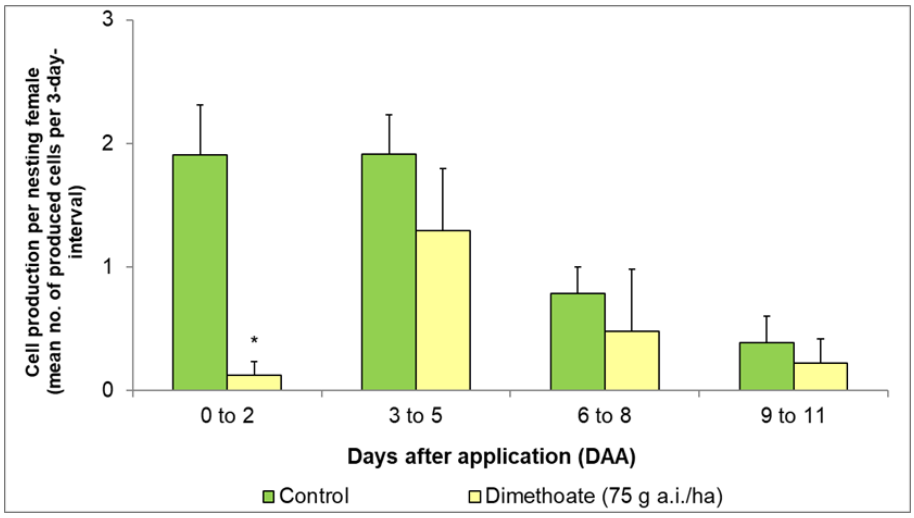

Figure 7 *Cell production per nesting female: Mean number of produced cells in a time interval of 3 days*

Table 5 *Calculations for the minimal detectable difference (MDD)*

Endpoint (Mean of exposure phase)	**Minimal Detectable Differnce (MDD)**
Flight activity	**17.7**
Nest occupation	**12.8**
Cell production (total no. per nesting unit)	**30.2**
Cell production (total no. per female)	**29.6**

Conclusions

Considering the presented results so far, it can be stated that the test design for mason bees (*Osmia* sp.) developed and recommended by the ICPPR non-Apis working group, is principally also a suitable design for semi-field trails with *Megachile rotundata* leafcutter bees. The test design was adapted with regard to the biology of the bees (i.e. incubation methodology, release technique etc.). All endpoints that have been recorded during the study provide a solid dataset for a valid evaluation of the results and demonstrate the potential and flexibility of this test design to open further

opportunities and provide solutions for a more reliable higher Tier II risk assessment of solitary bees, in particular leafcutter bee species.

References

EFSA (2013): Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal (2013) 11(7):3295, 266 pp.

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.

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.

MADER, E., SPIVAK, M., EVANS, E., 2010: Managing Alternative Pollinators: A Handbook for Beekeepers, Growers, and Conservationists, SARE Handbook 11, NRAES-186, co published by SARE and NRAES.

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.

Poster

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

Poster

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

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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

Poster

Method development for the acute contact test on the solitary bee *Megachiles rotundata*. – LD_{50} 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.

Poster

Section 2 - Risk Assessment/ Microbials

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

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Abstract

Semi-field studies of honey bee (*Apis mellifera* L.) colonies provide an important mean of assessing the effect of chemical exposure on brood development. Brood termination rate (BTR) is a common metric for evaluating effects of exposure; however, the index can be variable thereby limiting the extent to which studies can detect treatment-related effects. This study evaluated whether BTR in successive brood cycles differs between the enclosure phase vs monitoring phase of semi-field studies. The data indicate that for controls, differences were not statistically different; however, for colonies exposed to the reference toxicant fenoxycarb, BTR was significantly ($p < 0.05$) higher during the enclosure phase compared to the monitoring phase.

Keywords: honey bee, brood termination rate, BTR, consecutive brood cycles

Introduction

The potential impact of pesticides on developing honey bee (*Apis mellifera* L.) eggs, larvae and pupae (*i.e.*, brood) is often investigated under semi-field, worst-case exposure conditions, according to OECD GD 75 (OECD 2007), 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 high variability in the BTRs within the untreated control groups (Pistorius *et al.* 2012, Becker *et al*. 2015, Szczesniak *et al.* 2018). In contrast, control BTRs recorded in similar studies run under field conditions with free-flying honey bees are substantially lower and less variable (Lückmann & Tänzler 2020).

The current analysis by the International Commission for Plant Pollinator Relationships (ICP-PR) Bee Brood Working Group investigated the magnitude and variability of BTRs for a negative control and a reference chemical (*i.e.*, the insect growth regulator [IGR] fenoxycarb) over two subsequent brood cycles. The first started under semi-field conditions (*i.e.*, confinement of colonies in the tunnels), while the second was initiated under full-field conditions after completion of the first brood cycle when colonies have been removed from the tunnels to a monitoring site. In addition, the results obtained for the reference chemical fenoxycarb provide insight into the duration of effects caused by this chemical, an insect growth regulator (IGR) known to affect larval development. The results are discussed regarding the interpretation of BTRs gathered from such bee studies.

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Material and methods

For the evaluation, data from ten semi-field bee brood studies comprising a total of 44 control and 40 reference item nucleus colonies (application rate: 300 g fenoxycarb/ha, one study with 480 g fenoxycarb/ha) were available. The BTRs of marked eggs (BTR_{eggs}) at the end of the 1st (~BFD22) and 2nd brood cycle (~ BFD44) were analysed. The studies were conducted according to OECD GD 75 (OECD 2007) and current improvements (Pistorius *et al*., 2012, Becker *et al.* 2015) under Good Laboratory Practice (GLP) standards in Germany and Switzerland. A bee-attractive crop (*i.e*., *Phacelia tanacetifolia*) was used during the tunnel phase with an area between 82 m² and 126.5 m². The studies were performed between 2015 and 2020.

The statistical analysis was performed for a comparison of the BTRs of the 1st vs. 2nd brood cycle for each treatment group (two-sided) and for a comparison of the 1st and 2nd brood cycle for the control vs. fenoxycarb (one-sided greater). Normal distribution was tested with Shapiro-Wilk test, followed by Wilcoxon rank sum test with continuity correction for not normally distributed data, $\alpha = 0.05$. Program: R, version 4.0.5 (2021).

Results

The results (BTRS) are summarised in Table 1 and graphically illustrated in Figure 1.

Control:

In the 1st brood cycle, the mean BTR of 31.7% and proportion of colonies with BTRs ≤ 30% / ≤ 40% (*i.e*., 61% / 75%) were similar to historical control data as described by Becker *et al*. (2015) and Szczesniak *et al*. (2018) with a mean BTR of about 30% and proportions of about 61.5% to 65% and 77%, respectively.

In the 2nd brood cycle, the mean BTR of 22.1% was lower compared to the 1st brood cycle, but was not statistically significantly different. The mean BTR and proportion of colonies with BTRs ≤ 30% / ≤ 40% (*i.e*., 75% / 86%) were comparable to levels observed in free flying colonies as described by Lückmann & Tänzler (2020) with a mean BTR between 16% to 20% and proportions of about 80% to 90% and 87% to 90%, respectively. Finally, 86% of the colonies displayed a decrease in the BTR or the BTR remained at an already low level.

Reference item:

In the 1st brood cycle, the mean BTR of 71.4% was comparable with levele observed for historical reference item data as described by Becker *et a*l. (2015) (*i.e.,* 71%). Also, the proportion of colonies with BTRs $\geq$ 70% was comparable to historical data (*i.e*., 60%) compared to 58% (ICPPR unpubl.). The studies indicate that 13% of the colonies displayed BTRs $\leq$ 30% and 20% of the colonies had BTRs $\leq$ 40%.

In the 2nd brood cycle, the mean BTR of 26.4% and the proportion of colonies with BTRs $\leq$ 30% / $\leq$ 40% were similar to the control level. Almost no colonies with BTRs $\geq$ 70% were observed. The studies indicate that 83% of the colonies displayed a decrease in BTR and 55% of the colonies reached the control level of the 2nd brood cycle.

Figure 1 depicts box plots of control and reference colony BTRs for both brood cycles. The statistical analysis displayed a significant difference between the 1st and 2nd brood cycle ($p < 0.001$) within reference item group and between the control vs reference item for the 1st brood cycle ($p < 0.001$) but not for the 2nd cycle.

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Table 1 *Descriptive statistics of brood termination rate for honey bee eggs (BTR_{eggs}) in the control and reference item (fenoxycarb) group at two subsequent brood cycles.*

BTR_{eggs}°	Control		Reference item	
	1st brood cycle	2nd brood cycle	1st brood cycle	2nd brood cycle
Minimum [%]	3.7	3.3	32.8	3.4
Mean ± SD [%]	31.7 ± 28.4	22.1 ± 18.1	71.4 ± 29.6*,**	26.4 ± 19.2
Maximum [%]	100	46.0	100	90.1
Proportions of replicates with BTR_{eggs} ≤ 30% / ≤ 40%	61 / 75	75 / 86	13 / 20	65 / 80
Proportions of repl. with BTR_{eggs} ≥ 70%	11	5	60	3

*°calculated from all replicates; * = statistically significant different from the control (1st brood cycle), p <0.001; ** = statistically significant different between 1st and 2nd brood cycle (reference), p <0.001*

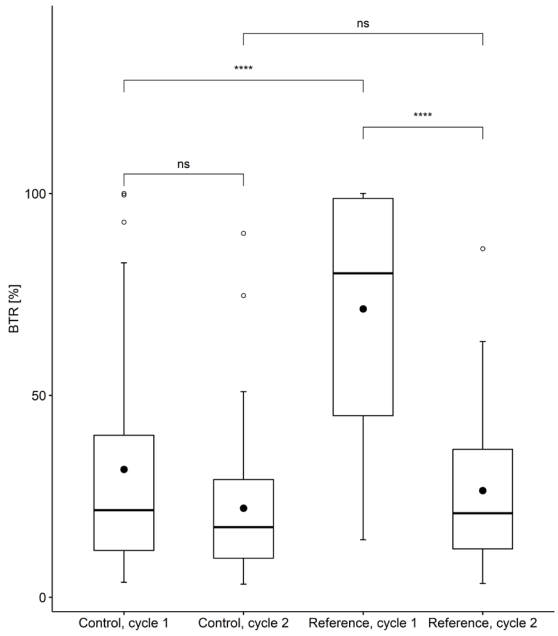

Figure 1 *Box plot of brood termination rate (BTR) for marked eggs in the control and reference item (fenoxycarb) group at two subsequent brood cycles (filled dots = mean, solid line = median, unfilled dots = outliers, ns = not statistically significant different, **** = statistically significant different with p <0.001)*

Discussion and Conclusion

The findings indicate that:

- the caging effect dissipates when honey bee colonies are removed from the tunnels

- the effects on the bee brood in the fenoxycarb group generally lasts for one brood cycle, dissipating in the subsequent cycle

- further investigations are needed (*e.g.*, on setups with chemicals that have proven long-lasting effects or effects persisting beyond the 1st brood cycle; reversed setup with 1st brood cycle started outside the tunnels followed by the 2nd brood cycle with a brood fixing evaluation under semi-field conditions)

- based on the available data, it is sufficient to analyse the detailed brood development during one brood cycle

- however, to broaden the database, more companies are asked to contribute their data sets for further evaluation.

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References

BECKER, R., LÜCKMANN, J., PISTORIUS, J., 2015: Effectiveness of method improvements of OECD GD 75 – Evaluation of the ICP-PR Bee Brood Working Group. Julius-Kühn-Archiv **450**, 83-92.

LÜCKMANN, J., TÄNZLER, V., 2020: Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate? Julius-Kühn-Archiv **465**, 91-95.

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

PISTORIUS, J., BECKER, R., LÜCKMANN, J., SCHUR, A., BARTH, M., JEKER. L., SCHMITZER. S. VON DER OHE. W., 2012: Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions. Julius-Kühn-Archiv **437**: 115-120.

SZCZESNIAK, B., PILLING, E., BOCKSCH, S., BECKER, R., LÜCKMANN, J., 2018: ICP-PR Bee Brood Working Group – Variability of brood termination rates in reference to validity criteria and limited effectiveness of method improvement in honeybee semi-field studies (OECD GD 75). Julius-Kühn-Archiv **462**: 111-115.

Poster

Effects of brood termination rate on colony viability – A BEEHAVE modelling study how timing, magnitude and duration of effects determine colony strength

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Abstract

The brood termination rate (BTR) investigated in higher-tier bee brood studies for plant protection product risk assessment is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult. It influences colony strength, and in turn pollination services, hive products and colony viability. According to the EFSA Bee GD (2013), a honey bee colony is regarded viable, if at least 5000 worker bees are recorded prior to hibernation. We investigate how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and therefore viability. For this purpose, we modified and applied BEEHAVE, a computer model to simulate honey bee colony dynamics. Our modifications allowed for in silico representations of higher-tier bee brood studies under semi-field conditions with the option to follow bee colony dynamics until the end of the season. We have found that bee colonies are rather resilient to an increased BTR, such that under common experimental conditions, the number of brood cells as well as the colony size can recover over time. Yet, if BTR was above ≥ 70% (approximately the effect size caused by the reference item fenoxycarb) for a long period of 20 days or the brood study was started late in the season (1st August), recovery was slow. Nevertheless, only if modelled experiments were started late in the season (1st August), there was a risk of colony sizes below 5000 worker bees before winter (31st October). This risk was found for treatments and control due to the seasonally reduced egg laying rate of the queen. Compared to the control the risk was only relevantly enhanced, if BTR was ≥ 70% for the entire brood cycle.

Keywords: Honey bees, BEEHAVE, model simulation, brood termination rate, colony strength

Introduction

The brood termination rate (BTR) investigated in higher-tier bee brood studies (such as the feeding studies according to Oomen *et al.* 1992 or Lückmann & Schmitzer 2019 as well as semi-field studies according to OECD GD 75 2007) for plant protection product risk assessment (RA) is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult. It influences colony strength, and in turn pollination services, hive products and colony viability. According to the EFSA Bee GD (2013) 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. We investigate how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and therefore viability.

Material and methods

The honey bee model BEEHAVE (Becher *et al*. 2014) simulates the colony dynamics in a bee hive in relation to the resource availability in the landscape. We adjusted the model (version 2016) to

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explicitly analyse the impact of BTRs at different magnitudes, at different starting times in the season and for different durations on the amount of bee brood shortly after the start of brood termination and on the colony strength after two brood cycles as well as shortly before overwintering (Table 1). In this context, we aimed at keeping the modelling study qualitatively comparable to typical field or semi-field toxicity test scenarios.

Table 1 *Full factorial design of parameter variation for BEEHAVE simulations.*

Parameter	Values	Rational
BTR [%]	Control	Default values from BEEHAVE
	0	No BTR (even less than control)
	20	Average BTR of the control in field experiments*
	30	Average BTR of the control in tunnel experiments*
	50	-
	70	Average BTR of positive reference in tunnel experiments**
	100	BTR removing entire brood
Starting time of BTR modification [day of year]	1st June	Typical start date of tunnel experiments
	1st July	Typical start date of tunnel experiments
	1st August	Late start date of tunnel experiments
Duration of modified BTR [d]	10	Covering egg and larval feeding stage
	20	Covering almost one full brood cycle; duration of effects caused by the insect growth regulator fenoxycarb **
Time of measurement [days after application]	5	Covering development of egg and young larvae
	20	Covering almost one brood cycle
	44	Covering two brood cycles
	31st October	Before hibernation

* see Lückmann & Tänzler (2020); ** see Lückmann et al. (2023)

We accounted for test scenarios in terms of firstly setting a fixed day in the season, when the test started. On this day, we adjusted the size of the colony to approximately 6000 bees, which is the minimum colony strength according to OECD GD 75 (2007). Simulation models as well as natural systems respond to abrupt artificial changes, such as the reduction of colony sizes. To ensure natural colony composition and to minimize disturbance of the colony dynamics, we applied a stepwise approach. Firstly, we started BEEHAVE with slightly modified standard settings (without *Varroa* infestation or bee keeping activities such as honey harvesting, colony merging, bee feeding – see Table 2). The modifications helped to isolate our study target, the effect of BTR, from other complex processes and their interactions. Secondly, we calculated the reduction factor *rf* as the ratio of 6000 bees and the simulated total hive size at BTR start. Thirdly, the number of in-hive individuals at each age stage was multiplied with *rf*, which proportionally reduced the number of larvae, pupae, nurse bees and drones. To adjust the number of forager squadrons, a proportion of *rf* squadrons was randomly selected. Also honey and pollen stores were adapted by multiplication with factor *rf (*see also Preuss *et al.* 2022*)*. The procedure resulted in hives of approximately 6000 worker bees. In order to account for variability in beehive dynamics, we repeated the procedure eight times. Each replicate provides slightly different initial conditions for the BTR simulation experiments. Practically the replicates can be considered as different test hives in an experiment.

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Table 2 *Changes to BEEHAVE default settings*

Parameter	BTR analysis	Default settings
allowreinfestation	FALSE	TRUE
dronebroodremoval	FALSE	TRUE
efficiencyphoretic	0	0.05
honeyharvesting	FALSE	TRUE
mergeweakcolonies	FALSE	TRUE
n_initial_mites_healthy	0	10
n_initial_mites_infected	0	10
rand_seed	0	1
stopdead	TRUE	FALSE
swarming	No swarming	Swarming (parental colony)
treatmentday	0	180
treatmentduration	0	20
x_days	151	161

BTR was modelled as a daily egg mortality at the day of egg-laying. This approach ignores that in reality bee brood might be terminated at any point during the twentyone-day brood cycle, and a later termination is connected to a higher loss of nursing investment. However, in the context of plant protection product application, the assumption was deemed appropriate, because compounds predominately affect the uncapped young larvae (see Lückmann & Schmitzer 2019). We considered effects of lasting increased BTR by applying the egg mortality for several days. This is a conservative assumption in the context of plant protection product applications, because usually effect strength declines over time.

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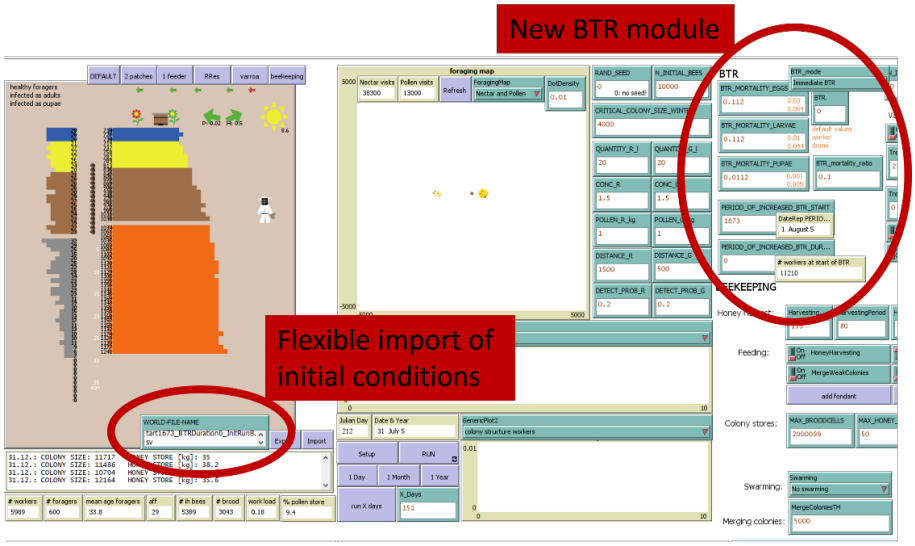

Figure 1 *Screenshot of the modified BEEHAVE software. Red circles indicate the new brood termination rate module and the possibility to flexibly import pre-defined initial conditions via NetLogo world files.*

The simulation experiments were conducted in Netlogo (Wilensky 1999), the programming platform, in which the BEEHAVE software is implemented. Netlogo provides the tool 'Behaviour space', which supports parameter sweeping, *i.e.*, the program automatically varies specified parameters. We varied the start and duration of a BTR period as well as the BTR strength in a full-factorial design. The tested parameter values are described in Table 1. In the model simulations, we accounted for natural variability in two ways: (1) We used the eight replicates of starting conditions for the simulation experiments. (2) We repeated the experiment for each parameter set 10 times, to account for the variability during and after the experiment. This resulted in 8 × 10 = 80 replicates per parameter set.

As the 'Behaviour space' cannot directly accommodate for the variation of initial conditions, we inserted the varied parameters in the so-called NetLogo world, using statistical software R (R Core Team 2022). Each of the modified worlds was then imported by 'Behaviour space' and automatically processed.

For the analysis, we monitored the simulated amount of bee brood shortly after the start of the impact *(i.e.*, after 5 and 20 days) and the colony strength after two brood cycles (*i.e.*, 44 days) as well as shortly before overwintering (*i.e.*, at the end of October). Finally, we estimated the proportion of colonies with a colony size lower than 5000 workers at the end of October. The proportion was calculated as the number of replicates with colony size below 5000 on 31st of October divided by the 80 replicates.

Results

Impact on the brood

Simulated BTR reduced the number of brood cells (including open and capped cells) in a colony in an effect size staggered way (Figure 2). With higher BTR, the brood cell reduction was stronger.

Five days after the start of the BTR manipulation period (Figure 2, left panel), the absolute reduction of the median number of brood cells started from different levels, amounted approximately 4000 cells (difference between minimum and maximum number) and was indedependent from the season, when the increased BTR was simulated (upper to lower rows).

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At day 20 (Figure 2, right panel), the number of brood cells was still decreased with increasing BTR. Yet, seasonality and duration of BTR increase became more influential. With the shorter BTR increase period of 10 days (Figure 2, left figure column) and low to intermediate BTR strength (*i.e.*, 20 to 50%), the number of brood cells was similar to control (except at a study start on 1st August), which reflects that the colony fully recovered the brood losses within the ten days after the end of the brood reduction period. The potential for recovery was slightly higher when BTR increased earlier in the season compared to an increase starting at the beginning of August.

For the longer BTR period (20 days – Figure 2, right figure columns), the reduction of the number of brood cells was stronger when brood was counted after 20 compared to an evaluation after 5 days. Thus, brood reduction accumulated.

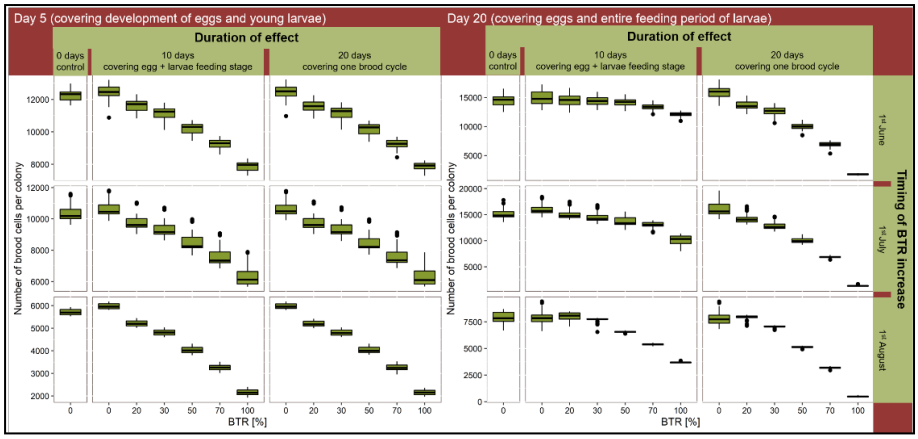

Figure 2 *Sensitivity of the number of brood cells to BTR counted at day 5 (left pannel) and at day 20 (right panel) of the onset of changed BTR. Figure columns show BTR increase periods of 10 days (left column) and 20 days (right column). Figure rows indicate the start day of the BTR increase period (upper row 1st June, middle row 1st July, bottom row 1st August). Boxes show median (central line) and span the 50% quantiles, whiskers roughly indicate the the 95% confidence interval and dots the extremes. Note that the number of brood cells is slightly higher with a BTR of 0% than in control, which reflects that natural brood mortality was ignored in BTR manipulations compared to control.*

Impact on the colony strength

BTR increase could affect colony size over intermediate time periods and to a minor exent until the end of season (Figure 3).

At day 44, *i.e.*, two brood cycles after the start of BTR increase (Figure 3, left panel) the number of worker bees was considerably lower than in control, if for a period of 20 days BTR was intermediate or high, independently when the increase of BTR started. A shorter period of 10 days of BTR increase reduced the number of worker bees only, if BTR increased intermediately or highly late in the season (1st August). Instead, almost no effects were found for a 10-day increased BTR starting at 1st June or 1st July, which means that colonies recovered from even severe impacts within two brood cycles (1.5 months).

Consequently, hardly any effects from periods of increased BTR up to 70% on colony size were found by the end of the season (1st October). Only from BTRs of 100% over 10 days slight differences occurred and from high BTRs of ≥ 70% over 20 days the colonies did not recover.

If studies started 1st June or 1st July, all colonies displayed a strength ≥ 5000 workes bees, independent the magnitude and duration of increased BTRs. But colonies remained particularly small

in the control and the treatment if studies started 1st of August, due to the seasonally reduced egg laying rate of the queen.

There was a risk that colony sizes dropped below 5000 worker bees (dotted line in Figure 3), which is an assumed realistic threshold for viable hibernation (see EFSA 2013). For studies which started 1st of June or July no colony displayed a strength below the value, irrespectively the size and duration of the BTR. For studies which started 1st of August, the proportion of colonies below this strength was low (*i.e.*, < 10%) for the control, if the BTR increase lasted for only 10 days (irrespective its magnitude) or if BTR increase lasted for 20 days but its magnitude was equal or below 30% (Table 3). Lower proportions at higher BTRs in these cases were due to to chance. However, the proportion of colonies with less than 5000 worker bees increased strongly up to 80% if the magnitude of the BTR was 50% or above for 20 days.

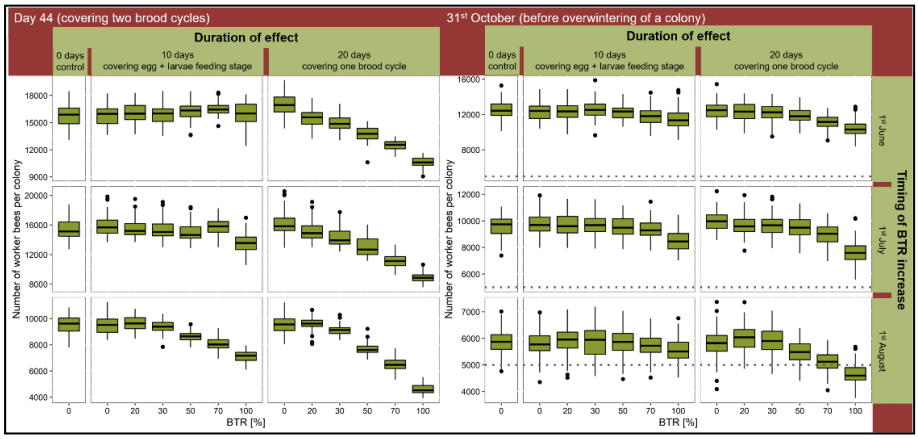

Figure 3 *Sensitivity of the number of worker bees to a BTR modification at day 44 of the onset of changed BTR (left panel) and on 31st October (right panel). Figure columns show BTR increase periods of 10 days (left column) and 20 days (right column). Figure rows indicate the start day of the BTR increase period (upper row 1st June, middle row 1st July, bottom row 1st August). Boxes show median (central line) and span the 50% quantiles, whiskers roughly indicate the 95% confidence interval and dots the extremes. The horizontal dotted line marks the threshold of 5000 worker bees for a viable overwintering colony size.*

Table 2 *Risk that colony size had dropped below 5000 worker bees before hibernation on October 31st.*

Starting date	BTR	Proportion of replicates with less than 5000 worker bees [%]	
		10 d BTR duration	20 d BTR duration
1st June	**all**	0.0	
1st July	**all**	0.0	
1st August	**CONTROL**	3.8	
	0	7.5	3.8
	20	7.5	2.5
	30	5.0	1.3
	50	8.8	13.8
	70	8.8	36.3
	100	8.8	80.0

Poster

Discussion and Conclusion

The BTR is currently one of the most important endpoints in higher tier (semi-) field studies for assessing plant protection product risk to honey bee brood according to Oomen et al. (1992), Lückmann & Schmitzer (2019) and OECD GD 75 (2007). However, the duration of these tests is restricted to roughly 1 month and does not cover an entire summer season. Thus, the meaning of measured BTRs in terms of colony dynamics and viability for the whole season is not well understood.

With the aim to understand the impact of different BTR magnitudes and durations on hibernation viability of bees, we in silico mimicked an OECD GD 75 (2007) test but continued simulations until onset of the hibernation period on 31st of October. We simulated the impact of an increase in BTR on the size of honey bee colonies across time scales with a customized version of the well established hive simulator BEEHAVE (Becher *et al.* 2014). In a full-factorial sensitivity analysis, we considered aspects of BTR strength and timing, as typical determinants of brood manipulation experiments.

During a period of increased BTR, we found quick exertion of effects, such that honey bee brood had strongly decreased after 5 or 20 days. Nevertheless, when the period of increased BTR ended, the impact on the brood ceased, and recovery of the hive started immediately. For example, 10 days after a 10-day period of weakly to intermediately increased BTR (*i.e.*, 20 to 50%), the number of brood cells had recovered to the control level.

However, if BTR was strong, even after the shorter 10-day period, the number of brood cells had not fully recovered. Particularly, later in the year, recovery seemed slower, probably due to the seasonally reduced egg laying rate of the queen. The seasonal pattern in the number of brood cells is also reflected in the number of worker bees one and a half months after the onset of BTR increase (*i.e.*, after two completed brood cycles).

Yet, if the intermediate or strong BTR duration already spanned over one brood cycle, the remaining brood cycle was insufficient to exert recovery at the level of worker bees. This can be understood in the extreme case of BTR = 100%, where brood of one complete cycle was terminated prematurely. Starting with the following cycle, brood cells were quickly filled, and this new brood developed normally. By the end of the second cycle, only the oldest of that new generation just matured and were counted as workers. Others were still in development. Therefore, compensation of adult mortality that occurred during the cycle, when no brood survived, can only be observed later (not shown here).

Hives usually recovered until the end of season to similar size as controls. Only at high BTR of ≥ 70%, rates known for highly toxic reference test compounds, minor effects still persisted.

Our results indicate that the timing of experiments is the most critical factor. Particularly, if the experiment was started late in the season (here 1st August), the colonies were small before hibernation. These small hive sizes were a result of the reduction of the colonies to 6000 worker bees at the beginning of the experiment late in the season, when egg laying of the queen has already been seasonally reduced. This combination of an initially small colony with low growth kept colonies considerably smaller than when experiments were started earlier in the season. That is why the preparation of new nucleus colonies normally takes place between April and beginning of July, which provides them enough time to develop to sufficiently large colonies before overwintering. Addtionally, bee keeping practice has demonstrated that summer brood interruption for subsequent

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Varroa treatment is a well know tool without impacting the colony strength before overwintering to a critical level (Büchler *et al.* 2020).

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References

BECHER, M., 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.

BÜCHLER, R., A. UZUNOV , M. KOVAČIĆ , J. PREŠERN , M. PIETROPAOLI , F. HATJINA , B. PAVLOV , L. CHARISTOS , G. FORMATO , E. GALARZA , D. GERULA , A. GREGORC , V. MALAGNINI , M. MEIXNER , N. NEDIĆ , Z. PUŠKADIJA , J. RIVERA-GOMIS , M. R. JENKO , M. I. SMODIŠ ŠKERL , J. VALLON , D. VOJT , J. WILDE, A. NANETTI, 2020: Summer brood interruption as integrated management strategy for effective *Varroa* control in Europe. Journal of Apicultural Research, **59** (5), 764-773.

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

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

LÜCKMANN, J., V. TÄNZLER, 2020: Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate? Julius-Kühn-Archiv **465**, 91-95.

LÜCKMANN, J., W. BLUHM, S. KIMMEL, T. STEEGER, S. WILKINS, 2023: Brood termination rate in honey bees in two consecutive brood cycles. Julius-Kühn-Archiv **474**, 100-103.

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

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

PREUSS, T. G., AGATZ, A., GOUSSEN, B., ROEBEN, V., RUMKEE, J., ZAKHAROVA, L., THORBECK, P., 2022: The $BEEHAVE_{ecotox}$ Model-Integrating a Mechanistic Effect Module into the Honeybee Colony Model. Environmental Toxicology and Chemistry 41: 2870 – 2882.

R CORE TEAM, 2022: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

WILENSKY, U., 1999: NetLogo. Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL. URL http://ccl.northwestern.edu/netlogo/.

Poster

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.

Poster

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

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Abstract

A data evaluation was conducted 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 sets for contact toxicity. For data sets with unbound honey bee LD_{50} values the data indicated similar or lower sensitivity of bumblebees versus honey bees by contact or oral exposure for all fungicides and herbicides. Likewise, similar or lower contact sensitivity of bumblebees than honey bees was found for all insecticides with definite honey bee endpoints. For oral exposure, this was also the case except for 5 active substances. Overall, the data supports for a wide range of chemistry that the honey bee is a sensitive surrogate test species for bumblebees. Therefore, routine application of a standard safety factor on honey bee endpoints in context of plant protection product risk assessment to cover bumblebee sensitivity is not justified. At present, honey bees seem to be an appropriate surrogate species to cover acute bumblebee sensitivity.

Keywords: honey bee, bumblebee, oral/contact toxicity, sensitivity ratio

Introduction

For decades, the honey bee, *Apis mellifera* L., has been used as standard test species in the context of registration of plant protection products in Europe and worldwide. According to the 'EFSA Guidance Document on the risk assessment of plant protection on bees' (EFSA 2013) and the new draft Guidance Document (EFSA 2022) there is now a requirement to also conduct studies with bumblebees and solitary bees (at least in acute oral and contact toxicity tests) and that these species should be considered in the risk assessment alongside honey bees.

However, the knowledge regarding the honey bee sensitivity versus the sensitivity of other bee species to plant protection products is limited in scientific publications, both in terms of numbers and types of active substances, and bee species (Arena & Sgolastra 2014; Devillers *et al.* 2003; Lewis & Tzilivakis 2019; Scott-Dupree *et al.* 2009; Thompson 2001; Thompson 2015; Uhl *et al*. 2019). In 2018, a preliminary data evaluation of 75 data sets for acute contact tests and 52 data sets for acute oral tests was conducted by CropLife Europe (previously ECPA) to compare the sensitivity of bumblebees

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(*Bombus terrestris* L.) to the sensitivity of honey bees (*Apis mellifera* L.). Using reliable honey bee and bumblebee data, the aim was to determine whether routine testing of *Bombus* species for all active substances and plant protections products was necessary (Dinter *et al*. 2018). The data sets comprised insecticides, fungicides, and herbicides in approximately equal numbers. Results indicated similar or lower contact and oral sensitivity of bumblebees compared to honey bees with one exception (an insecticide). For this insecticide, higher tier data under more realistic semi-field conditions indicated no negative impact on bumblebees at the maximum intended use rate. Thus, the authors conclude that in the majority of cases bumblebees are less sensitive than honey bees based on the acute toxicity assessment.

To support the findings from the initial work conducted by Dinter *et al.* (2018), the evaluation was repeated with more data generated since the last publication to increase the data set. The results are presented in this paper.

Material and methods

For this evaluation 97 data sets (i.e., endpoints for both bee species exist) were available for acute oral toxicity and 108 data sets for acute contact toxicity, derived from laboratory studies generated under Good Laboratory Practice (GLP). As most of the data is currently being used by the CropLife Europe companies in ongoing registrations efforts, the data for the different active substances and plant protection products cannot be made publically accessible at this time. Anonymised data are presented in the Appendix (Table 3, 4 and 5).

The data comprised 27 and 29 data sets for the oral and contact exposure to fungicides (e.g. benzamides, morpholines, organophosphates, pyrazoliums, strobilurins, triazolinthiones, triazoles), 42 and 41 for the oral and contact exposure to herbicides (incl. one plant growth regulator (PGR), e.g. amides, benzamides, benzofurans, carbamates, carboxamides, chloroacetamides, diphenyl ethers, hydroxyanilides, hydroxybenzonitriles, imidazoles, organophosphates, oxyacetamides, phenylureas, pyridazinones, pyrimidinylsulfonylureas, strobilurins, sulfonylureas, triazinones, triazolopyrimidines, triketones and uracils), and 28 and 38 for the oral and contact exposure respectively to insecticides (incl. one nematicide, e.g. anthranilic diamides, carbamates, neonicotinoids, organophosphates, oxadiazines, pyrazoliums, pyrethroids, spinosyns) (Table 1). Attribution of test substances was done according to 'Pesticide Properties Data Base' of the University of Hertfordshire, UK (https://sitem.herts.ac.uk/aeru/ppdb/).

Overall, 56 different active substances (15 fungicides, 30 herbicides/PGR, 11 insecticides) and 47 formulated plant protection products (12 fungicides, 12 herbicides, 23 insecticides) were tested. For 7 test substances (2 fungicides, 1 herbicide, 3 insecticides/1 nematicide) an attribution to these classes was not possible due to a lack of information. Formulated mixtures of fungicides with insecticides were attributed to insecticides as they drive the toxicity. Studies on the acute oral and contact toxicity of honey bees were conducted according to OECD test guideline 213 and 214 (OECD 1998a; OECD 1998b). Bumblebee studies were conducted according to OECD test tuideline 246 and 247 (OECD 2017a; OECD 2017b) or draft versions of the test guidelines before formal adoption by OECD. To analyse the sensitivity of bumblebees in comparison to honey bees the sensitivity ratio (SR) of the honey bee LD_{50} value divided by the bumblebee LD_{50} value for each test substance was calculated. A SR <1.0 indicates lower sensitivity of bumblebees compared to honey bees while a SR >1.0 reveals a greater sensitivity of bumblebees. This approach was only applied for data sets with definitive endpoints for honey bees, irrespective whether the corresponding bumblebee endpoint

was discrete or unbound ('>') (Table 1). For insecticides, 85.7% of the oral endpoints and 84.2% of the contact endpoints fulfilled this criterion. Furthermore, in 87.5% and 81.3% of these cases for the oral and contact endpoints also the corresponding bumblebee endpoints displayed to be definite LD_{50} endpoints.

In contrast, for fungicides and herbicides very few honey bee data reported discrete endpoints. For those few values the corresponding bumblebee endpoints were unbound values. The same observation was made for all other honey bee endpoints, except two, one oral fungicide endpoint and one oral herbicide endpoint. As SRs for honey bees based on unbound values were regarded of limited value, conclusion from these 'greater than' results were rather drawn by assigning all LD_{50} values of the honey bee and bumblebees to toxicity classes, i.e., endpoint up to <2, 2 to <11.0, 11 to <50.0, 50 to <100 and ≥100 µg a.s./bee. This approach is similar to USEPA, Health Canada, California Department of Pesticide Regulation (2014) toxicity ratings based on the contact LD_{50} value (practically non-toxic (LD_{50} ≥11 µg/bee), moderately toxic (10.9 > LD_{50} >2 µg/bee), or highly toxic (<2 µg/bee).

Table 1 *Number of data sets for acute oral and contact toxicity studies with definite and unbounded '>'endpoints for honey bees.*

Use type	**Number of data sets [n]**		**Number of data sets with ‚=' endpoint for honey bees [n]**		**Number of data sets with '>' endpoint for honey bees [n]**	
	Oral toxicity	**Contact toxicity**	**Oral toxicity**	**Contact toxicity**	**Oral toxicity**	**Contact toxicity**
Fungicide	27	29	2	5	25	24
Herbicide	42*	41*	2	2	40*	39*
Insecticide	28°	38°	24	32	4°	6°
Total	97	108	28	39	69	69

* including data of one plant growth regulator, ° including data of one nematicide

Results

Sensitivity ratios for data sets with definitive endpoints for honey bees

For the limited number of fungicides (2 data sets for oral and 5 data sets for contact toxicity) and herbicides (2 data sets for oral and contact toxicity, each), all ratios indicated lower oral or contact sensitivity of bumblebees with SRs ≤ 0.4 (Table 2). Based on the calculated SRs for insecticides with definitive LD_{50} values for honeybees, 71% (17 out of 24) of the oral data sets and 100% (32 out of 32) of the contact data sets displayed similar or lower sensitivity of bumblebees compared to honey bees, i.e. SR ≤ 1.0 (Table 2, Figure 1). The maximum observed SR for the oral toxicity of insecticides was 5.1 with a 95th percentile of 4.9, for the contact toxicity it was 1.0 and 0.8, respectively. For the oral comparison, 7 out of the 24 data sets displayed SRs higher than 1.0. From these, 3 data sets only slightly exceeded the SR of 1.0 with values of 1.1, 1.3, and 2.0. Furthermore, 3 of the 7 data sets belong to the same active substance with two different formulations and with SRs between 3.3 to 5.1, and for one data set the ratio was 5.0 (Table 2).

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Overall, for 75% (21 out of 28) of the oral data with definitive endpoints for honey bees, and 100% of the contact data (n = 39), SRs were ≤ 1.0 indicating lower sensitivity of bumblebees. The 95th percentile for the first were 4.7 and for the latter 0.7.

Table 2 *Descriptive statistics of oral and contact sensitivity ratios (SR) of honey bee LD_{50} / bumblebee LD_{50} (SR <1.0 indicates lower sensitivity of bumblebees compared to honey bees.)*

Parameter	SR of honey bee LD_{50} /bumblebee LD_{50}							
	Fungicides		Herbicides		Insecticides		Overall	
	Oral ratio	Contact ratio	Oral ratio	Contact ratio	Oral ratio	Contact ratio	Oral ratio	Oontact ratio
95th percentile	0.4	0.4	0.4	0.4	4.9	0.8	4.7	0.7
median	0.3	0.3	0.3	0.4	0.6	<0.1	0.4	0.1
mean	0.3	0.3	0.3	0.4	1.2	0.2	1.0	0.2
max	0.4	0.4	0.4	0.4	5.1	1.0	5.1	1.0
min	0.3	<0.1	0.1	0.3	<0.1	<0.1	<0.1	<0.1
proportion of data sets with SR ≤ 1.0 [%]	100	100	100	100	71	100	75	100
data sets [n]	2	5	2	2	24	32	28	39

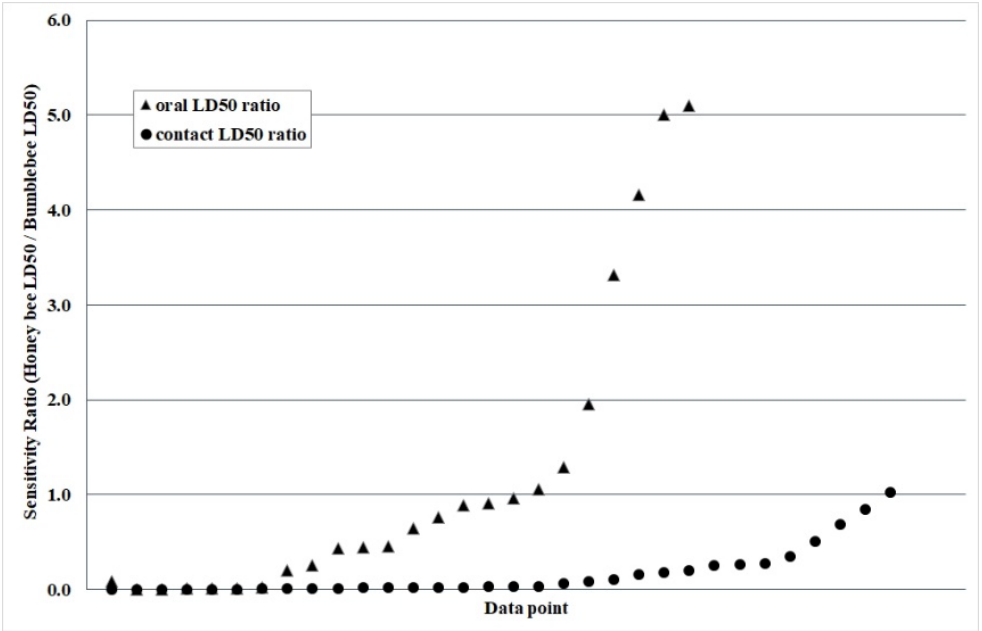

Figure 1 *Sensitivity ratio (SR) of honey bee oral and contact LD_{50} divided by oral or contact bumblebee LD_{50} for insecticides (n = 24 for oral and n = 32 for contact exposure). Data points at SR <1.0 indicate lower sensitivity of bumblebees compared to honey bees.*

Distribution of data if honey bee endpoint was unbound

For honey bee studies with unbound endpoints almost all corresponding bumblebee LD_{50} values were also above their maximum dose tested. Exceptions were observed for one fungicide (oral exposure), one herbicide (oral exposure) and three insecticides (two oral and one contact exposure), where definite endpoints were obtained. For the fungicide and the three insecticides, the LD_{50} values generated in the bumblebee tests were below those generated for honey bees.

Poster

The attribution of the fungicide and herbicide LD_{50} values to toxicity classes shows that a very high proportion of the endpoints was assigned to be '≥ 100 µg a.s./bee' for both bee groups (Figure 2). In more detail, the majority of oral and contact honey bee testing of fungicides and herbicides was performed at up to approximately 100 µg a.s./bee and a few at up to approximately 200 µg a.s./bee. In contrast, about 30 to 50% of bumblebee testing was performed at doses higher than 100 µg a.s./bee with highest doses up to approximately 400 µg a.s./bee.

For insecticides, four oral data sets and six contact data sets reported unbound values for honey bees, including the nematicide. Whereas for the contact exposure of bumblebees, the maximum dose tested was similar or higher compared to honey bees, i.e., SRs ranged between 0.5 and 1.1. Two oral data sets (belonging to different formulations of the same active substance) reported distinctly lower bumblebee LD_{50} values compared to those for honey bees. If these endpoints would be considered as definite values, the SRs would be 93.1 and 98.4.

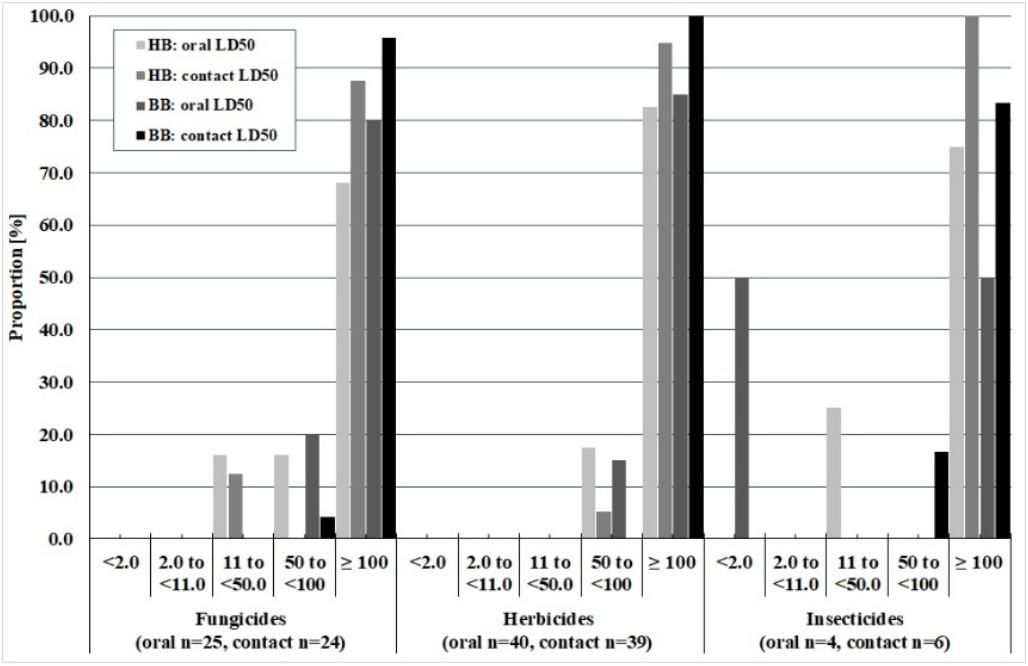

Figure 2 *Distribution of oral and contact honey bee (HB) and bumblebee (BB) LD_{50} values (in µg a.i./bee) to toxicity classes for fungicides, herbicides/PGR and insecticides/nematicide if the HB endpoint was unbounded. (Toxicity classes similar to USEPA, Health Canada, California Department of Pesticide Regulation, 2014 toxicity ratings based on the contact LD_{50} value (practically non-toxic (LD_{50} ≥11 µg/bee), moderately toxic (10.9 > LD_{50} >2 µg/bee), or highly toxic (<2 µg/bee)).*

Discussion

Across all available data sets of fungicides and herbicides, all contact data of insecticides and for most orally applied insecticides SRs ≤ 1.0 were calculated. Therefore, it can be concluded that in the majority of the available data sets bumblebees are similar or even less susceptible to plant protection products than honey bees, following acute exposure to the active substances and products tested, i.e., for the classes represented. In some cases, for insecticides with discrete LD_{50} endpoints, bumblebees may be up to five times more sensitive when exposed orally compared to honey bees. In case of the insecticide with unbound honey bee endpoints and assumed oral SRs of approximately 95, higher tier semi-field data with *B. terrestris* are available which indicate no negative impact on bumblebees or their colony development at the maximum intended use rate.

Poster

The distribution of LD_{50} values of fungicides and herbicides to toxicity classes showed that the majority of honey bee test have been performed at approximately 100 µg a.s./bee whereas a distinct number of bumblebee studies was performed at doses higher than this, even up to 400 µg a.s./bee. While the testing of 100 µg a.s./honey bee was in line with current OECD test guideline 213 and 214 (OECD 1998a; OECD 1998b) and high enough to cover most risk assessments according to EPPO (2010) more recent toxicity testing of bumblebees was often performed at higher doses to address and cover the conservative risk assessment proposals by EFSA (2013).

The body weight of an individual bee is an important factor influencing the LD_{50} and it is often negatively correlated with the sensitivity. Van der Steen (1994) has shown for bumblebees (B. terrestris) a correlation of the acute contact and oral toxicity of dimethoate with the bumblebee weight. The body weight of honey bee worker bees is about 100 mg (Schreiner 2012) while the body weight of bumblebee workers of B. terrestris may be up to 3-times higher (Van der Steen 2001). Therefore, the lower sensitivity of bumblebee workers compared to honey bee workers is likely to be influenced by the different body weight of the two bee species tested.

Our results are in line with several other findings. For example, Thompson & Hunt (1999) and Thompson (2001) showed for a limited number of data, in terms of number and type of insecticides, that insecticide toxicity was generally lower for bumblebees than honey bees, even when expressed based on body weight. This is supported by Devillers *et al*. (2003) who compared the effects of 32 pesticides and found no difference in sensitivity between *A. mellifera* and *Bombus* spp.. Lewis & Tzilivakis (2019) collated a dataset on basis of the Pesticide Properties Database (PPDB) from regulatory and peer reviewed articles covering 142 acute toxicity records for bumblebees and honey bees. They found that for the majority of the contact acute toxicity data pairs honey bees were indeed equal to or more sensitive to pesticides than bumblebees (84.6%). In terms of oral toxicity, for 75.8% of the data pairs, honey bees were more sensitive than bumblebees.

In a literature review, Arena & Sgolastra (2014) compared the pesticide sensitivity of the honey bee *A. mellifera* to 19 other bee species in the laboratory. This comparison included two other honey bee species (*A. cerana* F. and *A. florea* F.) and 17 non-*Apis* species from six systematic groups, covering social (five *Bombus* species and 7 species from the non-European stingless Meliponini bees) and non-social species. The majority of the 53 pesticides tested in this meta-study were insecticides, while only 2 were fungicides, and no data was available on herbicides. For their review the authors calculated the sensitivity ratio (R) between the endpoint (contact or oral LD_{50} or LC_{50} values) for *A. mellifera* and the other bee species in a total of 150 case studies. For five bumblebee species (*B. agrorum* F., *B. lapidaries* L., *B. lucorum* L., *B. terrestris* and *B. terricola Kirby*) the median R was 0.21 (analysed cases: 45) and the 95th percentile was 4.20 with a minimum of 0.001 and a maximum of 25.88 (oral and contact analysis combined). The median R for B. terrestris was 0.20 (analysed cases: 32) with a 95th percentile being 3.02, a minimum of 0.001 and a maximum of 25.88. Berenbaum & Johnson (2015) pointed out in their literature review the general deficit of detoxification genes for the honey bee *A. mellifera* relative to most other insect genomes which may be an evolutionary consequence of eusociality of the honey bee. This is likely to contribute to relative sensitivity of bees and i.e., the honey bee *A. mellifera* to different toxicants versus other bees and supports the regulatory use of the honey bee as a sensitive test species. At the same time the relative sensitivity of the honey bee verus other bee species may compensate for some of the differences in biology and exposure routes (e.g. potential exposure of bumblebee queens to soil residues to which honey bees get rarely exposed) that have been reviewed in detail by Gradish *et al.* (2019). During the last few years, a couple of higher tier testing approaches were investigated for bumblebees. Smagghe *et al.*

(2013) determined in a worst-case laboratory chronic feeding study – with test concentrations above predicted environmental concentrations – with microcolonies consisting of five newly eclosed workers of *B. terrestris* that the insecticide chlorantraniliprole had sublethal effects on reproduction (reduced drone production). Whereas semi-field tests under worst-case conditions at field rate with queen-right colonies of B. terrestris clearly demonstrated no effects of chlorantraniliprole on B. terrestris colonies including queen/male production, adult and larval survival and forager flight activity (Dinter & Brugger 2015; Dinter & Samel 2020). Overall, it can be concluded that the bumblebee contact sensitivity - when acutely exposed to fungicides, herbicides and insecticides - was lower in all cases compared to the honey bee. Also, in most cases the bumblebee oral sensitivity was lower than for the honey bee. For one active substance (with unbound oral endpoints) with SRs of approximately 95, higher tier semi-field data with *B. terrestris* did not indicate any negative impact on bumblebees and their colony development at the maximum intended use rate.

Therefore, routine regulatory testing of non-*Apis* bees i.e. of the bumblebee (*B. terrestris*) in context of registration of plant protection products for all type of active substances/products and/or using a standard safety of 10 on basis of honey bee endpoints is not justified on basis of available data review for a wide range of plant protection active substances or their plant proection products investigated. Similarly, a recent research paper indicates that also the solitary bees *Osmia bicornis* (L.) (also being proposed for standard regulatory testing by EFSA 2013 and EFSA 2022) seems not be significantly more sensitive via contact exposure than the honey bee when weight is considered (Uhl *et al*. 2019).

Conclusion

Overall, the current data supports that, for the currently registered classes of chemistry, the honey bee is a sensitive surrogate test species for bumblebees for the acute toxicity testing of plant protection products for which also a range of validated OECD test guidelines/guidances is available allowing for routine testing of chronic oral adult (OECD 2017c) and larval (OECD 2016) toxicity. Corresponding guidelines for chronic toxicity feeding testing of bumblebees are currently under development by ICPPR (International Commission for Plant-Pollinator Relationships) and will be available after the ongoing ring-tests demonstrate their reproducibility and repeatability at determining accurate endpoints.

References

ARENA, M., SGOLASTRA, F., 2014: A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology **23**, 324–334.

BERENBAUM, M.R., JOHNSON, R.M., 2015: Xenobiotic detoxification pathways in honey bees. Current Opinion in Insect Science **10**, 51-58.

DEVILLERS, J., DECOURTYE, A., BUDZINSKI, H., PHAM-DELEGUE, M.H., CLUZEAU, S., MAURIN, G., 2003: Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. SAR and QSAR in Environmental Research **14** (5–6), 389–403.

DINTER, A., BRUGGER, K.E., 2015: Chlorantraniliprole: Lack of effects on the bumblebee (*Bombus terrestris*) under semi-field conditions in *Phacelia tanacetifolia*. Hazards of pesticides to bees – Proceedings of the 12th International Symposium of the ICPPR Protection Group. Julius-Kühn-Archiv **450**, 208-217.

Poster

DINTER, A., SAMEL, A., 2020: Recent experiences with bumblebee (*Bombus terrestris*) semi-field tunnel testing following ICPPR Non-*Apis* 2016 and 2017 workshop recommendations to investigate the insecticide chlorantraniliprole. Hazards of pesticides to bees – Proceedings of the 14th International Symposium of the ICPPR Protection Group. Julius-Kühn-Archiv **465**, 113-121.

DINTER, A., ALIX, A., BECKER, R., CAMPBELL, P., MILES, M., PILLING, E., RUDDLE, N., SHARPLES, A., WEYMAN, G., OGER, L., 2018: Non-*Apis* (*Bombus terrestris*) versus honeybee (*Apis mellifera*) acute oral and contact sensitivity – Preliminary results of ECPA company data evaluation. Proceedings of the 13th International symposium of the International commission for plant-pollinator relationships (ICP-PR) Bee protection group, Valencia (Spain) 2017, Julius-Kühn-Archiv **462**, 170-172.

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

EFSA, 2022: Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bomus* spp. and solitary bees. DOI: 10.2903/j.efsa.201Y.xxxx10.2903/j.efsa.201Y.xxxx

EPPO (EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION), 2010: Environmental risk assessment scheme for plant protection products, chapter 10: honeybees. EPPO Bulletin **40**, 323-331.

GRADISH, A.E., VAN DER STEEN, J., SCOTT-DUPREE, C.D., CABRERA, A.R., CUTLER, G.C., GOULSON, D., KLEIN, O., LEHMANN, D.M., LÜCKMANN, L., O'NEILL, B., RAINE, N.E., SHARMA, B., THOMPSON, H.,2019: Comparison of pesticide exposure in honey bees (Hymenoptera: Apidae) and bumble bees (Hymenoptera: Apidae): Implications for risk assessments. Environmental Entomology **48**(1), 12–21.

LEWIS, K.A., TZILIVAKIS, J., 2019: Wild bee toxicity data for pesticide risk assessments. Data 2019, **4**(3), 98.

OECD, 1998a: Test No. 213: Honeybees, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 1998b: Test No. 214: Honeybees, Acute Contact Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2016: Guidance document on honey bee larval toxicity test following repeated exposure. Series of testing and assessment, Number 239. ENV/JM/MONO 34: 41, URL: https://one.oecd.org/document/ENV/JM/MONO(2016)34/en/pdf.

OECD, 2017a: Test No. 246: Bumblebee, Acute Contact Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2017b: Test No. 247: Bumblebee, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2017c: Test No. 245: Honey Bee (*Apis Mellifera* L.), Chronic Oral Toxicity Test (10-Day Feeding), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

SCHEINER, K., 2012: Birth weight and sucrose responsiveness predict cognitive skills of honeybee foragers. Animal Behaviour **84,** 305-308.

SCOTT-DUPREE, C.D., CONROY, L., HARRIS, C.R., 2009: Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). Journal of Economic Entomology **102**(1), 177-182.

SMAGGHE, G., DEKNOPPER, J., MEEUS, I., MOMMAERTS, V., 2013: Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. Pest Management Science **69**, 787-791.

Poster

THOMPSON, H.M., HUNT, L. V., 1999: Extrapolating from Honeybees to Bumblebees in Pesticide Risk Assessment. Ecotoxicology **8**(3), 147-166.

THOMPSON, H.M., 2001: Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus sp.*). Apidologie **32**(4), 305-321.

THOMPSON, H.M., 2015: Extrapolation of acute toxicity across bee species. Integrated Environmental Assessment and Management **12**(4), 622-626.

UHL, P., AWANBOR, O., SCHULZ, R.S., BRÜHL, C.A., 2019: Is *Osmia bicornis* an adequate regulatory surrogate? Comparing its acute contact sensitivity to *Apis mellifera*. PLOS ONE **14**(8), e0201081.

USEPA, HEALTH CANADA, CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION, 2014: Guidance for Assessing Pesticide Risk to Bees. 59 pages. URL: https://www.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

VAN DER STEHEN, J.J.M., 1994: Method development for the determination of the contact LD_{50} of pesticides to bumblebees (*Bombus terrestris* L.). Apidologie **25**, 463-465.

VAN DER STEEN, J.J.M., 2001: The effect of the size of the bumblebee (*Bombus terrestris* L.) on the susceptibility to the pesticide dimethoate 40%. Proceedings of the 7th International Symposium of the ICPBR Bee Protection Group, 7–9 September 1999, Avignon, France. Les Colloques de l'INRA **98**, 213–216.

Poster

Appendix

Table 2 Anonymised endpoints of oral and contact toxicity of insecticides including one nematicide for honey bees (HB) and bumblebees (BB) including sensitivity ratios (SR) of honey bee LD_{50}/bumblebee LD_{50}.

Type of test item tested	Endpoint unit	Oral LD_{50}		Contact LD_{50}		Sensitivity ratio	
		HB	BB	HB	BB	Oral	Contact
product	µg a.i./bee	= 0.045	= 0.103	= 170	= 0.15	0.00	0.69
product	µg a.i./bee	= 0.056	= 0.076	= 170	= 0.15	0.00	0.51
product	µg a.i./bee	= 0.17	= 0.08	> 26.4	= 0.9	0.01	0.09
	µg a.i./bee	= 0.28	= 0.16	= 34.4	= 36.4	0.01	0.00
product	µg a.i./bee	= 0.39	= 0.65	= 46.00	= 92.52	0.01	0.01
a.i.	µg a.i./bee	= 0.0037	no data	= 0.15	no data	0.02	n.a.
product	µg a.i./bee	= 1.8	= 1.9	= 22.3	> 100.0	0.08	0.02
	µg a.i./bee*	> 19.62	> 200	> 176	> 200	0.11	1.00
product	µg a.i./bee	= 0.118	= 0.151	= 0.58	= 5.6	0.20	0.03
a.i.	µg a.i./bee	= 0.01	= 0.41	= 0.04	= 21.89	0.25	0.02
a.i.	µg a.i./bee	= 0.06	= 0.045	= 0.14	= 1.75	0.43	0.03
a.i.	µg a.i./bee	= 0.08	= 0.43	= 0.18	= 19.3	0.44	0.02
	µg a.i./bee	= 0.25	= 0.03	= 0.54	= 0.29	0.46	0.10
a.i.	µg a.i./bee	= 0.11	= 0.024	= 0.17	= 1.83	0.65	0.01
product	µg a.i./bee	> 120	> 100	> 160	> 200	0.75	0.50
product	µg a.i./bee	= 0.404	= 0.659	> 0.53	> 100	0.76	0.01
	µg a.i./bee	= 0.0178	= 0.093	= 0.02	= 0.11	0.89	0.85
product	µg a.i./bee	= 0.1	= 0.08	= 0.11	= 0.32	0.91	0.25
product	µg a.i./bee	= 0.048	= 0.44	= 0.05	= 93.52	0.96	0.00
	µg a.i./bee	= 0.38	= 0.47	= 0.36	= 39.3	1.06	0.01
a.i.	µg a.i./bee	= 0.0025	= 0.0389	= 0.001943	= 0.1451	1.29	0.27
product	µg a.i./bee	= 0.92	= 2.78	> 0.47	> 100	1.96	0.03
a.i.	µg a.i./bee	= 0.232	= 0.0682	= 0.07	= 0.25	3.31	0.27
product	µg a.i./bee	= 0.32	= 0.11	= 0.077	= 0.551	4.16	0.20
product	µg a.i./bee	= 1.5	= 0.0049	= 0.3	= 0.25	5.00	0.02
product	µg a.i./bee	= 0.464	= 0.629	= 0.091	= 0.617	5.10	1.02
product	µg a.i./bee	> 119.19	> 100	= 1.28	= 87.8	93.12	1.14
product	µg a.i./bee	> 114.1	> 100	= 1.16	> 200	98.36	0.50
product	µg a.i./bee	no data	= 0.0308	no data	= 0.2	n.a.	0.15
product	µg a.i./bee	no data	= 0.29	no data	> 54.9	n.a.	0.01
product	µg a.i./bee	= 0.0132	= 0.183	no data	= 4.9	n.a.	0.04
a.i.	µg a.i./bee	no data	= 0.11	no data	= 36	n.a.	0.00
a.i.	µg a.i./bee	no data	= 0.28	no data	= 1.59	n.a.	0.18
product	µg a.i./bee	no data	= 35.3	no data	> 100	n.a.	0.35
product	µg a.i./bee	no data	= 0.01428	no data	= 85.3	n.a.	0.00
product	µg a.i./bee	no data	= 0.04	no data	= 28.1	n.a.	0.00
a.i.	µg a.i./bee	no data	> 100	no data	> 100	n.a.	1.00
a.i.	µg a.i./bee	no data	> 100	no data	> 100	n.a.	1.00
product	µg a.i./bee	no data	= 5.92	no data	> 100	n.a.	0.06

*nematicide, a.i.: active ingredient, n.a.: not applicable

Poster

Table 3 Anonymised endpoints of oral and contact toxicity of herbicides including one plant growth regulator for honey bees (HB) and bumblebees (BB) including sensitivity ratios (SR) of honey bee LD_{50}/bumblebee LD_{50}.

Type of test item tested	Endpoint unit	Oral LD_{50}		Contact LD_{50}		Sensitivity ratio	
		HB	BB	HB	BB	Oral	Contact
product	µg a.i./bee	> 122.4	> 50.3	> 481.5	> 326	0.25	0.15
product	µg a.i./bee	> 108.5	> 100	= 396.7	> 400	0.27	0.25
a.i.	µg a.i./bee	> 159.8	= 74.8	> 218	> 250	0.73	0.30
	µg a.i./bee	> 103.46	> 100	> 223.6	> 250	0.46	0.40
product	µg a.i./bee	= 74.7	= 84.6	> 182.9	> 200	0.41	0.42
product	µg a.i./bee	> 66.5	> 60.2	> 141.5	> 120.4	0.47	0.50
n.a.	µg a.i./bee	> 98.5	> 100	> 200.3	> 200	0.49	0.50
a.i.	µg a.i./bee	> 101.7	> 100	> 176	> 200	0.58	0.50
a.i.	µg a.i./bee	> 101.2	> 100	> 193.9	> 200	0.52	0.50
product	µg a.i./bee	> 94.76	> 100	> 193.3	> 200	0.49	0.50
product	µg a.i./bee	> 89.58	> 100	> 86.9	> 200	1.03	0.50
product	µg a.i./bee	> 207.58	> 213.3	> 392.3	> 400	0.53	0.53
product	µg a.i./bee	> 142.96	> 122.7	> 211.9	> 196.4	0.67	0.62
a.i.	µg a.i./bee	> 106.8	> 100	> 130.4	> 150	0.82	0.67
a.i.	µg a.i./bee	> 109.2	> 100	> 203	> 100	0.54	1.00
a.i.	µg a.i./bee	> 100.6	> 100	> 169	> 100	0.60	1.00
a.i.	µg a.i./bee	> 107.4	> 100	> 122	> 100	0.88	1.00
a.i.	µg a.i./bee	> 106.3	> 100	> 222	> 100	0.48	1.00
a.i.	µg a.i./bee	> 108.7	> 100	> 241.6	> 100	0.45	1.00
a.i.	µg a.i./bee	> 109.5	> 100	> 124.47	> 100	0.88	1.00
a.i.	µg a.i./bee	> 109.2	> 100	> 133	> 100	0.82	1.00
a.i.	µg a.i./bee	> 107.3	> 100	> 87.3	> 100	1.23	1.00
a.i.	µg a.i./bee	> 129.1	> 100	> 209	> 100	0.62	1.00
a.i.	µg a.i./bee	> 105.1	> 100	> 231	> 100	0.45	1.00
product	µg a.i./bee	> 108.4	> 100	> 222	> 100	0.49	1.00
a.i.	µg a.i./bee	> 107.6	> 100	> 118.8	> 100	0.91	1.00
a.i.	µg a.i./bee	> 102.4	> 100	> 93	> 100	1.10	1.00
a.i.	µg a.i./bee	> 108.9	> 100	> 129.1	> 100	0.84	1.00
a.i.	µg a.i./bee	> 76.7	> 100	> 140	> 100	0.55	1.00
a.i.	µg a.i./bee	> 108.2	> 100	> 109	> 100	0.99	1.00
a.i.	µg a.i./bee	> 122.1	> 100	> 198.7	> 100	0.61	1.00
product	µg a.i./bee	> 112.3	> 100	no data	> 100	n.a.	1.00
a.i.	µg a.i./bee	> 72.05	> 100	> 98.7	> 100	0.73	1.00
product	µg a.i./bee	> 216.7	> 200	> 190.1	> 200	1.14	1.00
a.i.	µg a.i./bee	> 72.19	> 100	> 90.6	> 100	0.80	1.00
a.i.	µg a.i./bee	> 200	> 200	> 194.5	> 200	1.03	1.00
a.i.*	µg a.i./bee*	> 111	> 100	> 167	> 100	0.66	1.00
product	µg a.i./bee	> 227.2	> 206.2	> 195.4	> 200	1.16	1.03
a.i.	µg a.i./bee	> 200	> 200	> 115.9	> 100	1.73	2.00
a.i.	µg a.i./bee	> 200	> 200	> 201	> 100	1.00	2.00
a.i.	µg a.i./bee	= 10.9	> 200.8	> 119	> 100	0.09	2.01
a.i.	µg a.i./bee	> 110.1	no data	> 233	no data	0.47	n.a.
a.i.	µg a.i./bee	> 105.9	no data	> 83.9	no data	1.26	n.a.

*plant growth regulator, a.i.: active ingredient, n.a.: not applicable

Poster

Table 4 Anonymised endpoints of oral and contact toxicity of fungicides for honey bees (HB) and bumblebees (BB) including sensitivity ratios (SR) of honey bee LD_{50}/bumblebee LD_{50}.

Type of test item tested	Endpoint unit	Oral LD_{50}		Contact LD_{50}		Sensitivity ratio	
		HB	BB	HB	BB	Oral	Contact
	µg a.i./bee	> 63	> 101	> 387	> 376	0.16	0.27
product	µg a.i./bee	> 23.895	> 22.5	> 95.85	> 180	0.25	0.13
product	µg a.i./bee	> 29.29	> 27.3	> 114.7	> 218	0.26	0.13
a.i.	µg a.i./bee	> 25.4	> 100	> 98.2	> 100	0.26	1.00
product	µg a.i./bee	> 100	> 100	> 382.2	> 400	0.26	0.25
product	µg a.i./bee	> 110.10	> 100	> 374	> 400	0.29	0.25
product	µg a.i./bee	= 50.1	= 66.4	> 165.5	> 200	0.30	0.33
product	µg a.i./bee	> 101	> 100	> 283.2	> 300	0.36	0.33
product	µg a.i./bee	= 105.9	= 116.2	> 278.1	> 444.7	0.38	0.26
	µg a.i./bee	> 40.26	> 100	> 103.5	> 100	0.39	1.00
product	µg a.i./bee	> 52.5	> 47.8	> 112.1	> 95.6	0.47	0.50
a.i.	µg a.i./bee	> 105.1	> 100	> 214.32	> 100	0.49	1.00
a.i.	µg a.i./bee	> 100	> 100	> 195.4	> 200	0.51	0.50
a.i.	µg a.i./bee	> 108.5	> 100	> 206.49	> 250	0.53	0.40
a.i.	µg a.i./bee	> 121.4	> 100	> 229.9	> 200	0.53	0.50
a.i.	µg a.i./bee	> 106.3	> 100	> 200.2	> 100	0.53	1.00
a.i.	µg a.i./bee	> 83.05	> 200	> 154.47	> 200	0.54	1.00
product	µg a.i./bee	> 120	> 100	> 176.3	> 200	0.68	0.50
a.i.	µg a.i./bee	> 85	> 100	> 111.1	> 100	0.77	1.00
a.i.	µg a.i./bee	> 217.6	> 200	> 231.6	> 200	0.94	1.00
a.i.	µg a.i./bee	> 298.3	> 300	> 293.9	> 300	1.01	1.00
a.i.	µg a.i./bee	> 102.3	> 100	> 92.5	> 100	1.11	1.00
a.i.	µg a.i./bee	> 110	> 100	> 97.2	> 100	1.13	1.00
product	µg a.i./bee	> 125.4	= 70.7	> 92.1	> 207.2	1.36	0.34
a.i.	µg a.i./bee	> 224.8	> 200	> 132.6	> 100	1.70	2.00
product	µg a.i./bee	> 117.7	= 68.23	> 65.89	> 194.8	1.79	0.35
product	µg a.i./bee	> 120	> 120	= 62	> 400	1.94	0.30
a.i.	µg a.i./bee	no data	> 100	no data	> 100	n.a.	1.00
a.i.	µg a.i./bee	no data	= 4.2	no data	> 100	n.a.	0.04

a.i.: active ingredient, n.a.: not applicable

Poster

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.

Poster

Access: 29. July 2022, URL: https://www.epa.gov/sites/default/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

Poster

Section 3 - Laboratory/Semi-field/Field

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).

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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 $2^{-\Delta\Delta CT}$ 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	500	0	0	0	0	14.5	21.8	29.1	36.4	40.0	40.0

item	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.

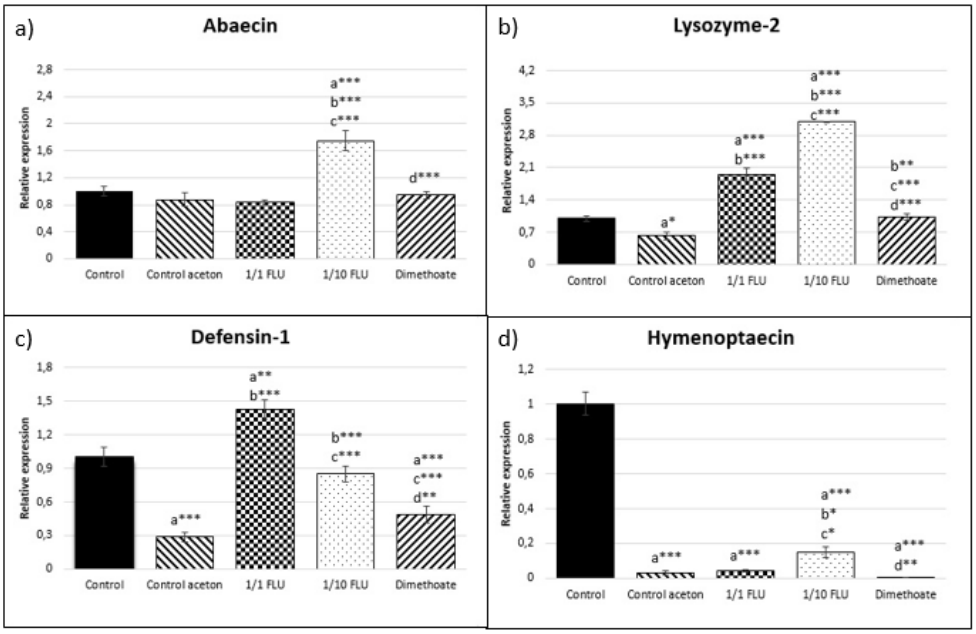

Figure 1 *The effect of tau-fluvalinate on the gene expression of bee immunologically important molecules: a) Abaecin, b) Lysozyme-2, c) Defensin-1, d) Hymenoptaecin. a – significantly different from Control; b – significantly different from Control aceton; c – significantly different from 1/1 Fluvalinate;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

Poster

Acknowledgements

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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

Poster

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.

Poster

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.

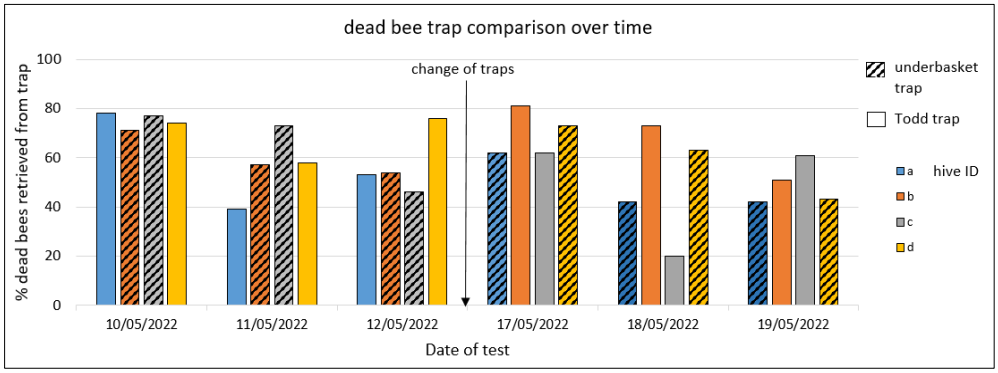

Figure 2 *Recovery of stained dead bees in the dead bee trap summed up over 24 hours.*

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.

Poster

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

Poster

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.

Poster

Section 4 - Monitoring

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

Poster

Section 5 - Microbials

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 studies 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

Poster

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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ICP-PR Honey Bee Protection Group

The ICP-PR Bee Protection Group held its first meeting in Wageningen in 1980 and over the subsequent 40 years it has become the established expert forum for discussing the risk of pesticides to bees and developing solutions how to assess and manage this risk. In recent years, the Bee Protection Group has enlarged its scope of interest from honey bees to many other pollinating insects, such as wild bees including bumble bees. The group organizes international scientific symposia, usually once in every three years. These are open to everyone interested. The group tries to involve as many countries as possible, by organizing symposia each time in another European country. It operates with working groups studying specific problems and proposing solutions that are subsequently discussed in plenary symposia. A wide range of experts active in this field drawn from regulatory authorities, industry, universities and research institutes participate in the discussions.

In the past decade the symposium has largely extended beyond Europe, and is established as the international expert forum with participants from several continents

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

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