INTERNATIONAL COMMISSION FOR PLANT-POLLINATOR RELATIONSHIPS (ICP-PR)

Bee Protection Group

14th INTERNATIONAL SYMPOSIUM

Bern, Switzerland October 23 – 25, 2019

HAZARDS OF PESTICIDES TO BEES

Location: Zentrum Paul Klee (www.zpk.org),
Monument im Fruchtland 3, 3006 Bern, Switzerland

Cover photo: Julius Kühn-Institut (JKI) - Institute for Bee Protection

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**

Organising committee 14 th conference:

Dr. Jens Pistorius	(Julius Kühn-Institut, Germany)
Dr. Anne Alix	(Corteva Agrisciences, United Kingdom)
Mr. Lukas Jeker	(Zentrum für Bienenforschung Agroscope, Switzerland)
Dr. Thomas Steeger	(US Enviromental Protection Agency, USA)
Dr. Guy Smagghe	(Ghent University, Belgium)
Dr. Klaus Wallner	(Hohenheim University, Germany)

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1. General Information

The Symposium will take place in:

Zentrum Paul Klee (www.zpk.org),
Monument im Fruchtland 3, 3006 Bern, Switzerland

Contact local organiser:

Mr. Lukas Jeker
Agroscope, Swiss Bee Research Center
Lukas.jeker@agroscope.admin.ch
Tel. +41 (0)58 463 51 85

Mrs. Daniela Grossar
Agroscope, Swiss Bee research Center
daniela.grossar@gmail.com
Tel.:+41 (0)58 466 77 00

Travel information:
Information on public transport: www.bernmobil.ch

Social Event:
On the nearby hill Gurten in Bern with diner at a traditional swiss farm.
(https://highland-gurten.ch/cms/)

highland-gurten.ch

Own notes:

Program:

Day 1 – Wednesday 23 th October 2019

No.	Start	End	Title	Presenting Author
8:00 - 8:45			**Welcome coffee and registration**	
8:45 – 9:00			Introduction: Jens Pistorius	
9:00 – 9:10			Conference Opening: Eva Reinhard (Head of Agroscope)	
9:10 – 9:20			Swiss Bee Research Centre – Agroscope: Jean Daniel Charrière	
9:20 – 9:40			Measures taken – the Swiss national action plan for bee health: Katja Knauer (Abstract 4.7.)	
9:40 – 10:00			Overview on the OECD activities with respect to Bees and Pollinators: Leon van der Wal	
10:00 – 10:10			EFSA bee guidance document 2.0: Csaba Szentes (4.8.)	
1. Session – Lab-, Semi-Field- and Field-Studies (Oral Presentations)				**Chair: Anne Alix**
1.1	10:10	10:30	Current experimental advances from the French Methodological Bee Group. New improvement for future repro-toxicity tests.	Hervé Giffard
1.2	10:30	10:50	The homing flight method to assess the effect of sublethal doses of plant protection products on the honey bee in field conditions: results of the ring tests and proposal of a new OECD TG	Julie Fourrier
10:50 - 11:20			**Coffee and Tea Break**	
1.3	11:20	11:40	Disturbed energy metabolism after neonicotinoid exposure as cause of altered homing flight activity of honey bees	Verena Christen
1.4	11:40	12:00	Gene expression analysis in honey bees as novel tool for assessing effects of plant protection products	Karl Fent
1.5	12:00	12:20	Practical experiences with a syrup feeding study design based on a new guideline SANTE11956/2016 rev.9 (2018)	Christian Berg
1.6	12:20	12:40	Impact of an Oomen feeding with a neonicotinoid on daily activity and colony	Gundula Gonsior

No.	Start	End	Title	Presenting Author
			development of honeybees assessed with an AI based monitoring device	
1.7	12:40	13:00	Consequences of a short term, sub lethal pesticide exposure early in life on survival and immunity in the honeybee (*Apis mellifera*)	Yahya Al Naggar
13:00 - 14:00			**Lunch**	
1. Session – Lab-, Semi-Field- and Field-Studies (Oral Presentations)			**Chair: Ivo Roessink & Nicole Hanewald**	
1.8	14:00	14:20	How does the novel insecticide flupyradifurone affect honeybee longevity and behavior?	Ricarda Scheiner
1.9	14:20	14:40	Dust drift from treated seeds during seed drilling: comparison of residue deposition in soil and plants	André Krahner
1.10	14:40	15:00	Coumaphos residues in beeswax after a single application of CheckMite® affect larval development *in vitro*.	Christina Kast
1.11	15:00	15:20	Exposure following pre-flowering insecticide applications to pollinators	Edward Pilling
1.12	15:20	15:40	Assessing effects of insecticide seed treatments on pollinators in oilseed rape and maize	Edward Pilling
15:40 - 16:10			**Coffee and Tea Break**	
1.13	16:10	16:30	Conservation and creation of multi-functional margins to maintain and increase the pollinator biodiversity in agricultural environments (d)	Francisco Javier Peris-Felipo
1.14	16:30	16:50	Applied statistics in field and semi-field studies with bees (honey bees, bumblebees and solitary bees)	Ulrich Zumkier
16:50 - 17:10			Discussion on oral presentations lab/semi-field/field (20 mins)	
17.10 - 18.20			ICPPR WG Semi-field and field Report and Discussion	
18:20 – 18:25			Organizational instructions: Lukas Jeker	
18:25			End of Scientific Program day 1	

Day 2 – Thursday 24th October 2019

No.	Start	End	Title	Presenting Author
2. Session – Non-Apis (Oral Presentations)			**Chair: Ivo Roessink & Nicole Hanewald**	
2.1	8:00	8:20	Summary of an ICPPR Non-*Apis* workshop – Subgroup higher tier (bumble bees and solitary bees) with recommendations for a semi-field experimental design	Silvio Knäbe
2.2	8:20	8:40	Progress on the Osmia acute oral test - findings of the ICPPR Non-*Apis* subgroup solitary bee laboratory testing	Ivo Roessink
2.3	8:40	9:00	Stingless bee ring test: acute contact toxicity test	Roberta Nocelli
2.4	9:00	9:20	Standardization of an *in vitro* rearing method for the stingless bee species *Scaptotrigona postica* larvae and its application for determining the toxicity of dimethoate on the larval phase	Anneliese Rosa-Fontana
2.5	9:20	9:40	Effects of chemical and biological Plant Protection Products on R&D colonies of the Buff-Tailed Bumblebee *Bombus terrestris*	Guido Sterk
2.6	9:40	10:00	Predicting wild bee sensitivity to insecticides utilizing phylogenetically controlled inter-species correlation models	Tobias Pamminger
10:00 - 10:20			Plenary discussion on talks on non-apis	
10:20 - 10:50			**Coffee and Tea Break**	
10:50 – 11:00			Update on Non-*Apis* WG	Nicole Hanewald
11:00 - 11:40			WG Discussion Non-*Apis* - Report and Discussion	
3. Session – Monitoring (Oral Presentations)			**Chair: Anne Alix**	
3.1	11:40	12:00	Lethality of Imidacloprid and Fipronil on *Apis mellifera*: a retrospective on the French case	Isaac Mestres Lóbez
3.2	12:00	12:20	Pesticide Residues and Transformation Products in Greek Honey, Pollen and Bee-bread	Konstantinos Kasiotis
12:20 - 13:20			**Lunch**	

No.	Start	End	Title	Presenting Author
3. 3	13:20	13:40	Impact of the use of plant protection products harmful to bees on bee colonies during spring: Results of a monitoring programme in apple orchards in South Tyrol (2014-2017)	Benjamin Mair
13:40 – 14:00			Discussion of oral talks on Monitoring (and WG discussion)	
5. Session – Other (Oral Presentations)			**Chair: Sjef van der Steen**	
a	14:00	14:20	Applying the mechanistic honey bee colony model BEEHAVE to inform test designs of Large-Scale Colony Feeding Study (LCFS)	Silvia Hinarejos
b	14:20	14:40	BEEHAVE validation and resulting insights for the design of field studies with bees	Annika Agatz
14:40 – 15:50			Plenary Discussion on ICPPR matters	
			End of Scientific Program day 2	
			Thereafter – Social Program	

Day 3 – Friday 25th October 2019

No.	Start	End	Title	Presenting Author
4. Session – Section Risk Assessment (Oral Presentations)			**Chair: Jacoba Wassenberg**	
4.1	8:30	8:50	Risk of exposure in soil and sublethal effects of systemic insecticides on ground-nesting hoary squash bees.	Susan Chan
4.2	8:50	9:10	Biopesticides and Pollinators – Examples and requirements on risk assessment from a technical perspective	Stefan Kimmel
4.3	9:10	9:30	Bumblebee (*Bombus terrestris*) versus honey bee (*Apis mellifera*) acute sensitivity – Final results of an ECPA data evaluation	Axel Dinter
4.4	9:30	9:50	Proposed decision tree to evaluate the potential risk of plant protection products to bees via succeeding crops	Anne Alix
9:50 - 10:20			**Coffee and Tea Break**	
4.5	10:20	10:40	Are flowering weeds in agricultural treated fields a significant exposure route for risk assessment?	Natalie Ruddle
4.6	10:40	11:00	Guttation as an exposure route in the risk assessment for plant protection products – Review of available data	Mark Miles
11:00 – 11:40			Discussion of oral presentations and general Risk Assessment issues (pot. to be continued in Working group discussions starting 14:15)	
5. Session – Other (Oral Presentations)			**Chair: Sjef van der Steen**	
c	11:40	12:00	Bee pollinator toxicogenomics: an interdisciplinary approach to unravel molecular determinants of insecticide selectivity	Marion Zaworra
12:00 - 13:00			**Lunch**	
e	13:00	13:20	Introducing the INSIGNIA project: Environmental monitoring of pesticide use through honey bees	Sjef van der Steen
f	13:20	13:40	Bee-o-meter	Johannes Meleschnig
13:40**– 14:05**			**WG Bee Brood Group discussions (g)**	
14:05 – 15:10			**Working Group discussions - status quo and next steps**	
15:10 – 15:40			**Conclusions from chairs and awards**	

No.	Start	End	Title	Presenting Author
15:40– 16:10			Concluding words and announcement next symposium	
16:10 – 16:40			Farewell Tea and Coffee	
END of 14th Symposium				

Program: Posters

1. Session – Risk Assessment/Risk Management (Posters)		
	Title	Presenting Author
1.1.	Precision farming – consideration of reduced exposure in the pollinator risk assessment	Johannes Lückmann, Sibylle Kaiser; Felix von Blankenhagen
1.2.	Evaluation of honey bee larvae data: sensitivity to PPPs and impact analysis of EFSA Bee GD	Johannes Lückmann; Roland Becker; Mark Miles; Anne Alix; Axel Dinter; Stefan Kroder; Ed Pilling; Natalie Ruddle[7]; Christof Schneider; Amanda Sharples; Laurent Oger
1.3.	Chronic oral exposure of adult honey bees to PPPs: sensitivity and impact analysis of EFSA Bee GD	Johannes Lückmann; Mark Miles; Roland Becker; Anne Alix; Axel Dinter; Stefan Kroder; Ed Pilling; Natalie Ruddle; Christof Schneider; Amanda Sharples; Laurent Oger
1.4.	Realistic exposure estimates of bees via the oral route using robust resource quality estimates for pollen and nectar	Tobias Pamminger, Christof Schneider, Matthias Bergtold
2. Session – Honeybee Brood (Posters)		
	Title	Presenting Author
2.1.	Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate?	Johannes Lückmann; Verena Tänzler
2.2.	Toxicity of oxalic acid on in vitro reared honeybee larvae	L. Sabová, M. Staroň, A. Sobeková, D. Staroňová, J. Legáth, R. Sabo
3. Session – Laboratory/Semi-field/Field (Posters)		
	Title	Presenting Author
3.1.	Do pollen foragers represent a more homogenous test unit for the RFID homing test, when using group-feeding?	Michael Eyer, Daniela Grossar, Lukas Jeker
3.2.	Digital Farming & evaluation of side effects on honey bees – first experiences within the Digital Beehive project	Catherine Borrek, Simon Hoff, Ulrich Krieg, Volkmar Krieg, Philipp Senger, Marc Schwering, Silke Andree-Labsch

	Title	Presenting Author
3.3.	Bee colony assessments with the Liebefeld method: How do individual beekeepers influence results and are photo assessments a possibility to reduce variability?	Holger Bargen, Aline Fauser, Heike Gaetschenberger, Gundula Gonsior & Silvio Knaebe
3.4.	Practical and regulatory experience in the conduct of bee residue trials	Silke Peterek; Elizabeth Collison; Vincent Ortoli; Alexia Faure
4. Session - Non-*Apis* Bees (Posters)		
	Title	Presenting Author
4.1.	Interactive effects of the neonicotinoid Thiacloprid and two common fungicides on foraging performance and reproductive success of the solitary bee Osmia bicornis under field conditions	Danja Bättig, Matthias Albrecht; Anina Knauer
4.2.	The use of toxic reference chemicals in solitary bee larval bioassays	Anja Quambusch; Nina Exeler
4.3.	Laboratory Contact Toxicity Test with the Leafcutter Bee Megachile	Annette Kling, Christian Maisch & Anna Maria Friedrich
4.4.	Recent experiences with bumblebee (Bombus terrestris) semi-field tunnel testing following ICPPR Non-Apis 2016 and 2017 workshop recommendations to investigate the insecticide chlorantraniliprole	A. Dinter, A. Samel
4.5.	Sensitivity of the honey bee and different wild bee species to plant protection products – two years of comparative laboratory studies	Tobias Jütte, Anna Wernecke and Jens Pistorius
4.6.	Honeybee viruses in novel hosts – Studying agrochemical-pathogen stress combination in wild bees.	Sara Hellström; Karsten Seidelmann; Robert J. Paxton
4.7.	Is Apis mellifera a good model for toxicity tests in Brazil?	Thaisa C. Roat, Lucas Miotelo, Roberta C. F. Nocelli and Osmar Malaspina
4.8.	Current achievements and future developments of a novel AI based visual monitoring of beehives in ecotoxicology and for the monitoring of landscape structures	Frederic Tausch, Matthias Diehl und Katharina Schmidt
4.9.	Pollinator monitoring for evaluation of potential exposure and assessment of effects	Julian Fricke, Olaf Klein, & Silvio Knäbe

	Title	Presenting Author
4.10.	Development and validation of a bumble bee adult chronic oral test	N. Exeler, A. Quambusch, N. Hanewald, A. Zicot, E. Soler, A. Kling, S. Vinall, K. Dressler, V. Tänzler, S. Kimmel, D. M. Lehmann, M. Patnaude, A. R. Cabrera
4.11	Method development for a larval test design for the solitary bee Osmia cornuta - First experiences with different larval pollen provisions	Nina Exeler; Anja Quambusch
4.12	Interactions between Bombus terrestris and glyphosate-treated plants: are bees at risk of herbicide exposure?	Linzi J. Thompson, Jane C. Stout, Dara A. Stanley
5. Session – Monitoring (Posters)		
	Title	Presenting Author
5.1.	Pesticide Residues and Transformation Products in Honeybees: A 2018 mid-2019 Appraisal	Konstantinos M. Kasiotis; Effrosyni Zafeiraki; Pelagia Anastasiadou; Electra Manea-Karga and Kyriaki Machera
6. Session – Microbials (Posters)		
	Title	Presenting Author
6.1.	Assessment of the impact of microbial plant protection products containing Bacillus thuringiensis on the survival of adult and larval honeybees (Apis mellifera, L.)	Charlotte Steinigeweg, Abdulrahim T. Alkassab, Jakob Eckert, Dania Richter, and Jens Pistorius
7. Session – Other (Posters)		
	Title	Presenting Author
7.1.	Investigating the transfer of acaricides from beeswax into honey, nectar, bee bread, royal jelly and worker jelly	Jakob H. Eckert; Lara Lindermann; Abdulrahim Alkassab; Gabriela Bischoff; Robert Kreuzig and Jens Pistorius

Abstracts: Oral Presentations

(in order of program)

1. Session – Laboratory/Semi-field/Field

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

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

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

The French Methodological Bee Group was re-initiated in 2006 during neonicotinoïds assessments by the authorities. Formerly managed under the Ministry of Agriculture (CEB), it is now committed to provide guidance and protocols to assessors about local or international methodologies. Public and private researchers work together with beekeepers, industrials and CRO's in the aim of providing adapted protocols to the honeybee.

Laboratory LD50 tests and Semi-Field experiments were set up during the 70s' and review regularly under CEB 230, while new guidelines were initiated because of needs for new assessments.

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

More recently the homing flight test was initiated in 2011 (ITSAP) and actually under ring-testing within 7 European laboratories.

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. Moreover it was discussed to apprehend the lifespan of bees, drones and queens. As it is a too large investment for a single methodology, we now focus on the drone fertility for a first step. Later on the lifespan of forager honeybees would be checked as a hypothesis of the decrease of the honey production if it is reduced by several days. Moreover the duration of queens will induce multiyear observations and difficulties to run under GLP.

Drone fertility.

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

The current design uses laboratory and semi-field conditions for the exposure and assessments of the drone development. This two-way assessment is necessary to choose the most efficient method to collect sexually mature drones.

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

In laboratory conditions the exposure begins with the feeding of nurse bees (syrup at different concentrations + water and pollen ad libitum) during 20 days similarly to LD50 exposure. In semi-field conditions the exposure begins with the introduction under tunnels where a feeder is daily supplied in each modality during 20 days.

In 2019 the protocol is not yet finalized but the collection of mature drone is efficient and the validity criteria are still under discussion. A guidance document is expected in 2021, then it could be transferred for ring-testing at OECD level. Results may help to determine if an expected concentration of chemicals in realistic exposure has an effect on the sexual maturation of honeybee drones.

1.2. The homing flight method to assess the effect of sublethal doses of plant protection products on the honey bee in field conditions: results of the ring tests and proposal of a new OECD TG

Julie Fourrier [1], Carole Moreau-Vauzelle [2], Colombe Chevallerau [2], Pierrick Aupinel [2], Mickaël Henry [3], Cyril Vidau [1], Axel Decourtye [1]

[1] ITSAP-Institut de l'Abeille, INRA UR 406 A&E, Domaine Saint Paul, 84914 Avignon Cedex 9, France
[2] INRA UE APIS, 17700 Surgères, France
[3] INRA UR 406 A&E, Domaine Saint Paul, 84914 Avignon Cedex 9, France
E-Mail: julie.fourrier@itsap.asso.fr

The evaluation of the potential effects of plants protection products on honeybee behavior is considered as part of the risk assessment according to Regulation (EC) No 1107/2009 and the EFSA Guidance document (EFSA 2013). But no standardized and validated method is still available. With current revisions of plant protection product risk assessment on the honeybee, a European ring test is conducted since 2015 with 11 voluntary laboratories to test a methodology assessing the effects of sublethal doses of a plant protection product administered in controlled conditions on the homing capacity of forager bees in the field. Homing success is measured by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology.

Main experimental steps are:

-The capture at the hive entrance of foragers coming from a known site located at 1 km (+/- 100 m) away from the experimental colony, to ensure that the foragers have a prior knowledge of the pathway back to the colony.

-The oral exposure of RFID-tagged bees to 3 sublethal dosing solutions of the reference item thiamethoxam, or to a control in laboratory. To do so, the dosing solutions are collectively administered to the honeybees with 20 µl per bee of a 30% sucrose solution (w/v).

-The release of the RFID-tagged foragers on the known site and the record of the homing success at the hive entrance with RFID system for 24 hours after release.

In the first ring test year (2015), already 7 laboratories out of 10 conducted the test and found a common No-Observed Effect Dose (NOED) on the homing success of 0.33 ng per bee, as a test endpoint. The test protocol evolved over time, taking into account methodological adjustments that increased labs test performance. For all control and exposed groups of bees, mortality before release decreased as a whole to ≤ 15 %. A dose with effect of 1 to 1.5 ng per bee was found for a majority of labs from 2015 to 2019. The factors due to the protocol and context (e.g. temperature, varroa infestation) that could modulate homing performances, especially in exposed bees, were considered.

The results showed as a whole the sensitivity of the method to detect the effects of low doses on homing success of foragers. This year (2019) is the last ring test year before documents submission to OECD. The validity criterion corresponding to the minimum and acceptable

homing success in control bees will be definitely set in accordance with the ring test results and expertise.

Acknowledgments

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- Participating laboratories: Agroscope, BioChem agrar GmbH, Biotecnologie BT S.r.l, CREA-AA, Eurofins Agroscience Services Ecotox GmbH, FERA, ibacon GmbH, IES Ltd, INRA Le Magneraud, LAVES-IBCE, TESTAPI

1.3. Disturbed energy metabolism after neonicotinoid exposure as cause of altered homing flight activity of honey bees

Verena Christen[1]; Lukas Jeker[2]

[1]University of Applied Sciences Northwestern Switzerland, Hofackerstrasse 30, 4132 Muttenz, Switzerland,
[2]Agroscope, Swiss Bee Research, 3003 Bern, Switzerland
E-Mail: verena.christen@fhnw.ch, lukas.jeker@agroscope.admin.ch

Neonicotinoids are implicated in the decline of honey bee populations. As nicotinic acetylcholine receptor agonists they disturb acetylcholine receptor signalling, leading to neurotoxicity. Several behavioural studies have shown links between neonicotinoid exposure and adverse effects on foraging activity, homing flight performance and reproduction but the molecular aspects underlying these effects are not well understood. We have elucidated the link between homing flight performance and expression of selected transcripts in the brain of honey bees. Besides possible neurotoxic effects of neonicotinoids leading to disturbed orientation and therefore prolongation of homing flight time, neonicotinoids may also disturb energy metabolism, also causing longer homing flight time. To test the second hypothesis, pollen foragers were fitted with RFID chips, exposed to 1 ng/bee thiamethoxam in single bee feeding and 10 bee-feeding settings and released 1km from the hive. The homing flight time was monitored. In the evening, all returned foragers were collected and stored at -80°C until further analysis. After homing flight data analysis, brain RNA of fast returning controls and slow returning exposed foragers of both feeding strategies was isolated and energy metabolism transcript expression was analysed using quantitative PCR. We analysed expression of *cox 5a*, *cox 5b*, *cox 6c* and *cox 17*, all transcripts of complex IV and *ndufb-7*, part of complex I of the oxidative phosphorylation. Comparing all generated expression data demonstrated that data of the 1 bee-feeding approach scatter less than data of the 10 bee-feeding approach. This finding clearly shows the unequal distribution of sugar syrup between caged honey bees due to trophallaxis. In addition, no significant changes were seen for all analysed transcripts of the 10 bee-feeding approach due to strong scattering of data and small sample size. In contrast, the expression of *cox 5a* and *cox 17* was significantly altered in foragers exposed to 1 ng/bee thiamethoxam in the single bee feeding approach and there was a strong correlation between the down-regulation of *cox 17* and the prolongation of homing flight time. In summary, this small study has two major findings. First, feeding strategy is very important as regards significant effects and single bee feeding approach should be used in fu

ture studies. Second, there is a clear link between prolongation of homing flight time and energy metabolism. Therefore, longer homing flight time may be not only due to disturbed orientation but also due to a lack of energy. Further studies are needed to analyse this point in more detail.

1.4. Gene expression analysis in honey bees as novel tool for assessing effects of plant protection products

Karl Fent[1]; Verena Christen[1]; Petra Kunz[2]

[1] University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Langackerstrasse 30, CH-4132 Muttenz, Switzerland; [2] Federal Office for the Environment, CH-3003 Bern;
E-Mail: karl.fent@bluewin.ch

To date, molecular approaches are not well established in bee research. This holds in particular for investigation into molecular adverse effects of plant protection products (PPPs). Furthermore, molecular tools in standardized, replicable experimental setups are not yet incorporated in standard protocols within the framework of OECD guidelines or other test guidelines for assessing effects and risks of PPPs. In the last few years, we applied gene expression analysis techniques, such as RT-qPCR and RNA-sequencing, to evaluate effects of a series of important PPPs, including insecticides, fungicides or PPPs used in organic farming. We performed short-term laboratory exposures of honey bee workers for 24 to 72 hours and assessed molecular responses in the brain. Our analyses demonstrate that environmental concentrations of PPPs cause significant alteration in gene expression of target genes that are associated with alteration of important physiological pathways. The presentation highlights effects of neonicotinoids, pyrethroids and additional PPPs with emphasis on endocrine disruptive activities of these compounds. Together, our studies indicate that molecular effects are highly sensitive tools that can be incorporated in existing or new test guidelines.

1.5. Practical experiences with a syrup feeding study design based on a new guideline SANTE11956/2016 rev.9 (2018)

Gundula Gonsior, Christian Berg, Yotam Cohen und Silvio Knäbe

Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
E-Mail: GundulaGonsior@eurofins.com

The new SANTE11956/2016 rev.9 (2018) guideline was established to determine the maximum residue levels (MRL) of plant protection products in honey. There are two study designs that have been used in the past, those being the field and semi-field option. In the guideline, a syrup feeding study design is also proposed as a worst case transfer of plant protection products into honey.

To get first hand experiences with this new proposed syrup feeding design, a study was performed in April 2019.

Four swarm units (10,000 bees) were prepared by the artificial swarm technique ("shook swarm method" add reference). The swarms (two with wax foundations and two with drawn out combs) were stored in a dark, cold place for up to 48 hours and fed with sugar solution before they were transferred into magazines and placed inside the tunnels.

Additionally, a colony (with drawn out combs) was placed in a tunnel at the same time as the swarms enter the dark, cold period. For the first 48 hours this colony was kept under normal conditions with brood and food storages. Once the four swarms were transferred into the magazines in the tunnels, the fifth colony was transferred into a new magazine with drawn combs.

The tunnels consisted of nonflowering vegetation with a size of 60 m^2.

Bees were feed with with sugar solution Apiinvert® (Südzucker AG, Germany / 39% fructose, 30% glucose and 31% sucrose (dry weight)) mixed with blue colour food additive (Figure 1). Feeders were placed inside the hives. During the first two feeding occasions 5% food dye sugar solution was provided. The following two feedings were done with 2.5% (w/v) dye sugar solution. Feeding continued with normal, uncoloured solution until artificial honey was available.

Honey was produced in all the hives. It took between 13 and 20 days until the harvest was possible. The fastest time was achieved if drawn out combs were used. The colour content was highest in the hives that produced earlier the honey. Concentration in the honey was about 50% higher than in the original feeding solution. The lowest concentration was found in a sample after 19 days in a hive with wax foundations where the concentration of the dye was only 1.73% compared to the 5% of the feeding solution.

The proposed feeding study design is considered suitable to produce honey.

Reference

SANTE11956/2016 rev.9 (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey

Figure 1 Frame with freshly stored syrup

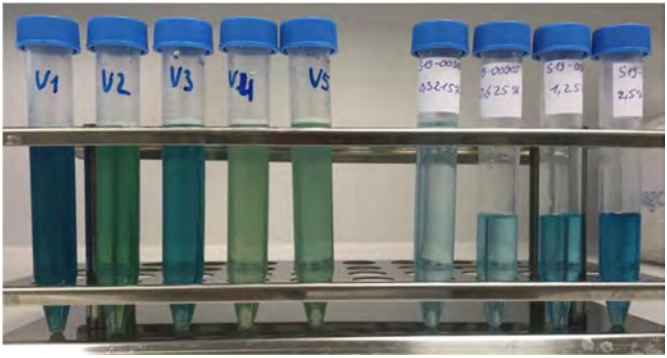

Figure 2: Sample before analysis with standard for calibration

1.6. Impact of an Oomen feeding with a neonicotinoid on daily activity and colony development of honeybees assessed with an AI based monitoring device

Gundula Gonsior[1], Frederic Tausch[2], Katharina Schmidt [2]& Silvio Knäbe[1]

[1]Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
[2]apic.ai GmbH Rintheimerstraße 31-33, 76131 Karlsruhe, Germany
E-Mail: GundulaGonsior@eurofins.com

In order to prove that a substance used in agriculture will bring no harm to pollinators, extensive testing must be performed on the active ingredients of plant protection products. There are several different testing protocols available. However, since there is a wide range of possible outside influences, tests run with free flying bees are always subject to uncertainty. One of the methods currently applied to compare bee mortality between different treatments is the use of dead bee traps. Regarding this method, assessors need to be aware of potential uncertainties e.g. in counting the number of dead bees carried out of the hives and the limited number of data sets which can be collected during testing. Furthermore, as the bees are foraging freely, it is not possible to determine their level of exposure. Therefore, a realistic dose response design is not possible. The only test design, which gives the possibility to test different rates in the same environmental conditions, is the Oomen test design.

The design presented was extended to include a digital hive monitoring device using computer vision and deep learning. The device records all bees entering and leaving their hives with a camera, thus enabling the constant near-time observation of hive development and bee activity throughout the year. Deep learning analysis of the footage recorded makes it possible to count the number of bees entering and leaving throughout the day and to calculate the losses of foragers over selected periods of time.

To test the applicability of the approach, the study compares the hive development and losses of foragers from hives exposed to a neonicotinoid with a control group. Out of eight hives monitored during the study, four were fed with 500 g of sugar solution with a concentration of 200 µg imidacloprid/ kg of sugar solution over a period of ten consecutive days. The control group was fed the same amount of sugar solution. Concurrently, assessments of brood, weight and daily mortality were made. The study will be finished in September after a post-monitoring period of several months.

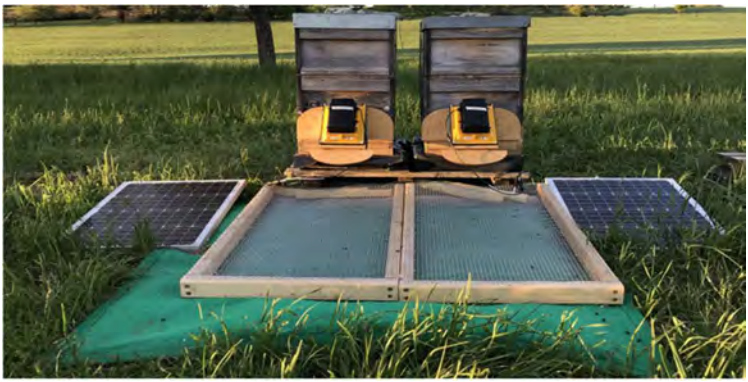

Figure 1: Hives with hardware system and bee monitoring

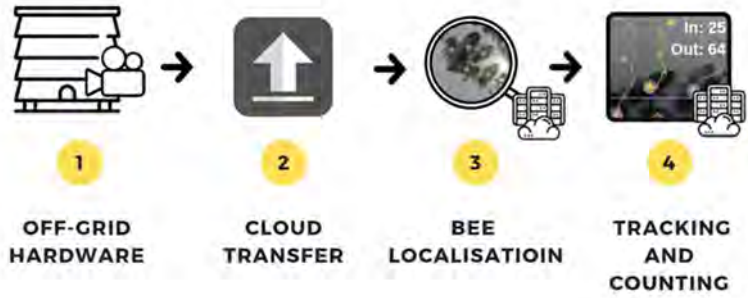

Figure 2: Operating principle of bee observation with digital monitoring device.

1.7. Consequences of a short term, sub lethal pesticide exposure early in life on survival and immunity in the honeybee (*Apis mellifera*)

Yahya Al Naggar[1,2,3]; Boris Baer[1]

[1]Center for Integrative Bee Research (CIBER), Department of Entomology, University of California Riverside, Riverside, CA 92507, USA. [2]Zoology Department, Faculty of Science, Tanta University31527, Tanta, Egypt. [3]General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany:
E-Mail: yehia.elnagar@science.tanta.edu.eg

Dramatic losses of pollinating insects have become of global concern, as they threaten their ecosystem services as well as human food production. Recent research provided evidence that interactions between ecological stressors are drivers of declining pollinator health and responsible for observed population collapses. We used the honeybee *Apis mellifera* and conducted a series of experiments to test for long-term effects of a single short exposure to the agricultural pesticide flupyradifurone to a second environmental stressor later in life. To

do this, we exposed individuals during their larval development or early adulthood to sublethal levels of flupyradifurone, either pure or as part of an agricultural formulation (Sivanto). We afterwards exposed bees to a second environmental stressor, infecting them with the fungal gut parasite *Nosema ceranae*. We found that pesticide exposures significantly reduced survival of bees and altered the expression of several immune and detoxification genes. The ability of bees to respond to these latter effects differed significantly between colonies, offering opportunities to breed bees with elevated levels of pesticide tolerance in the future. We conclude that short episodes of sublethal pesticide exposures during development are sufficient to trigger long lasting effects that could contribute to the widespread declines in bee health.

1.8. How does the novel insecticide flupyradifurone affect honeybee longevity and behavior?

Ricarda Scheiner, Antonia Schuhmann & Hannah Hesselbach

Universität Würzburg, Biocenter, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany
E-Mail: ricarda.scheiner@uni-wuerzburg.de

Flupyradifurone (4-[(2,2-difluoroethyl)amino]-2(5H)-furanone) is a new insecticide which was recently introduced to the market by the Bayer AG (Bayer AG, Crop Science Division, Monheim am Rhein, Germany). It belongs to Bayer's own new class of butenolides and is highly effective against sucking "pest" insects, especially white flies and aphids. Similar to the neonicotinoids, flupyradifurone binds to nicotinergic acetylcholine receptors in the insect brain and works as a reversible agonist.

So far, very little is known about sublethal effects of flupyradifurone on honeybees. We investigated the effect of this substance on honeybee longevity, sensory responsiveness, cognition, foraging initiation and flight behavior, behavioral rhythms and motor behavior. We analyzed both effects of acute treatment and of chronic exposure.

Interestingly, chronic application of flupyradifurone in low concentrations had no significant effect on survival of honeybees in cages of 30 individuals but significantly reduced survival of bees kept individually in activity monitors, indicating that additional stress through isolation might lead to synergistic effects. Further, in four out of eight replicates, flupyradifurone-treated bees did no longer display circadian rhythms in activity monitors compared to control animals.

When honeybees were treated chronically in the hive and their flight behavior was monitored using radio frequency identification (RFID), we measured a significantly earlier onset of foraging in the flupyradifurone group. Otherwise, flight activity did not seem to be affected.

Acute treatment with flupyradifurone reduced sensory responses and cognitive performance as well as motor behavior with typical indications of toxification such as walking in circles of falling on the back.

Generally, low concentrations of flupyradifurone had smaller effects on behavior than the hitherto frequently used neonicotinoids. However, we also see a negative impact of this novel insecticide on honeybees, even though it may sometimes only become apparent under stressed situations.

1.9. Dust drift from treated seeds during seed drilling: comparison of residue deposition in soil and plants

André Krahner[1]; Udo Heimbach[2]; Gabriela Bischoff[1]; Matthias Stähler[3] and Jens Pistorius[1]

[1]Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig, Germany
[2]Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11-12, 38104 Braunschweig, Germany
[3]Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Straße 19, 14195 Berlin, Germany
E-Mail: andre.krahner@julius-kuehn.de

Drilling of seeds treated with plant protection products leads to dust drift carrying active substances (a.s.) into adjacent areas. Since these residues potentially pose a risk for bees, standardised field experiments have been conducted between 2009 and 2017 to investigate the deposition pattern of a.s. and the potential bee exposure to a.s. The large resulting data set contains a lot of information that can be used to improve our understanding of how different parameters influence the deposition pattern of dust and a.s. of seed treatments. For the present analysis, residues sampled in different matrices were used, including Petri dishes placed on bare soil and within neighbouring cultures (oil seed rape and mustard) as well as plant material (divided into flowering and non-flowering plant parts). In a nested design, multiple samples were taken at each distance of 0, 1, 3 and 5 m from the field edge within a total of 6 blocks per trial. The a.s. content per sample was determined analytically, using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS).

By means of generalized linear mixed effect models (GLMM; R package 'lme4') and automated model selection (R package 'MuMIn'), the effects of environmental and drilling parameters, seed treatment quality and sampling matrix were analysed taking into account the information from multiple trials and thus allowing for analysing the effects independently from another. A high amount of variation cannot be explained by the resulting models, probably due to environmental factors not incorporated into the models, such as varying wind speed and direction as well was heterogeneous field characteristics (terrain, crop density). However, the incorporated fixed effects resulted to be relevant in the majority of the selected models. Overall, the dust-borne a.s. emission per hectar (Heubach value expressed as g a.s./ha) has a strong impact on the amount of residues, which decrease markedly within the observed distance of 5 m to the field edge. Comparing different sampling matrices, i.e. flowering plant parts and ground-based Petri dishes, a similar distance-related residue pattern was observed within the neighbouring crops. Based on field realistic data, the presented results will contribute to enabling a more precise risk assessment of seed treatment applications with regard to bees.

1.10. Coumaphos residues in beeswax after a single application of CheckMite® affect larval development *in vitro*.

Christina Kast; Verena Kilchenmann; Benoît Droz

Agroscope, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland
E-Mail: christina.kast@agroscope.admin.ch; verena.kilchenmann@agroscope.admin.ch; benoit.droz@agroscope.admin.ch

Coumaphos is an organophosphate insecticide used on bees for the control of the parasitic mite (*Varroa destructor*). We studied the distribution of coumaphos in beeswax after a single

application of CheckMite® and studied the effect of coumaphos in beeswax on larval development. Fifteen *Apis mellifera* colonies were treated with CheckMite® containing 2.72 g of coumaphos per application. During the following spring season, average coumaphos levels of 65 mg/kg were measured in combs that came into contact with the strips and average concentrations of 6.7 mg/kg were measured in combs that did not come into contact with the strips. Coumaphos was also detected in wax that was not present during the treatment, such as newly constructed wax, wax of honeycombs and capping wax, respectively. *In vitro* larval rearing in cups coated with beeswax containing coumaphos at a concentration of 70 mg/kg or 10 mg/kg demonstrated that coumaphos levels of 70 mg/kg in beeswax negatively affected larval development, while no differences to the controls were observed for larvae exposed to beeswax containing coumaphos at 10 mg/kg. Therefore, beeswax exposed to CheckMite should not be recycled in order to prevent elevated coumaphos residues in new foundations and hence to prevent honeybee larvae from being exposed to high residue levels.

1.11. Exposure following pre-flowering insecticide applications to pollinators

E. Pilling[1], J. Barnekow[1], V. Kramer[1], A. Alix[1], O. Klein[2], L. Franke[2], J. Fricke[2],

[1] Corteva Agriscience, [2] Eurofins Agroscience Services GmbH
E-Mail: edward.pilling@corteva.com

Applying insecticides pre-flowering can mitigate the risk to pollinators by significantly reducing exposure via both contact and dietary routes. Methods have been developed to quantify the exposure of foraging honeybees, bumblebees and solitary bees to insecticides following pre-flowering applications. The insecticide sulfoxaflor was applied pre-flowering at BBCH 55 to a variety of target crops at five different sites across Europe. The subsequent residue levels on foliage after application were determined to investigate the decline of residues prior to flowering. When the crop reached the flowering stage at BBCH 60, residue levels in pollen and nectar were determined to provide an estimate of potential maximum exposure to pollinators and rate of decline in pollen and nectar. Exposure levels were compared to results from effect studies with honeybees, bumblebees and solitary bees. With honey bees, effect assessments included mortality, foraging activity, behaviour and colony condition assessments. Nectar and pollen was sampled from forager bees, pollen traps and from combs to determine levels of dietary exposure. Effects on bumblebees were investigated by mortality assessments in the colony and tunnel, together with assessments of foraging activity, colony weight, queen production and brood assessments at the start and end of the study. Dietary exposure to bumblebees was determined by analysis of nectar and pollen collected from forager bees and in nectar and pollen pots in the colony. Effects on solitary bees (*Osmia bicornis)* were assessed following applications to oilseed rape in tunnels. Assessments included hatching rate, nest occupation, flight activity, cell and cocoon production and hatching success. Dietary exposure was determined in nectar and pollen collected from plants. Results from both exposure and effect studies will be presented together with a discussion on risk to pollinators and mitigation with pre-flowering applications.

1.12. Assessing effects of insecticide seed treatments on pollinators in oilseed rape and maize

E. Pilling[1], A. Alix[1], O. Klein[2], L. Franke[2], J. Fricke[2], M. Kleinholz[2] and H. Gätschenberger[2]

[1] Corteva Agriscience, [2] Eurofins Agroscience Services GmbH
E-Mail: edward.pilling@corteva.com

To fully assess the risk of insecticide seed treatments in oilseed rape and maize, methods have been development to investigate effects of seeds treated with cyantraniliprole on pollinators. Tunnel studies were conducted with oilseed rape grown from treated seed combining exposure and effects assessment on honey bees, bumblebees and solitary bees in Germany and Italy. With honey bees, effect assessments included mortality, foraging activity, behaviour and colony condition assessments. Nectar and pollen was sampled from forager bees, pollen traps and from combs to determine levels of exposure. Effects on bumblebees were investigated by mortality assessments in the colony and tunnel, foraging activity, colony weight, queen production and brood assessments at the start and end of the study. Exposure to bumblebees was determined by analysis of nectar and pollen collected from forager bees and in nectar and pollen pots in the colony. Effects on solitary bees were assessed with oilseed rape treated seed in tunnels with Osmia bicornis. Assessments included hatching rate, nest occupation, flight activity, cell and cocoon production and hatching success. Exposure was determined in nectar and pollen collected from plants. Honeybee field studies with cyantraniliprole treated maize seed were conducted in Germany and Italy. Colonies were placed in the fields prior to the onset of the guttation period at BBCH 10. Mortality, foraging activity on guttation fluid and colony condition assessments were made throughout the guttation period, together with residue analysis of the guttation fluid. Colonies were then exposed to maize pollen during flowering and similar assessments conducted plus residue analysis of pollen collected from pollen traps and combs. The abundance and species richness of naturally occurring wild bees in treated and untreated field plots of maize and adjacent field margins during pollen shedding were also investigated to gain further understanding of exposure and effects on wild pollinators in maize. To evaluate a wide range of wild bee species occurring at field sites during pollen shedding period, two methods were used: a non-selective method and a selective method. For the non-selective method two different types of traps were used. Vane traps and bee bowls were installed at three sampling areas: in the centre of the maize fields, at the borders of the fields (inside the maize crop) and outside at in the adjacent field margin. The selective sweep netting method was used in the crop centre and at the border of the fields (inside the maize crop) via transect walks in a defined distance and time interval. Additionally, nesting units were provided for solitary wild bee species that breed in woody cavities. The trap nests were set up at the centre and adjacent field margin and used for sampling of pollen to assess how attractive the maize pollen is to the cavity breeding species compared to other available pollen sources at the time of the year by pollen identification of pollen mass samples. In addition, residue analysis was performed with samples of pollen mass. Results from all the studies will be presented together with the risk of cyantraniliprole treated oilseed rape and maize seed to honeybees and wild pollinators.

1.13. Conservation and creation of multi-functional margins to maintain and increase the pollinator biodiversity in agricultural environments (d)

Peris-Felipo, F.J.[1], Aguado-Martín[2], L. O. and Miranda-Barroso[3], L.

[1] Syngenta Crop Protection AG, Rosentalstrasse 67, 4058 Basel (Switzerland);
[2] Andrena Iniciativas y Estudios Medioambiental S.L. c/. Gabilondo 16bis, 47007 Valladolid (Spain);
[3] Sustainable Agriculture Syngenta Spain, c/. Ribera del Loira, 8, 10, 28042 Madrid (Spain);
E-Mail: oscaraguado@lepidopteros.com; javier.peris@syngenta.com; luis.miranda@syngenta.com

When a natural ecosystem changes its use in agriculture, factors that greatly affect its fauna, especially insects, are introduced. This kind of land change, and especially intensive production models causes a clear loss of biodiversity, with a drastic decrease in the number of plant species that in turn affects the natural pollinator entomofauna.

In 2010, one of the main conclusions reached by the European Commission for the Conservation of the Environment was the need to promote research on the conservation, restoration and sustainable use of the diversity of pollinators in agriculture. This situation together with the climate change and the notable decrease in the number of wild pollinators has meant that the European Union, FAO (United Nations Food Organization) and other important international organizations have raised the alarm about the need to look for how to maintain and increase the presence of wild pollinators.

In order to find practical solutions, the company Syngenta Crop Protection launched the "Operation Pollinator (OP)" project in 2009, a European-level initiative launched in Britain as part of the EU action called EPI ("European Initiative on Pollinators"), whose main objective is to protect pollinators, increase their biodiversity and promote their presence and also other beneficial or auxiliary arthropods in the crops.

The present study collects the results obtained in different agricultural farms of the Iberian Peninsula, demonstrating how right agricultural practices can also help to maintain biodiversity and favour its rapid increase, both qualitatively and quantitatively.

1.14. Applied statistics in field and semi-field studies with bees (honey bees, bumblebees and solitary bees)

Ulrich Zumkier; Markus Persigehl; Andrea Roßbach; Ines Hotopp; Anja Ruß

tier3 solutions GmbH, Kolberger Str. 61-63, 51381 Leverkusen, Germany;
E-Mail: ulrich.zumkier@tier3.de

Field and semi-field studies are important tools in the ecotoxicological risk assessment of plant protection products (PPP) for bees (honey bees, bumblebees and solitary bees). While these studies represent far more realistic conditions than laboratory tests, they also present a challenge for the analysis and interpretation due to the large and complex datasets. There

fore, in order to correctly answer the underlying ecotoxicological questions, it is crucial that these studies are not only thoroughly planned and conducted, it is also important that they are subjected to adequate statistical analysis. The aim of this talk is to provide a better understanding on how to conduct and interpret statistical analyses in field and semi-field studies with bees made for regulatory purposes. An overview of how study design and statistics should be aligned with each other is given including the specific challenges of (semi-) field trials, as for instance how to address the problem of pseudoreplication if hives are regarded as experimental units. Different statistical tools are compared and their suitability for different data types and questions are discussed. Generalized Linear (Mixed) Models (GLMMs) are evaluated in more detail as they provide a flexible and robust tool for the analysis of honey bee (semi-) field data. Furthermore, some more light is shed on what p-values really tell us, how they can help to interpret data and how they should not be misinterpreted.

2. Session – Non-Apis bees

2.1. Summary of an ICPPR Non-Apis workshop – Subgroup higher tier (bumble bees and solitary bees) with recommendations for a semi-field experimental design

Silvio Knäbe[1],, Matthew J. Allan[2], Annika Alscher[3], Kristin Amseld, Christian Classen[3], Magdaléna Cornement[5], Charlotte Elston[8], Nina Exeler[7], Lea Franke[1], Malte Frommberger[8], Hervé Giffard[10], Juan Sorli Guerola[11], Sabine Hecht-Rost[3], Bettina Hodapp[5], Ines Hotopp[9], Carole Jenkins[12], Tobias Jütte[8], Stefan Kimmel[5], Olaf Klein[1], Britta Kullmann[9], Johannes Lückmann[3], Markus Persigehl[9], Ivo Roessink[13], Christof Schneider[14], Alexander Schnurr[4], Verena Tänzler[15], Sjef Van der Steen[16],

[1] Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany; [2]Atlantic Pollination Ltd., 18 North Street, Glenrothes, KY7 5NA, United Kingdom, [3]Rifcon GmbH, Goldbeckstr. 13, 69493 Hirschberg, Germany, [4] BioChem agrar GmbH, Kupferstraße 6, 04827 Machern/ OT Gerichshain, Germany, [5]Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland, [6]Syngenta Ltd, Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, United Kingdom, [7]Bayer CropScience, 40789 Monheim, Germany, [8]Institute for Bee Protection, Julius Kühn-Institut Federal Research Centre for Cultivated Plants, Messeweg 11-12, 38104 Braunschweig, Germany, [9]tier3 solutions GmbH, Kolberger Strasse 61-63, 51381 Leverkusen, Germany, [10]Testapi, 464 Sarré, Gennes, 49350 Gennes-Val-de-Loire, France, [11]Trialcamp SLU, Avda. Antic Regne de Valencia 25, 46290 Alcasser, Spain, [12]Envigo, Woolley Road, Alconbury, Huntingdon, Cambridgeshire PE28 4HS, United Kingdom, [13]WUR – Wageningen Environmental Research, Droevendaalsesteeg 3, 6708 PB Wageningen, The Netherlands, [14]BASF, 67117 Limburgerhof, Germany, [15]IBACON GmbH, Arheilger Weg 17, 64380 Roßdorf, Germany,[16]Alveus AB Consultancy, Oisterwijk, The Netherlands
E-Mail: silvioknaebe@eurofins.com

The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators highlighted that there are no study designs for non-Apis pollinators available. Since no official guidelines exist for semi-field testing at present, a protocol was proposed by the ICPPR non-Apis working group and two years of ring testing were conducted in 2016 and 2017 to develop a general test set-up. The ring-test design was based on the draft EFSA guidance document, OEPP/EPPO Guideline No. 170 and results of discussions regarding testing solitary bees during the meetings of the ICPPR non-Apis workgroup in 2015, 2016, 2017, 2018 and 2019 and an hands-on workshop in May 2017.

Ring-tests were conducted with two different test organisms, one representative of a social bumble bee species (*Bombus terrestris* L; Hymenoptera, Apidae) and one representative of a solitary bee species (*Osmia bicornis* L; Hymenoptera, Megachilidae). Both are polylectic and foraging on a diverse spectrum of flowering crops. In addition, they are common species in Europe, commercially available and widely used for pollination services. 16 laboratories participated in the higher-tier ring tests. 15 semi-field tests were conducted with bumble bees and 16 semi-field tests were accomplished with solitary bees in 2016 and 2017.

Two treatment groups were always included in the ring-tests: an untreated control (water treated) and dimethoate as a toxic reference item (optional other i.e. brood affecting substances (fenoxycarb, diflubenzuron). The toxic reference items were chosen based on their mode of action and long term experience in honey bee testing.

In the solitary bee study design adult bees (both sexes) were caged in tunnels containing a bee attractive flowering crop and exposed during their reproductive period. After the application of the respective reference items, the adult female bees collected the relevant food items from the treated crop, providing their offspring with exposed pollen and nectar as the only food source during brood development. In the test with solitary bees hatching success (1st generation) was assessed since it was the basis for later calculations of reproductive success and gives information on the quality of the cocoons. The evaluated endpoints were the establishment at the nesting units (nest occupation), flight activity, reproduction and hatching success (2nd generation).

In the bumble bee study design only the early part of the colony development took place during the exposure phase in the tunnels. After the exposure phase (at the end of flowering), the bumble bee colonies were transferred to a monitoring site until they produced queens and drones. In the bumble bee trials evaluated endpoints were brood development, colony weight and colony reproduction (production of sexuals).

The assessed endpoints were evaluated with respect to their potential for the use in risk assessment. A summary of the ring-test results will be given and the recommendations for the two semi-field test designs will be presented.

2.2. Progress on the Osmia acute oral test - findings of the ICPPR Non-Apis subgroup solitary bee laboratory testing

Ivo Roessink[1], Nicole Hanewald[2], Christof Schneider[2], Anja Quambusch[3], Nina Exeler[3], Ana Cabrera[4], AnnaMaria Molitor[5], Verena Taenzler[6], Bettina Hodapp[7], Matthias Albrecht[8], Annely Brandt[9], Steven Vinall[10], Anne-Kathrin Rathke[11], Hervé Giffard[12], Eugenia Soler[13], Alexander Schnurr[14], Michael Patnaude[15], Elodie Couture[16], David Lehman[17]

[1] Wageningen Environmental Research -The Netherlands, [2] BASF-Germany, [3] BAYER-Germany,[4] BAYER-United States, [5] Eurofins Agroscience Services Ecotox GmbH-Germany, [6] IBACON GmbH-Germany, [7] Innovative Environmental Services (IES) Ltd-Switzerland, [8] Agroscope-Switzerland,
[9] Landesbetrieb Landwirtschaft Hessen-Germany, [10] MamboTox-United Kingdom, [11] Noack laboratorien-Germany, [12] TestApi-France, [13] Trialcamp SLU-Spain, [14] Biochem Agrar GmbH-Germany, [15] Smithers-United States, [16] SynTech-France, [17] US Environmental Protection Agency-United States

The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators highlighted that there are no study designs for non-apis pollinators available. As a result the risk assessment of non-apis pollinators uses apis pollinator data with so-called assessment factors to compensate for the lack of knowledge on other species. To fill part of this knowlegde gap an acute oral test for solitary bees was developed within the ICPPR non-apis group.

Ringtests have been conducted in 2018 to validate and improve the suggested protocol. And in 2019 a standardized protocol has been tested by all participants once more. The tests have been performed with *Osmia bicornis*, *Osmia cornuta*, *Osmia lignaria* and *Osmia cornifrons*. A summary of the ringtest results of both years will be given and further recommendations will be presented.

2.3. Stingless bee ring test: acute contact toxicity test

Roberta C.F. Nocelli[1]; Thaisa C. Roat[2]; Lucas Miotelo[2]; Tauane A. Lima[1]; Aryadne G. Rodrigues[1]; Geovana M. Silva[1]; Osmar Malaspina[1]

[1]Agrarian Science Center – Federal University of São Carlos – Araras SP – Brazil – Rodovia Anhanguera, Km 174 – 13600-970 – Araras – SP – Brazil. [2]Social Insect Studies Center – São Paulo State University – Institute of Bioscience – Avenida 24A, 1515 – Bela Vista – 13506-900 - Rio Claro – SP.

E-Mail: roberta@ufscar.br; thaisaroat@yahoo.com; Lucke_Miotelo@hotmail.com; tauane.a.lima@hotmail.com; aryadne2212@gmail.com; geovanamaloni@gmail.com; osmar.malaspina@unesp.br

There is much discussion about the representativeness of *Apis mellifera* specie in relation to stingless bees and how protective the schemes are. Thus, since 2016 Brazil has been investing in the development of a method that can be applied to different species of stingless bees. Since 2017 Brazil has a new pesticide registration procedure, which includes the risk assessment process for bees. However, all required studies are still performed with the specie *Apis mellifera*, since there are no standardized protocols with native Brazilian species. In order to meet the growing demand for analysis and to ensure the availability of protocols that can answer the questions regarding the representativeness of *A. mellifera* in relation to the biodiversity of Brazilian bees, we have developed a stingless bees protocol for possible standardization and use in the risk assessment process. The protocol was developed from adaptations to OECD 214 protocol for *A. mellifera* and initially tested with the species *Scaptotrigona postica*. During its development, the best collection method, the most suitable experimental cage and anesthesia times were established. The proposed protocol was tested using the active ingredient dimethoate between October 2018 and March 2019. The contact LD_{50} were: 24h - 4.34 to 6.66 ng / µL; 48h - 3.08 to 5.39 ng / µL; 72h - 2.31 to 4.27 ng / µL; and 96h - 1.92 to 4.12 ng / µL. The method proved feasible and the protocol was presented during a workshop held in Rio Claro in January 2019 where a proposal for standardization throughout the national territory was presented. For the ring test the project has 13 laboratories: 7 universities, 3 research institutes and 3 private laboratories. Currently, the laboratories have been equipped and all involved are being trained to begin the first round of testing from September 2019. The Brazilian experience will be presented during the 13th SETAC Latin America for the exchange of experiences and discussion of more species-oriented methods from the tropical and subtropical regions of the Americas, with the aim of creating a network aimed at protecting local species.

2.4. Standardization of an *in vitro* rearing method for the stingless bee species *Scaptotrigona postica* larvae and its application for determining the toxicity of dimethoate on the larval phase

Annelise Rosa-Fontana [1*], Adna Dorigo[1], Juliana Stephanie Galaschi-Teixeira [2], Roberta Nocelli [3], Osmar Malaspina [1]

[1] State University of São Paulo, 24A Avenue 1515, Rio Claro, SP, Brazil
[2] Technological Institute of Vale – Boaventura da Silva St, 955, Belém, PA, Brazil
[3] Federal University of São Carlos, Anhanguera Road Km 174, Araras, SP, Brazil
E-mail contact: annesouzar@gmail.com

Currently, Brazil has a full framework for pesticide risk assessment established for *Apis mellifera*, based on North America's approach. However, the use of an exotic species as model-organism as a substitute for native species of Brazil (stingless bees) has been questioned. An *in vitro* larval rearing method has already been described for the Brazilian native *Melipona scutellaris* but, *Scaptotrigona postica* species has shown potential to be suitable for testing, mainly because its high numer of individuals per hive comparing to the other stingless bee species and for do not belongs to the list of endangedered species, like *M. scutellaris*. Thus, we aimed to establish an *in vitro* larval rearing method for *S. postica* and to apply it for determining the toxicity of dimethoate on larval phase. Larvae of 24 hours old were transferred to acrylic plates and five different procedures were carried out, considering the humidity control and the required fungus *Zygosaccharomyces* sp. as essential for the success of larval survivorship. Each replicated consisted of 100 larvae, totalizing 4.800 larvae. Mortality and emergence parameters of the individuals, as well as the progress of the larval development were assessed, in order to check the efficiency of these methods. The intertegular distance, head width and wings asymmetry were assessed from the individuals emerged from the most efficient method. The same parameters were checked on individuals emerged from *in vivo* brood combs. The chosen method consisted of the deposition of the pure larval food followed by adding KCl and NaCl solutions 72 and 120 hours after the larval transference, respectively. This procedure was applied to determine the lethal concentration 50% (LC_{50}) of dimethoathe, the standard active ingredient for toxicological tests, established by OECD. The active ingredient, obtained from Sigma-Aldrich (Pestanal), was directly diluted in the larval food, and successive subsequent dilutions were performed in the food, in order to reach the following concentrations to be offered to the larvae (in ng a.i./ larva): 250, 200, 150, 100, 50 and 25. Each bioassay was carried out 4 times (20 larvae/concentration in triplicates). The negative control consisted of the pure larval food. The dose-response data were assessed with binomial generalized linear models, using the cauchit function, for determining the LC_{50} for 24 and 48 hours. The analysis was performed in the R software (R Core Team). The best procedure indicated emergence/larvae, emergence/pupae and mortality/larvae of 93.44, 97.6 and 2.85%. The mean of intertegular distance for the *in vitro* method was 136.5 mm and for *in vivo* of 127.7 mm. For the head width, *in vitro* showed 92.58 mm and *in vivo* was 89.88 mm. The t test indicated no significative difference between the *in vivo* and *in vitro methods* ($p > 0.05$). Regarding the wings asymmetry, the ANOVA Procrustes indicated a significative difference in the centroid size only in the "individual effect", on individuals emerged from both *in vitro* ($F = 11.33$; $p < 0.0001$) and *in vivo* ($F = 38.35$; $p < 0.0001$) treatments, and in the wing venation pattern in the "individual effect" *in vitro* ($F = 12.03$; $p < 0.0001$) and *in vivo* ($F = 12.13$; $p < 0.0001$), and in the "size effect" on individuals emerged from the *in vivo* treatment ($F = 0.50$; $p < 0.0005$). The tests with dimethoathe indicated a LC_{50} (in ng a.i. /larva) of 172.48 and 156.33 for 24 and 48 hours, respectively. The mais points for the success of the *in vitro* rearing were the humidity control, the non-use of eggs for transference, and to the use of acrylic plates manufactured which the size simulates the real dimensions of brood cells. The differences showed in some patterns of the wings asymmetry on individuals emerged from *in vitro* treatment are considered normal, since we can observe also on *in vivo* emerged individuals. These little variations in morphology are common in the nature, especially because of environmental stresses. Thus, our results obtained *in vitro* may be used for representing *in*

vivo conditions. According to the OECD, to be possible carry out a toxicological comparison by LC and/or LD values, is necessary that the experimental method has been performed in the same way. This prevents, in a toxicological approach, to do a comparison between *A. mellifera* and stingless bees. While *A. mellifera* has a progressive feeding, stingless bees have en mass food deposition, making impossible the same way of exposure in the food. Anyway, it is important to consider an ecological approach, which indicates, although by different methods, a LC_{50} for *S. postica* 50 times more sensitive to dimethoathe than *A. mellifera*. This highlights the importance of inclusion of a native Brazilian species as model-organism for risk assessments studies, which may be extended for other areas of the Neotropical region. Our results are very useful for a validation of method through developing of ring tests, in accordance to OECD.

2.5. Effects of chemical and biological Plant Protection Products on R&D colonies of the Buff-Tailed Bumblebee Bombis terrestris

Guido Sterk., Paraskevi Kolokytha. and Janna Hanegraaf

IPM Impact Gierkensstraat 21, 3511 Hasselt, Belgium
E-Mail: Guido.Sterk@skynet.be

The effects of several plant protection products were tested on *Bombus terrestris dalmatinus*, using the new laboratory method on full standardised IPM Impact R&D colonies, starting with a mother queen and 20 callows as presented on the ICPPR meeting in Valencia in 2017. The maximum field recommended concentration (MFRC) of both biological and chemical products was applied in a series of tests through oral sugar water treatment as this is considered a worst case scenario for bumblebees. A sequential dilution testing scheme was used, by decreasing the dose rate with 1/10 of the MFRC concentration if triggered. The survival of the mother queen and initial workers, the total number of formed workers/drones at the end of the , the number of new born gynes and queen brood and the weight and volume of the colonies were determined as the most important end points. For the evaluation of the results the data were calculated and categorized in the IOBC side-effect classes, used for laboratory trials. For a very specific *Bacillus thuringiensis aizawai* strain, GC91, trade name Agree, the whole series of tests through spiked sugarwater, pollen and topical application was carried out.

2.6. Predicting wild bee sensitivity to insecticides utilizing phylogenetically controlled inter-species correlation models

Tobias Pamminger[1], Nicole Hanewald[1], Christof Schneider[1], Matthias Bergtold[1]

[1] BASF SE
E-Mail: tobias-pamminger@basf.com

Plant protection products (PPP), are a vital pillar of modern agricultural practice, but their potential adverse effect on bees has emerged as an intensively discussed topic. Historically, research on the effects of PPP on bees has focused on the honey bee (Apis mellifera), while non-Apis bee species remain largely understudied. This study is intended as a first step to address this obvious knowledge gap and hope that it may be used to facilitate the development and implementation of a scientifically sound wild bee risk assessment with limited additional testing needs. We have compiled a comparative data set on bee sensitivity (acute contact exposure) against Acetylcholine Esterase (AChE) inhibitors, pyrethroids, neonicotinoids, organochlorides and bee bodyweight, a trait likely influencing bee sensitivity to PPP exposure. In

total, we collected sensitivity data for up to 24 bee species per insecticide group covering five of seven bee families. Using this information, while controlling for their phylogenetic non-independence, we build inter species correlation models to predict bee sensitivity to PPPs belonging to different modes of action based on their bodyweight. We find that 1) bee weight is a robust predictor of bee resilience against insecticide exposure in many cases and 2) Apis is a particularly sensitive bee genus especially when body weight is taken into account. In contrast the currently proposed non-apis surrogate species (Bombus terrestris and Osmia sp.) for European risk assessment as well as many stingless bee species, are comparatively resilient to many classes of insecticides. We discuss the consequences of these findings in the context of the global non-Apis risk assessment debate in Europe and the Americas.

3. Session - Monitoring

3.1. Lethality of Imidacloprid and Fipronil on *Apis mellifera*: a retrospective on the French case

Isaac Mestres Lóbez

Solvay Brussels School, Université Libre de Bruxelles, Avenue Franklin D. Roosevelt 50/141, 1050 Brussel, Belgium
E-Mail: isaac.mestres@solvaypostgrad.net

The aim of this study is to draw a retrospective analysis on the lethality of Imidacloprid (Gaucho ®) and Fipronil (Régent TS ®) on *Apis mellifera* between 1992 and 2016 in France. Early monitoring reports in the 1992-2002 period notified these two embedded insecticides to be at the origin of massive colony collapse disorders. Ecotoxicological analyses based on the LD_{50} of Imidacloprid and Fipronil highlighted their differential lethality by both contact (Imidacloprid: 81 ng/honeybee vs Fipronil: 5,9 ng/honeybee) and ingestion (Imidacloprid: 3,7 ng/honeybee vs Fipronil: 4,2 ng/honeybee), but failed to point Imidacloprid's high solubility as a higher lethal agent. Chemical properties and action mode of these two insecticides originated neural disfunction in the case of Imidacloprid, and honeybee brood immune depression for Fipronil. Despite the conduction of these monitoring reports and laboratory researches, Fipronil was completely banned in 2005 but Imidacloprid only in 2016.

3.2. Pesticide Residues and Transformation Products in Greek Honey, Pollen and Beebread

Konstantinos M. Kasiotis; Effrosyni Zafeiraki; Pelagia Anastasiadou; Electra Manea-Karga and Kyriaki Machera

Benaki Phytopathological Institute, Laboratory of Pesticides' Toxicology, 8 St. Delta Street, Athens, Kifissia 14561, Greece
E-mail: K.Kasiotis@bpi.gr, K.Machera@bpi.gr

Apiculture products, to an extent, are considered as environmental pollution markers, since they tend to accumulate a plethora of contaminants. The latter come in contact or enter into bees during nectar and pollen collection and transferred inside the beehives. In addition, residual prevalence in honey, and beebread also reflects the chemical treatments that take place inside the beehives in order to mainly control the parasitic mite of Varroa destructor.

In this context, during the period of 2014-2018, 109 samples of honey, pollen, and beebread (63 honey and 46 pollen and beebread), including samples originated also from colonies in which honeybees' death incidents were recorded, were sent by authorities and individuals in Benaki Phytopathological Institute for the determination of pesticides and their transformation products. More than 130 analytes were investigated by applying two multi-residue methods (an HPLC-ESI-MS/MS and a GC-MS/MS), based on modified QuEChERS methodology using for clean-up Z-Sep, PSA, and C18 materials. In particular, the two analytical methods applied were validated according to the SANTE/11945/2015 and 11813/2017 guidelines. More specifically, the recoveries observed for the majority of the analytes ranged between 68 and 117%, while the relative standard deviations were below 19%. The calculated limits of

quantification (LOQs) ranged from 1 to 10 ng/g depending on the analyte. Other parameters, such as linearity, selectivity, precision and matrix effect were also validated.

Until the end of 2018, 37 determinations were registered in honey, resulting in a 38% of positive to at least one active substance in honey samples (16 active substances and transformation products were detected in total). The detected concentrations of pesticides and their transformation products ranged between 1.3 and 785 ng/g honey. In some cases, maximum residue limits (MRLs) violations were evidenced. Coumaphos, imidacloprid, acetamiprid, the transformation products of amitraz, DMF-DMPF, tau-fluvalinate and in limited cases metabolites of imidacloprid and coumaphos (its oxon metabolite), were the most predominant compounds detected in honey, while several pyrethroids such as λ-cyhalothrin, cypermethrin, and cyfluthrin were also found. In several honey samples, more than one active substance was detected, while the most common combination comprised of coumaphos, imidacloprid, and DMF. In pollen, and beebread more active substances were identified (21) with a comparative number of determinations (including a higher number of fungicides detected compared to honey), and a higher proportion of positive samples (65%).

Overall, this work aims to provide an overview of the current situation of pesticides and transformation products occurrence in honey, pollen, and beebread during the period of 2014-2018 in Greece.

3.3. Impact of the use of plant protection products harmful to bees on bee colonies during spring: Results of a monitoring programme in apple orchards in South Tyrol (2014-2017)

Benjamin Mair, Manfred Wolf

Versuchszentrum Laimburg

Ausgangspunkt für das Projekt Apistox waren die vermehrten Meldungen von Imkern aus dem Einzugsgebiet des Südtiroler Apfelanbaus über massive Flugbienenverluste und eine generell schleppende Volksentwicklung vor allem im Frühjahr 2013 und in wenigen Jahren zuvor. Es lag die Vermutung nahe, dass die Beobachtungen auf die Intensivierung der Bekämpfungsmaßnahmen gegen Apfeltriebsuchtvektoren (*Cacopsylla picta* und *C. melanoneura*) im Apfelanbau und dem damit einhergehenden intensiveren Einsatz von bienengefährlichen Pflanzenschutzmitteln vor und nach der Blüte des Apfels zurückzuführen sein könnten. Im Projekt wurden über drei Jahre (von 2014-2016) Bienenvölker im Einzugsgebiet des Südtiroler Apfelanbaus rund um die Obstbaumblüte beobachtet und beprobt. Es handelte sich um ein Monitoring (kein experimenteller Ansatz) in welchem Bienenvölker im Zeitraum der Vorblüte, während der Blüte und der Nachblüte des Apfels überwacht wurden (jährlicher Beobachtungszeitraum: ca. Ende März - Mitte Juni). Die untersuchten Standorte verteilten sich zum einen über diverse Höhenlagen (Apfelanbau wird hauptsächlich zwischen 200 und 800 m ü. N. N. betrieben) und zum anderen über Gebiete mit unterschiedlichen Insektizideinsätzen. Im Rahmen des Projekts wurden Untersuchungen zum Totenfall, der Volksentwicklung (Schätzmethode nach Liebefeld), der Flugaktivität und dem Eintrag von Pflanzenschutzmittelwirkstoffen über Pollenhöschen durchgeführt. Die Ergebnisse bestätigen einen Zusammenhang zwischen dem Einsatz von bienengefährlichen Pflanzenschutzmitteln und den beobachteten Totenfall-Anstiegen. In wenigen Fällen gingen auch Flugaktivitätsrückgänge damit einher. Teilweise ließ sich auch ein Zusammenhang zwischen erhöhtem und vermehrtem Totenfall mit einer geringeren Volksstärke erkennen. Darüber hinaus konnten im Bienenbrot und in gesammelten Pollenhöschen mitunter relevante Konzentrationen von bienengefährlichen Pflanzenschutzmittel über mehrere Wochen festgestellt werden. Die genaue Dynamik hinter diesen Einträgen wird in einem aktuell noch laufenden und separat angelegten Projekt (Apistox II) weiter untersucht.

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4. Session – Risk Assesment/Risk management

4.1. Risk of exposure in soil and sublethal effects of systemic insecticides on ground-nesting hoary squash bees.

D. Susan Willis Chan [1*], Ryan S. Prosser [1], Jose L. Rodríguez-Gil [2], Nigel E. Raine [1]

School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
[2]Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

Ground-nesting solitary bees comprise 70% of bee species in temperate climates. In these species, female bees contact relatively large amounts of soil as they excavate their nests. Using the hoary squash bee (*Peponapis pruinosa*) as a model species, we evaluated the risk to adult female ground-nesting bees of exposure to lethal doses of systemic insecticide residues (clothianidin, thiamethoxam, imidacloprid, chlorantraniliprole) in agricultural soil in Ontario, Canada. To do this, we gathered agricultural soil samples at biologically relevant depths both during the bee-active period (July/August) and before insecticide application was made. Samples were analyzed for insecticide residues, and the residue concentrations were fitted to a distribution curve relating concentration to probability of exposure. Three LD50 benchmarks were then applied to the distribution curve to determine the probability of exceeding these benchmarks. Our assessment demonstrated high risk to ground-nesting bees, of exposure to lethal doses of clothianidin, thiamethoxam, and imidacloprid residues in agricultural soil based on the hoary squash bee model. No exposure risk was found for chlorantraniliprole. In parallel to our risk assessment, we introduced mated adult female hoary squash bees into net-covered hoop-houses in which a squash crop had been treated with imidacloprid, thiamethoxam, or chlorantraniliprole or not treated to evaluate the effects of exposure to these insecticides on nest establishment, reproduction, and pollen harvest. Statistically significant sublethal effects on pollen harvest, nest establishment, and reproduction were found for bees exposed to imidacloprid-treated squash plants with no effects found for bees exposed to squash plants treated with thiamethoxam or chlorantraniliprole.

4.2. Biopesticides and Pollinators – Examples and requirements on risk assessment from a technical perspective

Stefan Kimmel

Innovative Environmental Services (IES) LTD, Benkenstr. 260, CH-4108 Witterswil
E-Mail: s.kimmel@ies-ltd.ch

Biopesticides such as plant extracts or microbial compounds are currently the fastest growing segment of the crop protection industry, making the need for a more structured and efficient risk assessment undisputable. Regulators and relevant authorities have started to work on binding documents and set requirements, but yet, navigating the regulatory pathway is still a challenge. Requirements, differ around the globe. As an example, in Europe, Biopesticides are treated similar to conventional plant protection products, whereas in the US a separate set of Requirements and partly also risk assessment is set up.

This presentation intends to show current legislative background and guidelines in place when it comes to risk assessment for pollinators concerning Biopesticides. Further on some examples from the daily Laboratory routine as well as differences between standard approaches for common plant protection products versus Biopesticides are shown. Overall the

need for a differentiated approach as well as adapted mechanisms and testing strategies for special type of biological active compounds shall be discussed.

4.3. Bumblebee (*Bombus terrestris*) versus honey bee (*Apis mellifera*) acute sensitivity – Final results of an ECPA data evaluation

A. Dinter[1], J. Lückmann[2], R. Becker[3], M. Miles[4], E. Pilling[5], N. Ruddle[6], A. Sharples[1], S. Kroder[7], L. Oger[8]

[1] FMC Agricultural Solutions,
[2]Rifcon GmbH, [3]BASF SE, [4]Bayer AG, [5]Corteva Agroscience, [6]Syngenta Ltd., [7]Adama, [8]ECPA
E-Mail: axel.dinter@fmc.com

A data evaluation was conducted by ECPA companies to compare the acute sensitivity of the bumblebee *Bombus terrestris* L. with that of the honey bee *Apis mellifera* L. to plant protection products. For the evaluation 97 data sets were available for oral toxicity and 108 data set for contact toxicity for both bee species. The data comprised 27 and 29 sets for oral and contact toxicity testing of fungicides, 42 and 41 for oral and contact exposure for herbicides (including one plant growth regulator), and 28 oral and 38 contact data sets for insecticides (including one nematicide), respectively. For data sets with definitive endpoints for honey bees (most insecticides), the sensitivity ratio (SR) was determined by dividing the honey bee LD_{50} by the bumblebee LD_{50} value. Endpoints of data sets with unbound '>' endpoints (most fungicides and herbicides) for honeybees were assigned to toxicity classes. For data sets with unbound honey bee LD_{50}-values the data evaluation indicated similar or lower sensitivity of bumblebees versus honeybees by contact or oral exposure for all fungicides and herbicides. Likewise, similar or lower contact sensitivity of bumblebees than honey bees was determined for all insecticidal data sets (including the nematicide) with definite honeybee endpoints. For the oral exposure this was also the case except for 5 active substances. For two insecticide active ingredients the SRs were between 3.3 and 5.1. For two insecticide formulations with the same active ingredient and with unbound LD50-values for honeybees which generated SRs of approximately 95, results of higher tier semi-field data do not indicate any negative impact on *B. terrestris* and their colony development under more realistic semi-field conditions. Overall, the current data supports that, for a wide range of chemistry, the honey bee is a sensitive surrogate test species for bumblebees based on acute toxicity testing of plant protection products. Therefore, routine regulatory testing of the bumblebee (*B. terrestris*) in context of registration of plant protection products and/or using a standard safety of 10 on basis of honey bee endpoints is not justified on basis of available data review.

4.4. Proposed decision tree to evaluate the potential risk of plant protection products to bees via succeeding crops

Anne Alix[1], Mark J. Miles[2]

[1]Corteva Agriscience, Abingdon, OX14 4RN, UK, [2]Bayer Crop Science Division, Cambridge, CB4 0WB, UK

The exposure of bees from residues in succeeding crops is included on the list of exposure scenarios to be considered in a risk assessment in the EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees) (EFSA, 2013). A stepwise approach is proposed which is based on the default assumption of exposure in the succeeding crops, which is further refined based on knowledge of the quantitative coverage by attractive crops in the crop cycle and modelling estimates of pollen and nectar residues. EFSA acknowledged the difficulty to assess the spatial distribution of succeeding crops as well as the relevance of the assumptions on active substance properties and residue calculations to properly run this exposure scenario, and recommended to perform field experiments to study transfer from soil pore water to bee-relevant matrices to develop targeted succeeding crops scenarios.

This presentation proposes to contribute to the definition of targeted exposure scenarios for exposure through succeeding crops by introducing properties of the active substance and its metabolite(s) into the scheme that dictate the likelihood of presence as quantifiable residues in succeeding crops. These parameters are derived from existing guidance documents in use to decide e.g. upon soil persistence or to define residues levels in honey (EC, 2018). The possibility to define endpoints that trigger a risk assessment from succeeding crops will be discussed.

4.5. Are flowering weeds in agricultural treated fields a sigificant exposure route for risk assessment?

Natalie Ruddle[1], Ed Pilling[2], Graeme Last[3], Gabor Pap[3], Gavin Lewis[3], Mark Miles[4], Christof Schneider[5], Roland Becker[5], Anne Alix[2], Axel Dinter[6], Stefan Kroder[7], Amanda Sharples[6], Laurent Oger[8]

[1] Syngenta Ltd, [2]Corteva Agriscience, [3]ERM, [4]Bayer, [5]BASF, [6]FMC, [7]Adama, [8]ECPA
E-Mail: natalie.ruddle@syngenta.com

As part of an industry led initiative, the European Crop Protection Association (ECPA) have used available industry efficacy trial data to check the hypothesis of significant exposure via 'weeds in the treated field' exposure scenario, referred to in the EFSA bee Guidance Document, which suggests that if <10% of the area of use contains attractive flowering weeds then the exposure route is not relevant.

Weed recordings from over 8500 industry herbicide efficacy trials from a range of arable (sunflower, maize, oilseed rape, cereals, sugar beet, potatoes, peas and beans) and permanent crops (orachards, citrus and gapes) were anlaysed to check the hypothesis of significant exposure route via weeds in the treated field. Information was extracted from efficacy trial control data to determine if the occurrence of attractive flowering weeds constitutes less than 10% of the area of use, thereby highlighting that attractive flowering weeds in treated agricultural fields are not applicable for many commercially grown crops.

Here we present the analysis on the presence of weed species, growth stage of the weed species, attractiveness to bees of the weed species, the ground coverage of the weed species, the trial location and dates and the crop growth stage in the trials. The most pertinent questions being asked were '*are attractive flowering weeds likely to be present in arable and permanent crop fields*?' and '*what percentage of the area of the treated field might be occupied by attractive flowering weeds*?'. The project builds on the initial work from Maynard *et al,* 2014.

4.6. Guttation as an exposure route in the risk assessment for plant protection products – Review of available data

M. Miles[1], U. Zumkier[2], A. Sharples[3], N. Ruddle[4], Anne Alix[5], C. Schneider[6], E. Pilling[5], A. Dinter[3], S. Kroder[7], L. Oger[8]

[1] Bayer AG, [2] Tier3 Solutions, [3] FMC Agricultural Solutions, [4] Syngenta Ltd, [5] Corteva Agroscience, [6]BASF,
[7] Adama, [8] ECPA
E-Mail: mark.miles@bayer.com

Based on increased concern and awareness of the risks to pollinators from exposure to plant protection products (ppp), focus has been drawn to additional potential routes of exposure other than *via* pollen/nectar and direct contact. One potential source being considered for risk assessment is exposure following collection of contaminated guttation droplets by honey bees, which are known to exploit different water sources to satisfy colony needs. A risk could occur from this source when residues of water-soluble/systemic substances applied to a crop are present in the guttation liquid at levels which could result in toxicity to exposed honey bee colonies. Whereas toxicity can be measured in standardised laboratory tests, potential exposure via guttation droplets is more complex and three elements need to be considered as follows:

1: The concentrations of residues occurring in guttation water following ppp application

2: The occurrence of guttation on a certain crop species

3: The extent to which honey bees are actively collecting water via guttation droplets

These three points were used as the basis of a review of available data, which included 25 extensive regulatory studies conducted by industry specifically to evaluate the risk to honey bees from the occurrence of guttation in different crops. Assessments included the collection of guttation droplets by honey bees and almost always the potential effects at the colony level and measurement of residues in guttation liquid. Additionally, a review of published literature was performed in which 16 relevant papers were identified. The aims were to determine a 90th percentile for occurrence of guttation on a certain crop and the 90th percentile for numbers of honey bees collecting guttation droplets, along with consideration of measured residue levels. Results of this evaluation are presented here in the context of the exposure risk from ppp residues in guttation droplets to honey bees at the colony level.

4.7. Measures taken - the Swiss national action plan for bee health

Katja Knauer

Federal office for agriculture, Schwarzenburgstrasse, 3003 Bern Schweiz,
E-Mail: katja.knauer@blw.admin.ch

The annual winter losses of honey bees in Switzerland vary between 9% and 23% during the years 2008 to 2019 and are exceeding the as normal defined 10% level. The causes for the losses can have several reasons. However, one of the main reasons is the infection of the honeybees with the Varroa mite. Therefore, a health services for bees was founded to offer education programs for beekeepers and to support beekeepers in preventing and combating diseases. Switzerland further decided in 2014 to implement an action plan to promote the health of bees. Measures have been taken in the areas of disease prevention, promotion of food supply and reduction of risks from plant protection products. Immediate measures have been implemented such as the inclusion of a flowering strip in the Direct Payments Ordinance and measures to protect bees from plant protection products. Switzerland is actively involved in the development of new OECD test guidelines to evaluate the acute and chronic risk to honey- and wild bees. Honey and wild bees play an important role in pollination of agricultural crops and wild plants. The current situation is currently evaluated to decide if further measures are needed.

4.8. EFSA bee guidance document 2.0

Csaba Szentes

European Food Safety Authority (EFSA), Via Carlo Magno, 1A, 43126 Parma PR, Italy;
E-Mail: csaba.szentes@efsa.europa.eu

In 2013, EFSA adopted a Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees), which so far has not been fully implemented due to some lack of consensus between Member States. In March 2019, the European Commission has mandated EFSA to revise this Guidance Document (SANTE/E4/SH/gb(2019)1623216). The work program of EFSA will have to take into account the on-going discussions initiated by the Commission on defining specific environmental protection goals. Also, available relevant guidance developments (e.g. draft Guidance Document on seed treatments) should be considered. In order to have a clear picture on the main procedural aspects and timelines, EFSA has published an outline paper (http://www.efsa.europa.eu/en/press/news/190705). As asked by the mandate, several stakeholder consultations and a public consultation are planned. For the execution of the mandate, EFSA has created a working group consisting of experts from academia, regulatory experts and EFSA staff. According to the mandate and the terms of reference, this revision should focus on several aspects for which new scientific evidence may have meanwhile become available. EFSA will review:

- the evidence as regards bee background mortality
- the different exposure routes
- the list of bee-attractive crops
- the methodology with regard to higher tier testing

5. Session – Other

a. Applying the mechanistic honey bee colony model BEEHAVE to inform test designs of Large-Scale Colony Feeding Study (LCFS)

Silvia Hinarejos[1], Farah Abi-Akar[2]; Nika Galic[3]; Amelie Schmolke[2]

[1]Sumitomo Chemical Co. Ltd, Saint Didier au Mont d'Or, France
[2]Waterborne Environmental, Inc., Leesburg, Virginia, USA
[3]Syngenta Crop Protection LLC, Greensboro, North Carolina, USA
E-Mail: silvia.hinarejos@sumitomo-chem.fr; abi-akarf@waterborne-env.com; schmolkea@waterborne-env.com; nika.galic@syngenta.com

In 2017 a new subgroup was established within the ICPPR Semi- and Full-field Testing Workgroup. This new subgroup was tasked to develop guidance for designing and conducting large-scale colony feeding studies (LSCFS). LSCFS are one type of Tier II studies designed to determine potential effects of pesticides on free-foraging whole colonies during and after dietary intake of a known pesticide concentration. Recently, regulatory authorities in North America have used the LSCFS in their pollinator risk assessments for neonicotinoid insecticides on honey bees and other active ingredients. The LSCFS design involves a relatively large number of replicates, treatment levels, and colony condition assessments, including overwintering. Despite its high cost and use in regulatory risk assessments, no formal regulatory protocol exists for conducting these studies. High overwintering losses of control hives have been observed in some LSCFS. Loss of control colonies indicates that stressors other than pesticides, e.g. resource availability, weather, diseases and beekeeping activities, likely influence colony overwintering survival, confounding the assessment of impacts caused by pesticides. Honey bee colony models have been gaining interest as tools in pesticide risk assessment to inform study design and ultimately, colony-level risks to honey bees. In the current project commissioned by the Pollinator Research Task Force, we assessed the study design and environmental conditions experienced by the untreated colonies of seven LSCFS. We applied the mechanistic colony model BEEHAVE to systematically assess the impact of study design and environmental conditions on control colonies. We first calibrated BEEHAVE to a subset of the studies, validated it with the remaining studies, and then used it to run simulations that changed only one variable at a time. The goal of the project was to inform study design that leads to increased likelihood of control colony overwintering success in LSCFS. From the simulations, the initial status of the colonies as well as the sugar feeding pattern were more important for fall colony condition than resource availability control colonies across seven LCFSs. Overwintering success in these control colonies differed considerably among the studies. In addition, the studies differed with respect to initial colony conditions, amount and timing of sugar feeding, landscape composition around study apiaries and weather in the landscape and weather. Larger honey stores present in the colonies at study initiation, greater feeding amounts and earlier supplemental feedings (beginning in late summer to early fall) were the main factors that led to larger colony sizes and honey stores in the fall. This information can be used to inform the standardization of a study design, which in turn can increase the likelihood of overwintering survival in untreated controls and help ensure that studies are comparable. This project demonstrates how a mechanistic model can be used to inform study designs for higher-tier effects studies. Mechanistic models like BEEHAVE could further be applied to supplement higher-tier risk assessments, for instance, by extrapolating to non-tested exposure scenarios and environmental conditions and therefore potentially reducing the number of higher-tier studies.

b. BEEHAVE validation and resulting insights for the design of field studies with bees

Annika Agatz[1], Mark Miles[2], Thorsten Schad[2], Thomas Preuss[2]

[1] ibacon GmbH, Arheilger Weg 17, D-64380 Rossdorf, Germany
[2] Bayer AG, Crop Science, Building 6692, 40789 Monheim, Germany
E-Mail: annika.agatz@ibacon.com

Factors affecting honey bee health are manifold (including diseases, parasites, pesticides, environment and socio economic factors). A lack of standard procedures for higher tier risk assessment of plant protection products for bees makes coherent availability of data, their interpretation, and their use for higher tier risk assessment challenging. Focus has therefore been given to the development of modelling approaches which in the future could fill this gap. BEEHAVE is the first model attempting to link two of the processes vital for the assessment of bee mortality; the within-hive dynamics for honey bee colonies and bee foraging in heterogeneous and dynamic landscapes.

Here we show results of several BEEHAVE validation studies conducted. We specifically focus on insights gathered through these modelling exercises for the design and the usability of field studies for further development, testing and validation of the BEEHAVE model.

Overall the model validation shows that predictions of bee hive dynamics fit observations of the total number of adult bees, the total number of offspring in the hive, and the production of drones well. This result underpins the results of the EFSA evaluation of the BEEHAVE model, that the most important inhive dynamics are represented and correctly implemented in the model, with empirical evidence. Agreement between data and model predictions is particularly high for the initial experimental phase prior the generally conducted relocation of the bee hive from the actual experimental landscape to an overwintering site. Increased discrepancy following the relocation is an artefact of lack of information on the landscape characterisation of the overwintering site for model parameterisation; leading to increased inaccuracy of the model prediction for pollen and nectar resources in the hive, that in turn determines the abundance of bees and thus the overwintering survival probability of the colony.

It is vital to redistribute experimental efforts allocated to a field study to better assess the suitability of using BEEHAVE for the prediction of bee colony overwintering survival as an important endpoint for higher tier risk assessment for bees. A more equal bee hive and landscape investigation throughout the entire field study, rather than a bias towards the actual exposure phase, is required to improve data availability for model validation.

c. Bee pollinator toxicogenomics: an interdisciplinary approach to unravel molecular determinants of insecticide selectivity

Marion Zaworra and Ralf Nauen

Bayer AG, Crop Science Division, Research and Development, Alfred-Nobel-Straße 50, 40789 Monheim am Rhein, Germany
E-Mail: marion.zaworra@bayer.com; ralf.nauen@bayer.com

A favorable bee profile is one of the key requirements in the development and (re)registration of insecticides. While the toxicity of insecticides to bees is routinely assessed according to officially published guidelines and guidance documents, their interactions with bees on the molecular and biochemical level have not been intensively studied, yet.

Thus, Bayer AG, Crop Science Division, initiated the project "Bee Pollinator Toxicogenomics" with the particular aim to elucidate the molecular basis of selectivity of insecticides against

bee pollinators with special reference to a comparative functional genomics approach covering different bee species in cooperation with external partners.

As a starting point, we performed toxicological studies with the *N*-cyano-substituted neonicotinoid insecticide thiacloprid and *N*-nitro-substituted compound imidacloprid to identify the reason(s) for the over 500-fold higher intrinsic toxicity of *N*-nitro-substituted compounds to the honey bee (*Apis mellifera*). Radioligandbinding assays revealed that both, thiacloprid and imidacloprid, display a similar nanomolar binding affinity to their target, the postsynaptic nicotinic acetylcholine receptor (nAChR). However, thiacloprid is significantly faster degraded by hydroxylation compared to imidacloprid providing evidence that cytochrome P450 monooxygenases (P450s) facilitate oxidative metabolism of this chemical class. Subsequently, a honey bee P450 expression library compromising all 27 clade 3 P450s was established and P450s belonging to CYP9Q-subfamily were identified to be involved in the rapid turnover of thiacloprid, mainly driven by CYP9Q3, but with a low turnover of imidacloprid. Beside the honey bee CYP9Q-family, we also identified in collaboration with external partners at Rothamsted Research and Exeter University the orthologous P450s CYP9Q4-6 in the bumblebee (*Bombus terrestris*) and CYP9BU1-2 in the red mason bee (*Osmia bicornis*) as key determinants of neonicotinoid selectivity. The knowledge obtained from this interdisciplinary approach is of high value to mechanistically understand the interaction of pesticides and bees beyond guideline studies and is further extended to gain insights in the molecular mechanism underlying bee-sensitivity in other pollinator species, i.e. the alfalfa leafcutter bee *Megachile rotundata*.

Moreover, the established molecular and biochemical tools are ready to be applied to address questions of fundamental research as well as in the targeted design of intrinsically bee-friendly insecticides.

d. Conservation and creation of multi-functional margins to maintain and increase the pollinator biodiversity in agricultural environments (1.13)

Peris-Felipo, F.J.[1], Aguado-Martín[2], L. O. and Miranda-Barroso[3], L.

[1] Syngenta Crop Protection AG, Rosentalstrasse 67, 4058 Basel (Switzerland);
[2] Andrena Iniciativas y Estudios Medioambiental S.L. c/. Gabilondo 16bis, 47007 Valladolid (Spain);
[3] Sustainable Agriculture Syngenta Spain, c/. Ribera del Loira, 8, 10, 28042 Madrid (Spain);
E-Mail: oscaraguado@lepidopteros.com; javier.peris@syngenta.com; luis.miranda@syngenta.com

When a natural ecosystem changes its use in agriculture, factors that greatly affect its fauna, especially insects, are introduced. This kind of land change, and especially intensive production models causes a clear loss of biodiversity, with a drastic decrease in the number of plant species that in turn affects the natural pollinator entomofauna.

In 2010, one of the main conclusions reached by the European Commission for the Conservation of the Environment was the need to promote research on the conservation, restoration and sustainable use of the diversity of pollinators in agriculture. This situation together with the climate change and the notable decrease in the number of wild pollinators has meant that the European Union, FAO (United Nations Food Organization) and other important international organizations have raised the alarm about the need to look for how to maintain and increase the presence of wild pollinators.

In order to find practical solutions, the company Syngenta Crop Protection launched the "Operation Pollinator (OP)" project in 2009, a European-level initiative launched in Britain as part of the EU action called EPI ("European Initiative on Pollinators"), whose main objective is to protect pollinators, increase their biodiversity and promote their presence and also other beneficial or auxiliary arthropods in the crops.

The present study collects the results obtained in different agricultural farms of the Iberian Peninsula, demonstrating how right agricultural practices can also help to maintain biodiversity and favour its rapid increase, both qualitatively and quantitatively.

e. Introducing the INSIGNIA project: Environmental monitoring of pesticide use through honey bees

Jozef J.M. van der Steen on behalf of the Insignia consortium:

Dr Jozef J.M. van der Steen, Alveus AB Consultancy, Oisterwijk , Netherlands; Dr Robert Brodschneider, Ms Kristina Gratzer, Ms Sarah Bieszczad, University of Graz, Graz, Austria; Dr Fani Hatjina, Dr Leonidas Charistos, Ellinikos Georgikos Organismos - Dimitra, Nea Moudania, Greece; Mr Norman L. Carreck, Carreck Consultancy Ltd, Shipley, UK; Dr Alison Gray, University Of Strathclyde, Glasgow, UK; Prof. M. Alice Pinto, Prof. Joana Amaral, Prof. José Rufino, Dr Andreia Quaresma, Instituto Politecnico De Braganca, Braganca, Portugal; Dr Ivo Roessink, Dr Hans Baveco, Wageningen Environmental Research, Wageningen, Netherlands; Dr Giovanni Formato, Dr Marco Pietropaoli, Instituto Zooprofilattico Sperimentale Delle Regioni Lazio E Toscana, Rome, Italy; Dr Konstantinos Kasiotis, Dr Christ Anagnostopoulos, Dr Effrosyni Zafeiraki, Benaki Phytopathological Institute, Athens, Greece; Prof. Amadeo Fernandez-Alba, Ms. Maria Murcia,
Universidad De Almeria, Almeria, Spain; Ms Caroline Eulderink, HKH Kwaliteit en Certificering, Veldhoven, Netherlands; MSc Flemming Vejsnæs, Dr Ole Kilpinen, Danish Beekeepers Association, Sorø, Denmark; Dr Mary Frances Coffey, University Of Limerick, Limerick, Ireland, Dr David , Biron, Centre National De La Recherche Scientifique CNRS, Aubière, France; Mr Valters Brusbardis, Latvian Beekeepers Association, Jelgava, Latvia; Prof. Dirk de Graaf, University of Gent, Gent, Belgium

INSIGNIA aims to design and test an innovative, non-invasive, scientifically proven citizen science environmental monitoring protocol for the detection of pesticides by honey bees. It is a 30-month pilot project initiated and financed by the EC (PP-1-1-2018; EC SANTE). The study is being carried out by a consortium of specialists in honey bees, apiculture, statistics, analytics, modelling, extension, social science and citizen science from twelve countries. Honey bee colonies are excellent bio-samplers of biological material such as nectar, pollen and plant pathogens, as well as non-biological material such as pesticides or airborne contamination. Honey bee colonies forage over a circle of 1 km radius, increasing to several km if required, depending on the availability and attractiveness of food. All material collected is accumulated in the hive.

The honey bee colony can provide four main matrices for environmental monitoring: bees, honey, pollen and wax. Because of the non-destructive remit of the project, for pesticides, pollen is the focal matrix and used as trapped pollen and beebread in this study. Although beeswax can be used as a passive sampler for pesticides, this matrix is not being used in INSIGNIA because of its polarity dependent absorbance, which limits the required wide range of pesticides to be monitored. Alternatively, two innovative non-biological matrices are being tested: i) the "Beehold tube", a tube lined with the generic absorbent polyethylene-glycol PEG, through which hive-entering bees are forced to pass, and ii) the "APIStrip" (Absorbing Pesticides In-hive Strips) with a specific pesticide absorbent which is hung between the bee combs.

Beebread and pollen collected in pollen traps are being sampled every two weeks to be analysed for pesticide residues and to record foraging conditions. Trapped pollen provides snapshots of the foraging conditions and contaminants on a single day. During the active season, the majority of beebread is consumed within days, so beebread provides recent, random sampling results. The Beehold tube and the APIStrips are present throughout the 2-weeks sampling periods in the beehive, absorbing and accumulating the incoming contaminants. The four matrices i.e. trapped pollen, beebread, Beehold tubes and APIStrips will be analysed for the presence of pesticides. The botanical origin of trapped pollen, beebread and pollen in the Beehold tubes will also be determined with an innovative molecular technique. Data on pollen and pesticide presence will then be combined to obtain information on foraging conditions and pesticide use, together with evaluation of the CORINE database for land use and pesticide legislation to model the exposure risks to honey bees and wild bees. All monitoring steps from sampling through to analysis will be studied and rigorously tested in four countries in Year 1, and the best practices will then be ring-tested in nine countries in Year 2. Information about the course of the project, its results and publications will be available on the INSIGNIA website www.insignia-bee.eu and via social media: on Facebook (https://www.facebook.com/insigniabee.eu/); Instagram (insignia_bee); and Twitter (insignia_bee). Although the analyses of pesticide residues and pollen identification will not be completed until December 2019, in my talk I will present preliminary results of the Year 1 sampling.

f. Bee-O-Meter

Johannes Meleschnig, PhD

IoT40 Systems GmbH, 9020 Klagenfurt, Lakesidepark B04;
E-Mail: meleschnig@iot40systems.com

schöbinger is an innovative cloud service that measures the ecological purity of our environment with the help of bee colonies. The measurement criterion is the bee loss rate, which results from the counted bee trips and returns to the hive. This measure is combined with other data from the hive and data from external stations. Based on AI (Artificial Intelligence) logic, various alarms are set, and a dashboard allows all data to be viewed.

The visual sensor recognizes the sexes, swarming, flight of a foreign queen, wasps and other insects, especially the prey beetle, from different bee species (e.g., Carnica Bee, Buckfast Bee). This is based on techniques from the field of ANN (artificial neural networks)

The Bee-o-Meter thus supports the individual beekeeper in the observation of his bee colonies.

By networking in the field, the Bee-o-Meter service also detects and localizes environmental changes such as Pesticides and other pollutants that harm the bees, and therefore also humans. The Bee-o-Meter thus enables an efficient biomonitoring of a region as well as the networking of Bee-o-Meter stations.

The Bee-o-Meter thus supports the individual beekeeper in the observation of his bee colonies.

And through the networking of Bee-o-Meter stations in the field, the service also recognizes and localizes environmental changes such as Pesticides and other pollutants that harm the bees, and therefore also humans. The Bee-o-Meter thus enables an efficient bio-monitoring of a region via the networking of Bee-o-Meter stations.

g. Report of the activities of the ICPPR Bee Brood Working Group

Matthew Allan, Markus Barth, Roland Becker, Sigrun Bocksch, Magdaléna Cornement, Jakob Eckert, Hervé Giffard, Bettina Hodapp, Lukas Jeker, Stefan Kimmel, Johannes Lückmann, Markus Persigehl, Ed Pilling, Natalie Ruddle, Rastislav Sabo, Christof Schneider, Stephan Schmitzer, Maryam Sultan, Verena Tänzler, Selwyn Wilkins

ICP-PR Bee Brood Working Group (WG)
Co-Chairs: Verena Tänzler (Ibacon), Lukas Jeker (Agroscope) and Selwyn Wilkins (Fera)

The ICP-PR Bee Brood Working Group (WG) was founded at the 9th Symposium held at York, UK, in 2005. It was chaired by Roland Becker (BASF) until the 13th Symposium in 2017 in Valencia, Spain; the WG is currently chaired by Verena Tänzler (Ibacon)), Lukas Jeker (Agroscope) and Selwyn Wilkins (Fera). The first WG meeting following Valencia was held in Amsterdam in March 2018. The first task was to identify WG priorities given recent regulatory developments and data requirements on higher-tier bee brood studies *i.e.* semi-field and field testing. The aim was to continue the previous work of the group toward improving and harmonizing the OECD 75[1] and Oomen *et al.* 1992[2] methods. A full review of the available test methods was undertaken, looking at the strengths and limitations of the semi-field and full-field brood testing methods. Additionally, one of the major issues noted was lack of a clear structure or guidance for progressing through the testing methods and under what circumstances should a particular test be considered? Based on this initial meeting and discussions, three subgroups were formed each working separately on their tasks and coming together at joint WG meetings to discuss their progress.

1. **Conceptual Framework sub-group (Maryam Sultan - Bayer)**
   Tasked by the WG to develop a conceptual framework (road map) in which OECD 75 and the Oomen *et al.* tests (both original and modified) may be improved and where the methods can be applied most effectively. A draft has been produced.
2. **OECD75 revision sub-group: (Verena Tänzler – Ibacon)**
   To review the OECD 75 method and to identify possible amendments to OECD Guidance Document (GD), and address issues associated with meeting validity criteria. Based on other guidance documents, the subgroup determined that there is sufficiently new information (*e.g.*, inclusion of new photographic methodologies) to recommend a revised OECD GD. The subgroup elected to present their thoughts and findings to ICP-PR and seek feedback.
3. **Oomen de Reuter sub-group (Johannes Lückmann – RIFCON)**
   To expand improve the method based upon recent developments (*e.g.*, including recommendations of ICPPR Bee Brood WG and papers of Lückmann and Schmitzer 2019[3] and AG Bienenschutz).

[1] OECD. 2007. Guidance document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions. Series on Testing and Assessment No. 75. ENV/JM/MONO(2007)22

[2] Oomen, P. A. A. De Ruijter and J. Van der Steen. 1992. Method for honey bee brood feeding tests with insect growth-regulating insecticides. Bul OEPP/EPPO Bulletin 22: 613 – 616.

[3] Lückmann, J. and S. Schmitzer. 2019. The Oomen bee brood test—revision of the method to current needs and developments. Bulletin OEPP 49(1): 137 – 146. https://doi.org/10.1111/epp.12553

Abstracts: Posters

1. Session – Risk Assessment/Risk management

1.1. Precision farming – consideration of reduced exposure in the pollinator risk assessment

Johannes Lückmann, Sibylle Kaiser; Felix von Blankenhagen

RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany;
E-Mail: Johannes.lueckmann@rifcon.de

In the course of the ongoing discussions on the desired reduction of plant protection products (PPP) and the protection of insects, especially pollinators, but also in the light of intended yield increase, process optimisation and cost reduction in agriculture, precision farming becomes more and more important. As there can be huge small-scale variability of insect, weed or fungal infestation of the crop, precision plant protection application will enable farmers an infestation-orientated and subarea-specific crop protection within a field.

Currently, developments and research activities are mainly focussed on technical optimisation such as spatial recording of pests (weed species, insect pests, crop relevant fungal diseases), data processing and analysis as well as on the use of this information for the operating of precision application equipment. Agricultural devices used in precision application is currently under development (mainly research-orientated), but a few models are already in practical use.

In principle, the use of precision techniques in plant protection should lead to reduced exposure of non-target organisms such as soil organisms, epigeous and epiphytic beneficial arthropods and bees. However, it is unclear to what extent this is the case for the different exposure pathways and where and how this can be taken into account in the environmental risk assessment of PPP.

We present exemplary precision application systems and discuss their potential influence on the exposure of honey bees and their colonies of partially treated fields. Furthermore, we will suggest how precision plant protection application can be included in the environmental risk assessment scheme and present ideas to verify the theoretical assumptions.

1.2. Evaluation of honey bee larvae data: sensitivity to PPPs and impact analysis of EFSA Bee GD

Johannes Lückmann [1]; Roland Becker[2]; Mark Miles[3]; Anne Alix[4]; Axel Dinter[5]; Stefan Kroder[6]; Ed Pilling[4]; Natalie Ruddle[7]; Christof Schneider[2]; Amanda Sharples[5]; Laurent Oger[8]

[1]RIFCON GmbH,
[2] BASF SE, [3]Bayer AG, [4]Corteva Agriscience, [5]FMC Agricultural Solutions, [6]ADAMA, [7]Syngenta Ltd, [8]ECPA
E-Mail: Johannes.lueckmann@rifcon.de

Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk on honeybee larvae or honeybee brood.

In July 2013 the European Food Safety Authority (EFSA) published a guidance document on the risk assessment of plant protection products on bees (EFSA 2013). This document is intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009 (EC 2009). Since the guidance was first published, honeybee larvae toxicity studies have been conducted for active substances and formulated products according to newly developed test methods

The first objective of this poster is to summarize all these available industry data on honeybee larvae testing according to OECD TG 237 and OECD GD 239, in order to gain an overview of these results and the selectivity of different product groups. The sensitivity of the endpoints are presented. In addition, endpoints obtained at different development stages after 8 and 22 days in OECD GD 239 studies are compared.

As a first step in the risk assessment, EFSA requires a screening step, which consists of the calculation of risk quotients (ETRs) for honeybee larvae. This considers exposure routes for the in-field (PPPs applied as sprays) and off-field (PPPs used as seed treatments and granules) scenarios. Where a use does not pass one of the screening level risk quotients, EFSA offers the possibility for refinement in a Tier 1 risk assessment. This includes the refinement of exposure estimates from the screening step and also additional exposure routes, such as the exposure to flowering plants in the field margin and adjacent crops. As worst-case scenarios, the risk of honeybee larvae being exposed to treated crops and weeds were assessed. The same approach as used for the honeybee was also conducted for bumblebees and solitary bees but with the application of specific short cut values (SV) from the EFSA guidance document. As no validated testing guidelines are available for bumblebees and solitary bees honey bee endpoints are used as a surrogate with an additional 10x safety factor applied to the endpoints.

The second objective of this poster is to evaluate the impact of the proposed screening and Tier 1 risk assessments on the pass rates of currently available active substances and formulated products, which is an ability of the scheme to correctly identify compounds of potential concerns and consequently screen out those of low concern. In addition, the outcome of an industry proposed alternative risk assessment as described by ECPA (2017) is presented.

1.3. Chronic oral exposure of adult honey bees to PPPs: sensitivity and impact analysis of EFSA Bee GD

Johannes Lückmann[1]; Mark Miles[2]; Roland Becker[3]; Anne Alix[4]; Axel Dinter[5]; Stefan Kroder[6]; Ed Pilling[4]; Natalie Ruddle[7]; Christof Schneider[3]; Amanda Sharples[5]; Laurent Oger[8]

[1] RIFCON GmbH; Johannes.lueckmann@rifcon.de
[2] Bayer AG, [3] BASF SE, [4] Corteva Agriscience, [5] FMC Agricultural Solutions, [6] ADAMA, [7] Syngenta Ltd, [8] ECPA

Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the chronic risk on adult honeybees.

In July 2013 the European Food Safety Authority (EFSA) published a guidance document on the risk assessment of plant protection products on bees (EFSA 2013). This document is intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009 (EC 2009).

The first aim of this poster is to summarize industry data based on studies conducted up to 2018, for active substances and formulated products on the chronic oral testing of adult honeybees according to OECD test guideline 245 and its previously drafts, in order to gain an overview of these results and the selectivity of different product groups.

As a first step in the risk assessment, EFSA requires a screening step which consists of the calculation of risk quotients (ETRs) for the chronic exposure based on the application rate, an application depending shortcut value, an exposure factor and the endpoint (LDD_{50}). This considers exposure routes for the in-field (PPPs

applied as sprays) and off-field (PPPs used as seed treatments and granules) scenarios. Where a use does not pass one of the screening level risk quotients, EFSA offers the possibility for refinement in a tier I risk assessment. This includes refinement of the exposure estimates from the screening step and also additional exposure routes, such as the exposure to flowering weeds in the field and adjacent flowering crops. Screening step and tier I risk assessment were also conducted for bumble bees and solitary bees, using 1/10 of the honeybee endpoint.

The second aim of this poster is to evaluate the impact of the proposed screening and tier I risk assessments on the pass rate of currently available active substances and formulated products, thereby testing the ability of the scheme to correctly identify compounds of potential concern and consequently screen out those of low concern. The third objective of this work is to present the outcome of alternative calculations as described by ECPA (2017).

The aforementioned analysis follows the principles described in the ECPA impact analysis (Alix et al. 2013) which used theoretical data due to lack of real data. The present analysis compares the pass rates from this first approach with the outcome based on real laboratory data which are now available.

1.4. Establishing realistic exposure estimates of solitary bee larvae via pollen using inter species correlation models

Tobias Pamminger[1], Christof Schneider[1], Matthias Bergtold[1]

[1] BASF SE
E-Mail: tobias.pamminger@basf.com

In recent years there is growing concern that some solitary bee populations are in decline, potentially compromising pollination security in agricultural and non-agricultural landscapes. Among the numerous causes associated with this trend bee exposure to plant protection products (PPP) in agricultural landscapes has been discussed. Bees can be exposed to PPP directly resulting from overspray and/or to residues in pollen and nectar. In the case of solitary bee larvae, the main exposure route is likely pollen and the amount consumed depends on the size of the bee larvae and the pollen composition and (e g. pollen protein concentration). So far exposure estimates for wild bee larvae for risk assessment purposes have often been based on a limited number of observations making their accuracy uncertain. As a first step to tackle this question we combine information on solitary bee ecology (plant preference), plant pollen quality (pollen protein concentration), bee larvae weight and pollen consumption to build a phylogenetically controlled inter species correlation model to estimate the protein/pollen needs of solitary bee larvae. We use this model to predict the protein/pollen needs of Osmia bees (the currently discussed solitary bee surrogate for EU risk assessment) and contrast our results with the proposed default pollen consumption estimates. We find that the currently used default pollen consumption values likely overestimate exposure and we discuss the implications of our findings for the future solitary bee risk assessment in Europe.

first step to tackle this question we combine information on solitary bee ecology (plant preference), plant pollen quality (pollen protein concentration), bee larvae weight and pollen consumption to build a phylogenetically controlled inter species correlation model to estimate the protein/pollen needs of solitary bee larvae. We use this model to predict the protein/pollen needs of Osmia bees (the currently discussed solitary bee surrogate for EU risk assessment) and contrast our results with the proposed default pollen consumption estimates. We find that the currently used default pollen consumption values likely overestimate exposure and we discuss the implications of our findings for the future solitary bee risk assessment in Europe.

2. Session – Honeybee Brood

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

Johannes Lückmann [1]; Verena Tänzler [2]

[1] Rifcon GmbH, 69493 Hirschberg, Germany
[2] ibacon GmbH, 64380 Rossdorf, Germany
E-Mail: johannes.lueckmann@rifcon.de, verena.taenzler@ibacon.com
* on behalf of the ICP-PR Bee Brood Working Group and the bee brood working group of the German AG Bienenschutz

Based on EU Regulation 1107/2009/EC the current regulatory risk assessment of plant protection products (PPP) on bees has to address the risk on honeybee larvae or honeybee brood. According to the 'EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)' (EFSA 2013), both, the Oomen bee brood feeding test (Oomen et al., 1992) as well as the OECD Guidance Document 75 (2007; OECD GD 75) are given as the two higher tier options to refine the risk on honeybee brood if concern is raised in tier 1.

Both methods focus on the brood termination rate (BTR) as the key endpoint. While the Oomen brood test investigates an artificial and worst case acute or chronic oral exposure scenario with a test item spiked feeding solution administered inside the hive (Lückmann & Schmitzer 2019) brood studies according to OECD GD 75 under semi-field conditions rely on a realistic contact and oral exposure scenario to bees comprising contaminated nectar and pollen after overspray of a bee attractive crop. But the evaluation of historical data from semi-field studies according to OECD GD 75 showed a strong variability of the control BTRs (e.g. Becker et. al 2015). Therefore, field studies according to EPPO 170 (2010) which comprise bee brood evaluations according to OECD GD 75 were regarded as an option to get more reliable BTR data (Becker et. al 2015, Giffard & Huart 2015).

The present poster compares control BTRs from acute and chronic Oomen feeding studies with BTRs obtained from OECD GD 75 semi-field trials and field trials. Moreover, the possibilities and limitations of the three methods will be discussed.

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

L. Sabová[1], M. Staroň[2], A. Sobeková[1], D. Staroňová[2], J. Legáth[1], R. Sabo[1]

[1]University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia
[2]Institute of Apiculture Liptovský Hrádok, Gašperíkova 599, 033 80 Liptovský Hrádok, Slovakia
Correspondence: rastislav.sabo@uvlf.sk

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

3. Session – Laboratory/Semi-field/Field

3.1. Do pollen foragers represent a more homogenous test unit for the RFID homing test, when using group-feeding?

Michael Eyer, Daniela Grossar, Lukas Jeker

Agroscope, Swiss Bee Research Center, 3003 Bern, Switzerland

The RFID homing ring test aims at developing a method, which can assess sublethal effects of xenobiotic substances on the navigation of foraging bees. Thereby, bee biology and corresponding behavioral processes might strongly influence the output of this test method. Accordingly, previous experiments demonstrated that the homing ability of nectar foragers differed between group- and single-bee-feeding, based on uneven crop content of returning bees and/or due to uneven food distribution via trophallaxis. Therefore, we here evaluated if pollen foragers represent a more homogenous test unit, when test item solutions are administered to groups of bees and thus are distributed between each other via trophallaxis. For this, we tested thiamethoxam and thiacloprid (both neonicotinoid insecticides) at field realistic doses by orally exposing tagged pollen foragers, either in groups of ten bees, or in single cages.

Our results demonstrate that the homing ability of thiamethoxam exposed pollen foragers was significantly different from the non-exposed control in the single-bee feeding approach, but not in the ten-bee feeding approach (using conservative bonferroni correction in nominal pairwise matrices). Similar tests with thiacloprid, revealed not such clear differences between the two feeding approaches. Thus, it seems that the effect of group size on the homing ability of pollen foragers seems to be compound/dose specific. Nevertheless, our results suggest that single-bee-feeding reveal biologically more robust results in context of homing ability compared to group feeding, which should be considered in the development of this new test guideline by ideally performing such tests with single-bee feeding. Moreover, pollen- instead of nectar foragers should be preferentially chosen, since they consumed the feeding solution quicker and more reliable compared to previous trials with nectar foragers.

3.2. Digital Farming & evaluation of side effects on honey bees – first experiences within the Digital Beehive project

Catherine Borrek, Simon Hoff, Ulrich Krieg, Volkmar Krieg, Philipp Senger, Marc Schwering, Silke Andree-Labsch

Bayer AG Division Crop Science, Versuchsgut Höfchen, 51399 Burscheid, Germany
E-Mail: catherine.borrek@bayer.com

Within the framework of the bee pollinators risk assessment of plant protection products, like honey bees (Apis mellifera), semi-field studies (in net houses) are conducted under worst-case exposure conditions to evaluate potential side-effects on the colony level.

Therefore, several parameters concerning the bees' health status, activity and behavior on the level of individual bees and the entire colony have to be assessed. These in situ observations and evaluations are necessary conducted by skilled investigators, who are experienced in both bee management and plant protection practices.

Furthermore, digital sensor technologies around the beehive can provide additional valuable information to better understand the assessed parameters. A clear advantage of such a digital

monitoring system is a continuous data acquisition, whereas the required manual assessments represent only short snapshots in time. Especially within the first hours after the application, when observations and assessments are limited for reasons of time and health protection, sensor technology can be utilized for observation of the bees' reaction to a test compound and thereby allows the detection of a potential repellent effect or similar. Additionally, digital sensors can be calibrated to ensure the accuracy of the measurements.

In several semi-field trials according to EPPO guideline No. 170 we compared two different digital monitoring systems (ApiSCAN® and Arnia remote hive monitoring™) and related the sensor-derived data with usual manual assessments. Based on our findings we want to highlight benefits and limitations of a digital beehive in context of the assessment of potential side-effects of plant protection products on pollinators.

3.3. Bee colony assessments with the Liebefeld method: How do individual beekeepers influence results and are photo assessments a possibility to reduce variability?

Holger Bargen, Aline Fauser, Heike Gaetschenberger, Gundula Gonsior & Silvio Knaebe

Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany,
E-Mail: GundulaGonsior@eurofins.com

Colony strength, food storage and brood development are a fundamental part of each honeybee field study. Colony assessments are used to compare and assess those for beehive over time. At present, most colony assessments are made by experienced beekeepers according to Liebefeld. This method is based on an estimation of areas covered by honeybees, food and brood stages on each side of a comb. Areas are counted from a grid separating the comb side into 8 sections which are protocolled with an accuracy of 0.5 sections. An assessment for a hive takes up to 20 min and even with two field locations, it is necessary to split assessments between beekeepers. So, it is important to make estimates as comparable as possible. For this purpose beekeepers practice the assessments on pre-determined photographs to "calibrate themselves". The advantage of the Liebefeld assessment is that the condition of bee hive is estimated with minimum disturbance of the bees. Digital photography is under discussion to gain data with high precision and accuracy with one major disadvantage. To be able to see food and brood stages in photographs, bees have to be removed from combs. This, however, results in a disturbance of the colony – especially if the assessments take place in short time intervals of 7 ± 1. An experiment was performed to evaluate the variation between individual beekeepers and to compare the results to data generated with photographs. For the experiment, five colonies were assessed each by five beekeepers independently according to Liebefeld method. Each comb side of the five colonies was photographed with and without honeybees sitting on it for precise analysis at the computer for a number of bees, nectar cells, pollen cells, eggs, open brood and capped brood. The number of bees and cells with the different contents were generated by an area-based assessment in ImageJ as well as a detailed counting with help of HiveAnalyzer® Software. Data from beekeeper estimations were then compared with assessments based on digital photography. With the results of the experiment, we tried to answer several questions. First of all, we wanted to determine the level of variation between the beekeepers for the live stages and food stores estimated. Furthermore,

we wanted to find out, if the photo assessment is such a precise method that it would justify a replacement of the well-established Liebefeld method despite the strong disturbance of the bees.

3.4. Practical and regulatory experience in the conduct of bee residue trials

Silke Peterek[1]; Elizabeth Collison [2]; Vincent Ortoli[3]; Alexia Faure[3]

[1] Staphyt GmbH, Langenburger Str. 35, 74572 Blaufelden, Germany
[2] APC Wetherby (part of Staphyt group), Cromwell Office Park, York Road, LS22 7SU Wetherby, United ingdom
[3] Staphyt and Staphyt Regulatory, 23 rue de Moeuvres, 62860 Inchy en Artois, France

To ensure the safe use of agrochemicals, today's regulatory system requires an assessment of the environmental risk to bees, as well as an assessment of the dietary risk to humans following the consumption of honey and other bee products. Field trials can provide valuable data to assess the potential exposure of foraging honey bees to agrochemical residues and hence the potential for residues to reach honey consumed by humans.

With increasing requests for pesticide residue data on honey and other bee products, field trial teams and risk assessors alike must find workable procedures to collect and analyse appropriate samples and understand how such data can be used in a regulatory context. For the past several years, Staphyt's field team has conducted experimental GLP field and tunnel residue trials, testing different methods for the collection of various apicultural matrices for subsequent residue analysis. These trials have included studies on primary and succeeding crops, across several Central and Southern European Member States, with collection of matrices including pollen and anthers, nectar, mature honey, soil cores and guttation fluid. Having gained (and continue to gain) considerable practical experience in the setup of these studies, here we will present our tested field methods to share our expertise. In particular, we will discuss the advantages and disadvantages of various sampling techniques, such as manual- versus honey bee-collected sampling. We will discuss practical considerations for trial sites distributed across different European zones, including the importance of uniformity of tunnel setup, equipment and sampling techniques, as well as the choice and cultivation (e.g. sowing time and irrigation) of appropriate pollinator-attractive crops.

With the combined expertise of Staphyt's Bee Team, consisting of regulatory, scientific and field specialists, together we aim to provide both a practical (field) and regulatory (consultancy) perspective on the conduct of pan-European field and tunnel residue studies for environmental and consumer risk assessments.

3.5. Establishment of honeybee brood studies under semi-field conditions in Korea

Kyongmi Chon[1*], Hwan Lee[1], Bo-Seun Kim[1], Yeon-Ki Park[1], Are-Sun You[1], Jin-A Oh[2], Yong-Soo Choi[3]

[1]Chemical Safety division, Department of Agro-food Safety and Crop Protection, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea;
[2]Agromaterial Assessment division, Department of Agro-food Safety and Crop Protection, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea
[3]Sericulture and Apiculture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea
E-Mail: kmchon6939@korea.kr

Honeybee brood studies under semi-field conditions were carried out to select appropriate toxic standards from 2016 to 2019 in Korea since fenoxycarb is banned for use because of regulations. The semi-field test tunnels were located in the field study area of the National Institute of Agricultural Sciences (NAS). The experiments included three treatment groups (control, toxic reference chemicals (dimethoate or diflubenzuron), and test materials), each with three replicate tunnels. The honey bee colonies were introduced in the tunnels with a size of 70m^2 containing flowering *Brassica napus.* The dimethoate emulsifiable concentrate (EC) 46% (400 g dimethoate a.i./ha) and diflubenzuron wettable powder (WP) 25% (600 g, 800g diflubenzuron a.i./ha.) were used as reference chemicals. The mortality of the honey bees, flight activity, condition of the colonies, and brood development were assessed during the 28 day testing period following BFD 0 (brood area fixing day 0). For the honey bee brood assessment, 200 cells containing eggs were selected and evaluated by the digital photo method. The mean brood termination rates (BTRs) ranged from 20.5 to 47.3% in the control groups from 2016 to 2019. The toxic reference treatment with dimethoate or diflubenzuron led to a drastic reduction in the brood development, resulting in BTRs ranging from 68.0 to 100.0%. Clear adverse effects were observed in the brood development of selected eggs after treatment with two toxic references. These two chemicals could be appropriate as toxic reference compounds, depending on the study aims, for semi-field tests in Korea. Recently, the method guideline of honeybee *(Apis Mellifera L.)* brood test under semi-field conditions has been published in the agricultural chemical regulation laws of Korea. In the near future, a ring test of the semi-field test among other companies and research centers will be performed to evaluate and validate the test method in Korea.

4. Session – Non-Apis bees

4.1. Interactive effects of the neonicotinoid Thiacloprid and two common fungicides on foraging performance and reproductive success of the solitary bee *Osmia bicornis* under field conditions

Danja kroder[1,2]; Matthias Albrecht[1]; Anina Knauer[1]

[1] Agroecology and Environment, Agroscope, Reckenholzstrasse 191, 8046 Zürich, Switzerland; [2] Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
E-Mail: danja.baettig@icloud.com; matthias.albrecht@agroscope.admin.ch; anina.knauer@agroscope.admin.ch

Bee pollinators are often exposed to pesticide mixtures in intensively managed agricultural landscapes. There is increasing evidence for synergistic sub-lethal effects of different agrochemicals on bees, such as insecticides and fungicides, potentially negatively affecting their orientation, foraging performance or reproduction. However, most of this evidence is based on laboratory studies, while much less is known about potential insecticide-fungicide interactive effects under field conditions, and particularly few is known about how they may impact forging performance and reproductive of solitary bees. We used a combined laboratory-field approach treating the solitary bee species *Osmia bicornis* with field-realistic doses of the neonicotinoid insecticide Thiacloprid (oral feeding), as well as the two fungicides Captan and Tebuconazole (contact treatment), individually and in combinations, and assessed impacts on foraging performance, orientation and reproductive success of nesting, *Osmia* under field conditions. We will present the study design and first results.

4.2. The use of toxic reference chemicals in solitary bee larval bioassays

Anja Quambusch, Nina Exeler

Bayer AG, Research & Development, Crop Science, Alfred-Nobel-Straße 50, 40789 Monheim am Rhein, Germany
E-Mail: anja.quambusch@bayer.com

In Europe, North America and Asia, several species of the genus Osmia are successfully reared and managed as pollinators for different crops. Many of these species are active in spring and recognized as important pollinators in orchards. Therefore, it is important to evaluate the exposure and potential risk of plant protection products not only to honey bees but also to other managed bees. New methodologies are under development to assess acute contact and oral toxicity of plant protection products to adult solitary bees (ICPPR non-Apis working group). One of the remaining challenges is to set-up a standardized study design to assess solitary bee larval development under laboratory conditions to contribute valuable information for a risk assessment. Such a laboratory test method should allow for a conservative, highly controlled, and standardized evaluation of the relationship between a test item dose and the organism response.

Based on the first results of a previous experiment, assessing the larval development of Osmia cornuta feeding on different larval diets, we designed an experiment to test the potential effects of different toxic reference chemicals, used in honey bee and bumble bee laboratory bioassays (i.e. Dimethoate, Fenoxycarb, Diflubenzuron), on the development of solitary bee larvae. Toxic reference items are used to demonstrate that the test system and conditions are responsive and reliable. We compared the larval development and mortality of different

treatment groups to untreated control groups and give first recommendations for this test design. Future work should address the robustness of endpoints and acceptable validity criteria.

4.3. Laboratory Contact Toxicity Test with the Leafcutter Bee *Megachile rotundata*

Annette Kling, Christian Maisch & Anna Maria Friedrich

Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
E-Mail: annettekling@eurofins.com

Testing of possible effects of Plant Protection Products (PPPs) on the honey bee *Apis mellifera* is an integral part of the current risk assessment. However, little is known about the toxicity of these PPPs to solitary bees other than *Osmia* spp. as well as the inter-species sensitivity differences.

Megachile rotundata is a commercially bred solitary bee which is used worldwide mainly for the pollination of alfalfa. In general, bees can be exposed to PPPs directly by contact spray application (overspray) or indirectly via nectar and pollen. The leafcutter bees have an additional exposure scenario by (possibly) contaminated leaf pieces which are used for the building of brood cells. Therefore, contact toxicity might be of major importance within the leafcutter bee species.

The aim of this study was to carry out first contact toxicity testing with *M. rotundata* based on the existing honey bee testing guideline OECD No. 214, to make a first step in the direction of the development of a standard test method and collect data for the comparison of inter- and intra-species contact toxicity sensitivity. The toxic reference item dimethoate was tested. Results will be compared to historical honey bee toxicity data.

4.4. Recent experiences with bumblebee (*Bombus terrestris*) semi-field tunnel testing following ICPPR Non-Apis 2016 and 2017 workshop recommendations to investigate the insecticide chlorantraniliprole

A. Dinter, A. Samel

FMC Agricultural Solutions,
E-Mail: axel.dinter@fmc.com

In 2019 a semi-field Phacelia tunnel test with bumble bee (Bombus terrestris L) was conducted to investigate the effects of the insecticide Chlorantraniliprole 20SC. The study protocol was based on general SETAC/ESCORT recommendations (BARRETT et al. 1994), EPPO Guideline No. 170 (4), (2010) and the ring-test protocols from the ICPPR Non-Apis workshops held in 2016 and 2017. In two treatment groups (T1 and T2) the test item was applied to soil and incorporated into the soil before Phacelia seeding to achieve a the predicted 20-year plateau concentration in 20 cm top soil. Additionally, in T1 two applications of 60 g a.s./ha during pre-flowering (BBCH 51-55 and BBCH 55-59) and in T2 two applications of 60 g a.s./ha, the first application before flowering (BBCH 55-59) and the 2nd application during flowering and bumble bee flight (BBCH 61-63) in P. tanacetifolia were applied. The application in the control C and reference item treatment R (dimethoate) was carried out during full flowering

and bumble bee flight on the same day as the 2nd application of T2. Available results (e.g. mortality, flight activity, colony development and queen reproduction) will be presented.

4.5. Sensitivity of the honey bee and different wild bee species to plant protection products – two years of comparative laboratory studies

Tobias Jütte*, Anna Wernecke and Jens Pistorius

Julius Kuehn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig, Germany
*corresponding author: tobias.juette@julius-kuehn.de

In the past years, potential effects of plant protection products (PPPs) on insects, especially on bees are increasingly being discussed in public. Currently, effects of PPPs and their active substances have been tested mainly on Apis mellifera, so that it is still unclear if and to which extent the sensitivity of honey bees, especially to PPPs is comparable to wild bee species.

Therefore, the response of different bee species including the honey bee (Apis mellifera, (Am)) and different wild bess species (Andrena vaga (Av), Bombus terrestris (Bt), Colletes cunicularius (Cc), Osmia bicornis (Ob), Osmia cornuta (Oc) and Megachile rotundata (Mr)) with various life history characteristcs, to a pyrethroid insecticide, containing lambda-cyhalothrin, was investigated in a series of studies under controlled laboratory conditions over the last two years.

The chosen insecticide is classified as harmless to bees (at the authorized application rate of 0.075 product L/ha) but known for transient effects under laboratory conditions. Here, a spray chamber with a flat spray nozzle was used to evaluate effects following contact exposure by typical field application rates.

After the application, mortality and behaviour of bees were monitored for at least 48 h following the OECD acute contact toxicity test (guideline No. 214) and were prolonged up to 10 days. The evaluation was made up to the day on the criteria for the control mortality was exceeded (≤ 15% honey bees; ≤ 15-20% wild bees). Furthermore, to investigate the natural detoxification process of active substances, living individuals of honey bees and three further wild bee species (bumble bee Bombus terrestris, mason bees Osmia bicornis and Osmia cornuta) were frozen at -20°C to different time points after the application. Residues were analysed using a multi-residue method. The residue level of lambda-cyhalothrin was quantified by use of gas chromatography/mass spectrometry (GC-MS).

The aim of the experiments was a comparative analysis of the potential effects of applied PPPs on the honey bee and wild bee species. Furthermore, it should be clarified to what extent the extrapolation from data of the honey bee, as representative organism in the registration processes and risk assessment of PPPs, to other wild bees is possible and which differences in sensitivity exist at the laboratory level.

In the last year of the laboratory tests, our investigations of the mortality show comparable results of the different bee species regarding their sensitivity in two out of three studies. The sensitivity could be ranked as follows (from less to more sensitive): 1st Ob / Bt < Cc / Av < Am; 2nd Am / Bt / Ob < Oc; 3rd Ob / Bt < Am < Mr. Overall, the leaf-cutting bee (Mr) was the most sensitive bee species. For the detoxification, comparable results regarding the half-life period of lambda-cyhalothrin and the residue degradation, respectively the influence of the metabolism, in the different bee species were detected.

This year, laboratory tests were performed to verify the previous results and further to optimize the methodology. The interim results let assume that potential differences in sensitivity can be reliable recorded for the already established bee species in the risk assessment of pesticides (Am, Bt and Ob / Oc), while for the other wild bee species (especially the two ground-

nesting, solitary species Av and Cc) an increased variability was observed, which so far does not allow a clear classification. Nevertheless, the following tendency (from less to more sensitive) could be deteced so far: 1st Bt < OB / Oc < Am; 2nd Bt < Ob / Av < Cc / Am; 3rd - .

The results of the last experiment of this series of laboratory studies as well as the overall conclusion will be presented as part of the poster presentation.

4.6. Honeybee viruses in novel hosts – Studying agrochemical-pathogen stress combination in wild bees.

Sara Hellström[1]; Karsten Seidelmann[2]; Robert J. Paxton[1]

[1] Institut für Biologie/Zoologie, Allgemeine Zoologie, Martin-Luther-Universität Halle, 06120 Halle (Saale), Germany; [2] Institut für Biologie/Zoologie, Abteilung Tierphysiologie, Martin-Luther-Universität Halle, 06120 Halle (Saale), Germany
E-Mail: sara.hellstroem@zoologie.uni-halle.de

It has been theorized that agrochemicals can impact the immune response in honeybees, leading to increased sensitivity to pathogens. The link between neonicotinoids and increased severity of gut-parasite *Nosema ceranae* infection has been experimentally established, while the link between viral pathogen infection outcome and agrochemical exposure remains unclear. Viruses first discovered in honeybees have been found in wild-caught individuals of a variety of bee species, proving the potential of spillover from honeybees to wild bees and may act as pathogens in these novel hosts. As wild solitary bees share the environment with honeybees, they are potentially exposed to similar combinations of pathogen and agrochemical stress. No study has so far tested the combined effects of agrochemical exposure and pathogen pressure on solitary bees. In order to study this relationship, experimental pathogen infection must first be established for the novel hosts. In this study, two wild bee species (*Osmia bicornis* and *Anthophora plumipes*) were injected with a fixed titre of three viral honeybee pathogens commonly found across Europe, with the aim to observe if the viruses would replicate in these novel hosts. This pathogenic stressor can then be experimentally combined with agrochemical exposure, in order to locate potential synergistic interaction between pathogen and pesticide. Further experiments will combine infection with gut parasites *Apicystis bombi* and *Crithidia mellificae* with exposure to the novel insecticide Sulfoxaflor to further evaluate the fitness effect of these combined stressors that wild bees encounter in the agricultural landscape.

4.7. Is *Apis mellifera* a good model for toxicity tests in Brazil?

Thaisa C. Roat, Lucas Miotelo, Roberta C. F. Nocelli and Osmar Malaspina

São Paulo State University (UNESP), CEIS-Center of Studies of Social Insects, Department of Biology, Institue of Biosciences, Avenida 24-a n. 1515 Bela Vista, Rio Claro/SP/Brazil
E-Mail: thaisaroat@yahoo.com.br

Exposure to pesticides are among the contributing factors related to the reducing pollinators. To register these molecules and release for them use in Brazil, the bee used in toxicity tests is the *A. mellifera* species, which is a non-native bee. There are questions about whether we should use this species as a model. Thus, it is important to establish the toxicity in different species of bees to verify whether there are differences in the sensitivity to these compounds among the bees. The present study compared oral toxicity (OECD, 213) of thiamethoxan among two species of stinglees bees (*Melipona scutellaris* and *Scaptotrigona postica*) and *A. mellifera* by determining the mean lethal concentration (LC_{50}). The results showed that the

stingless bees are more sensitive to the insecticide with a lower LC_{50} of 0.0543 ng active ingredient (a.i./µL) in *M. scutellaris*, 0.14 ng a.i/µL.in *S.* postica compared to 0.227 ng a.i./µL in *A. mellifera*. These results show that could be harmful to use *A. mellifera* as model for toxicity tests in Brazil. Thus, the current challenge is to establish the maximum concentrations or limits of environmental contaminants that protect the diversity of bee species in Brazil, comparing the data obtained for *A. mellifera* to stingless bees, and verify if toxicity tests for a model species are safe and effective at inferring effects on the ecosystem as a whole.

4.8. Current achievements and future developments of a novel AI based visual monitoring of beehives in ecotoxicology and for the monitoring of landscape structures

Frederic Tausch, Matthias Diehl und Katharina Schmidt

apic.ai GmbH Rintheimerstraße 31-33, 76131 Karlsruhe, Germany

Since there is close to no reliable data on the extent and interdependencies of the factors influencing insect mortality, many hypotheses stand unexamined. A system is presented that enables near-time scalable monitoring of beehives using networked sensor technology, computer vision and deep learning.

The monitoring system was used in a bee field study and runs in a present monitoring study. With the data present, possibilities of data capture and analysis are presented.

Future possibilities through development of the technologies will be discussed as well. In particular the potential to create a database that can be used to systematically verify assumptions by detecting causal relationships. With the help of the values measured with the AI based hive monitoring system, existing gaps in knowledge about the influencing factors of species loss could be closed. The technology has been or could be applied in various areas:

1. Precise quantitative assessment of forager loss following the contact with plant protection products and other environmental pollutants.
2. Detection of the abundance of pollen availability, foraging and locomotion behaviour.
3. Assessment of habitat biodiversity through differentiation of foraged pollen by color.

4.9. Pollinator monitoring for evaluation of potential exposure and assessment of effects

Julian Fricke, Olaf Klein, & Silvio Knäbe

Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
E-Mail: olafklein@eurofins.com

Pollinator monitoring studies focus on native bee communities occurring in agroecosystems and are performed under field conditions. They can either be used to evaluate potential exposure of pollinators to plant protection products in different crops or agricultural scenarios or, to assess possible effects of treatments at a larger scale taking into account other influencing parameters like the management intensity, landscape composition, growing season etc.

Generally, the abundance and species richness of naturally occurring pollinators in a crop and adjacent field margins will be investigated. For crops considered to be not attractive as foraging and nesting habitats for honey bees, wild bees and other pollinators, the comparison of in-field and off-crop abundance and richness can help to understand if pollinators are exposed to plant protection products or not. This might include temporal as well as spatial differences (timing of monitoring and placement of monitoring within the field and landscape).

To evaluate a wide range of pollinator species occurring in a specific crop several methods are available. We recommend using different types of sampling/assessment methods: non-selective methods and selective methods. For the non-selective methods two different types of traps might be used in combination: vane traps and bee bowls (pan traps). These traps can be installed at different locations: i.e. in the centre of the fields, at the borders of the fields and outside in the adjacent field margin. As a selective method sweep netting or observations can be used via transect walks in a defined distance and time interval.

In addition, trap nests can be provided for hypergeic (above-ground nesting) solitary wild bee species that breed in woody cavities. The trap nests can also be set up at the different locations and will be used for sampling of pollen to assess pollen sources by pollen identification of pollen mass samples. If required, residue analysis can performed with samples of pollen mass

4.10. Development and validation of a bumble bee adult chronic oral test

N. Exeler[1], A. Quambusch[1], N. Hanewald[2], A. Zicot[3], E. Soler[4], A. Kling[5], S. Vinall[6], K. Dressler[7], V. Tänzler[8], S. Kimmel[9], D. M. Lehmann[10], M. Patnaude[11] A. R. Cabrera[12]

[1]Bayer AG, Monheim am Rhein, Germany, [2]BASF SE, Limburgerhof, Germany, [3]SynTech, Nimes, France,
[4]Eurofins Trialcamp, Alcàsser-Valencia, Spain, [5]Eurofins Agroscience Services, Niefern-Öschelbronn, Germany, [6]Mambo-Tox Ltd., Southampton, UK, [7]BioChemagrar GmbH, Machern OT Gerichshain, Germany
[8]Ibacon, Rossdorf, Germany, [9]IES Ltd, Witterswil, Switzerland, [10]USEPA, Research Triangle Park, NC, USA
[11]Smithers Viscient US, Wareham, MA, USA, [12]Bayer CropScience LP, Chesterfield, MO, USA

The regulation of pesticide uses is based on the local Risk Assessment frameworks, including a specific framework for pollinators. These frameworks rely on data from honey bee toxicity in a three-tiered process, from laboratory to semi-field to field settings, and exposure estimates based on application rates or refined via residue levels in nectar and pollen. In recent years, concerns about the risk to other bees such as bumble bees have been the driver for the

development of new methods to address toxicity and exposure with selected surrogate species. Here, we present the results from the second international ring test for a bumble bee adult chronic oral test. Nine European laboratories conducted the 10-d test with *Bombus terrestris* workers while 3 US laboratories conducted the test with *B. impatiens*. Along with biological observations and consumption measurements, the stock solutions and feeding diets were confirmed for the concentration of dimethoate. There were 5 and 7 dimethoate test levels for the European and US ring test, respectively. The LC_{50} endpoints derived from these tests were on average 0.468 and 0.258 mg a.s./kg of diet for *B. terrestris* and *B. impatiens*, respectively. Similarly, the LDD_{50} endpoints derived from the tests were on average were 0.093 and 0.032 µg a.s./bee/d for *B. terrestris* and *B. impatiens*, respectively. Our results indicate the test design is robust and replicable, and after a two-year effort, a validation report is in preparation to initiate the process to develop it into an OECD Guideline document.

Disclaimer: This presentation does not represent U.S. EPA Policy

4.11. Method development for a larval test design for the solitary bee *Osmia cornuta* - First experiences with different larval pollen provisions

Nina Exeler; Anja Quambusch

Bayer AG, Research & Development, Crop Science, Alfred-Nobel-Straße 50, 40789 Monheim am Rhein, Germany
E-Mail: nina.exeler@bayer.com

The important role of bees for the pollination of agricultural crops is widely acknowledged. Besides the honey bee, other pollinators like bumble bees and solitary bees are used to support pollination services. Therefore, it is particularly important to understand the biology of these species to assess the potential exposure of managed non-Apis bees to plant protection products. Several initiatives support the development of new test methods for solitary bees. To gain a better understanding of the development of solitary bee larvae, we performed an experiment with the aim to develop a standardized larval test design for the solitary bee *Osmia cornuta* by combining semi-field and laboratory methods. To obtain a sufficient number of eggs of *O. cornuta*, adult bees in a colony size of 1250 individuals (sex ratio females:males 1:1,5) were established under confined conditions in oilseed rape. Nesting tubes with eggs and newly emerged larvae were transferred to the laboratory. Eggs and young larvae were carefully taken out of the nesting tube and transferred into 48-well culture plates either together with the pollen provision or without the pollen provision to artificial pollen provisions. The plates were checked daily for larval mortality. At the end of the larval period, the numbers of cocoons and offspring were assessed. The pupation rate of *O. cornuta* larvae was constantly high between 85 and 95% irrespective of the food source and the amount of food. There was no difference between the treatments: Oil seed rape pollen from nesting blocks, artificial pollen mix with 25 % sugar solution, artificial pollen mix with 15 % sugar solution, artificial pollen mix with 30 % Api-Invert. Even so, the hatching rate of *O. cornuta* was high, between 85 and 100%, the sex ratio was shifted towards an excess of male bees. This might reflect the artificial rearing conditions in a „semi-field" design and needs further method improvement and standardization.

4.12. Interactions between *Bombus terrestris* and glyphosate-treated plants: are bees at risk of herbicide exposure?

Linzi J. [1,2], Jane C. Stout[3], Dara A. Stanley[1,2]

[1]School of Agriculture and Food Science, University College Dublin, Dublin 4, Ireland, [2]Earth Institute, University College Dublin, Dublin 4, Ireland, [3]School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland; Linzi.Thompson@ucdconnect.ie

Exposure to agricultural pesticides is often cited as one of the primary drivers of pollinator decline. Most of the research has been focused on the impacts of insecticides but herbicides have been receiving more attention for their potential implications for bee health. However, little is known about how pollinators are being exposed to herbicides, whether it is through direct contact with herbicides during spraying, foraging on herbicide-treated plants or contact with herbicide residues within the wider environment. We examined the interactions between bumble bees (*Bombus terrestris*) and herbicide treated plants, comparing behavior of bees when offered a choice between glyphosate-treated and untreated plants. We aimed to determine whether bees avoid herbicide-treated plants, thus reducing their potential exposure to herbicides.

Individual foragers were released into an exclusion cage containing four *Phacelia tanacetifolia* plants: two sprayed with glyphosate and two untreated plants. We measured the frequency and duration of nectar feeding, pollen collecting and investigation (inspection but not foraging) of plants. We tested interactions between the bumble bees and plants which had been freshly sprayed (within 24 hours) and again once the glyphosate had begun to translocate within the plant – but before any significant physical effects began to appear (48 hours). Here, we present the preliminary results from this study.

5. Session - Monitoring

5.1. Pesticide Residues and Transformation Products in Honeybees: A 2018 mid-2019 Appraisal

Konstantinos M. Kasiotis; Effrosyni Zafeiraki; Pelagia Anastasiadou; Electra Manea-Karga and Kyriaki Machera

Benaki Phytopathological Institute, Laboratory of Pesticides' Toxicology, 8 St. Delta Street, Athens, Kifissia 14561, Greece
E-mail: K.Kasiotis@bpi.gr, E.Zafeiraki@bpi.gr, and K.Machera@bpi.gr

Due to the ongoing reports of numerous death incidents of honeybees, there is still an urge to assess the occurrence of pesticide residues and their transformation products in them. In this context, during the period of 2018 mid-2019, 82 honeybee samples were sent from several areas of Greece and analyzed for the determination of pesticide residues and transformation products. In particular, more than 130 analytes were incorporated and assessed by applying two multi-residue methods (HPLC-ESI-MS/MS and GC-MS/MS) based on modified QuEChERS methodology and clean-up with Z-Sep, PSA, and C18 materials. Both analytical methods were validated for repeatability, reproducibility, specificity, recovery and sensitivity according to SANTE/11813/2017 guideline. The confirmation of the analytes was based on the retention time (RT), retention time relative to the isotope labelled internal standards and ion-ratio of the quantifier and qualifier ion. The limit of quantification (LOQ) for the analytes of both methods were in the range of 1 to 10 ng/g. In addition, quality control (QC) standards (one blank and two honeybee samples spiked at LOQ and 10 LOQ) were analyzed in every batch of samples, controlling in this way the repeatability of the analytical method. The recoveries of the spiked analytes and of the mass-labeled internal standards, added to the sample prior to extraction, were monitored and ranged between 67 and 120% for the different analytes. Moreover, the uncertainty and the expanded uncertainty of the two methods were also assessed and calculated.

According to the results, 78% of the analyzed honeybee samples were contaminated with at least one active substance. In particular, neonicotinoids were the most frequently detected compounds during 2018, while pyrethroids, and especially cypermethrin, were the most predominant ones in the samples of 2019. The relatively high concentrations of cypermethrin (84.1 to 66288 ng/g $_{\text{bee body weight}}$), and in one case of λ-cyhalothrin (1259 ng/g $_{\text{bee body weight}}$) could be attributed to the misuse of plant protection products containing them. In addition, fungicides, such as difenoconazole, trifloxystrobin, cyprodinil, and carbendazim were also frequently detected, mainly in the samples analyzed until mid-2019, with concentrations ranging from 5 to196 ng/g $_{\text{bee body weight}}$. Apart from the aforementioned pesticide residues, transformation products of imidacloprid such as imidacloprid olefin and 5-hydroxy imidacloprid, the oxon metabolites of chlorpyrifos and coumaphos, and the metabolites of amitraz (DMF and DMPF) were also detected. Last but not least, in limited occasions, piperonyl butoxide, a known synergist component of pesticide formulations, was also quantified.

The above information reveals that honeybees frequently accumulate a broad range of concentrations of pesticide residues and their transformation products. To this end, this work's results, indicate that the extended use and the subsequent occurrence of pesticides in honeybees, could potentially cause or be implicated in severe health effects to the latter.

6. Session – Microbials

6.1. Assessment of the impact of microbial plant protection products containing *Bacillus thuringiensis* on the survival of adult and larval honeybees (*Apis mellifera*, L.)

Charlotte Steinigeweg [1,2], Abdulrahim T. Alkassab [1], Jakob Eckert [1], Dania Richter [2], and Jens Pistorius [1]

[1]Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig, Germany
[2]Technische Universität Braunschweig, Institute of Geoecology, Langer Kamp 19c, 38106 Braunschweig, Germany

Recently, the number of publications regarding the potential adverse effects of chemical plant protection products (PPPs) on insect pollinators including apis and non-apis bees and concerns of the public on the potential side effects greatly increased. On the other hand, the development of microbial plant protection products as substitutes for chemical PPPs is exalted. However, there are several knowledge gaps related to toxicity testing with microbial PPPs and risk assessment, e.g. quantitative assessment such as HQ calculation, common for chemical PPPs, can not be performed. Therefore, an evaluation of the appropriateness of available test guidelines, which are used for testing the toxicity of chemical PPPs, for testing of microbial PPPs should be conducted.

In the current study, we evaluated the effect of the product FlorBac®, with the active substance *Bacillus thuringiensis* ssp. aizawai (strain: ABTS-1857), on adult and larval honeybees (*Apis mellifera*) under laboratory conditions. The chronic oral toxicity tests on adult bees following the OECD guideline 245 and the larval toxicity tests with repeated exposure following the OECD guidance document 239 were conducted. Additionally, possible modifications of the chronic oral toxicity test, such as additional pollen feeding, were assessed.

Our results showed that the survival of adult bees was affected after chronic exposure to the tested product depending on the concentrations. The test duration seemed to play an important role, because the mortality of bees arose first after 96 h at the highest tested concentration. This indicates the limitations and/or inappropriateness of the duration of the acute tests, such as OECD 213, for testing the effect of microbials on bees, as these are usually terminated after 48h and may be extended to a maximum of 96h. Moreover, our results showed that the feeding of tested bees with pollen had a significant effect on the survival duration of the treated bees. Furthermore, the survival of treated larvae was significantly reduced at all tested concentrations, which indicated a higher sensitivity of the larval stage than of the adults to the tested microbial.

In conclusion, further studies are required to assess the side effects of microbial plant protection products on bees under realistic conditions. The current knowledge gaps regarding the realistic exposure duration, the quantitative exposure of larvae, life duration of different micro-organisms in different matrices within the hive, and their development under colony conditions need to be addressed.

7. Session – Other

7.1. Investigating the transfer of acaricides from beeswax into honey, nectar, bee bread, royal jelly and worker jelly

Jakob H. Eckert[1]; Lara Lindermann[2]; Abdulrahim Alkassab[1]; Gabriela Bischoff[1]; Robert Kreuzig[3] and Jens Pistorius[1]

[1]Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig, Germany
[2]Technische Universität Braunschweig, Institute for Geoecology, Langer Kamp 19c, 38106 Braunschweig, Germany
[3]Technische Universität Braunschweig, Institute of Environmental and Sustainable Chemistry, Hagenring 30, 38106 Braunschweig, Germany
E-Mail: jakob.eckert@julius-kuehn.de

A main source of beeswax contaminants are acaricides which are used to control Varroa destructor. Since it is common practice to recycle wax, acaricides can accumulate in beeswax due to their fat-soluble properties. The purpose of this study was to compare contamination levels in different types of bee products depending on their chemical properties and their storage duration in-hive. Wax foundations were poured with a mix of nine different acaricides that had been most frequently detected in commercial beeswax and subsequently processed into honeycombs by bees. The used initial concentration mirrored field-realistic maximum concentrations. The bee products honey, nectar, bee bread, royal and worker jelly were manually applied to treated combs and incubated at in-hive conditions in the laboratory. The incubation time ranged from a few days for nectar and larval food up to two months for honey and bee bread, mimicking natural processing conditions in a hive. Samples were analysed by liquid and gas chromatography linked with mass spectrometry.

Results showed a negligible transfer of the active substances bromopropylate, chlorpyrifos, fenpyroximate, hexythiazox, tetramethrin and amitraz from beeswax into the tested bee products due to their low initial concentrations and degradation processes. In contrast, a significant transfer into bee bread, worker jelly and royal jelly was found for tau-fluvalinate, coumaphos and propargite, which occur at relatively high concentrations in beeswax at field-realistic conditions. Based on the initial maximum concentration in beeswax and the detected residues of tau-fluvalinate, coumaphos and propargite in bee bread, royal jelly, worker jelly, honey and nectar, maximum transfer rates of 6.9 %, 3.4 % 1.6 %, 0.2 % and 0.03 % could be calculated, respectively. Transfer rates of the tested acaricides were found to be dependent on the initial concentration in beeswax, the storage duration and the lipid/water content of the bee products. A biologically relevant exposure of bees at field realistic concentrations was classified as unlikely.

List of participants:

Name	Affiliation	Country	E-mail
Addai, Richard	Federation of Ghana Bee-keepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Agatz, Annika	ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf	Germany	annika.agatz@ ibacon.com
Aguilar, Josep	Eurofins Trialcamp, Av Antic Regne Valencia, 25, 46290 Alcasser	Spain	josepantoni-aguilaralberola@ eurofins.com
Agyei Poku, Vida	Federation of Ghana Bee-keepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Albercht, Matthias	Agroscope, Agroecology and Environment, Reckenholzstrasse 191, 8049 Zürich	Switzerland	matthias.albrecht@ agroscope.admin.ch
Alix, Anne	Corteva Agriscience, 3 Milton park, OX14 4RN Abingdon	United King-dom	anne.alix@corteva.com
Alkassab, Abdul-rahim	Julius Kühn-Institut (JKI), Institute for bee protection Messeweg 11-12, 38104 Braunschweig	Germany	abdulrahim.alkassab @julius-kuehn.de
Allan, Matthew	41 George Street, SO50 9BT Eastleigh	Great Britain	mattallan@aol.com
Almanza, Maria Teresa	Bayer	Germany	mariateresa.al-manza@bayer.com
Al Naggar, Yahya	Martin-Luther-Universität Halle-Wittenberg, Institut für Biologie, Le-hrstuhl Allgemeine Zoolo Hoher Weg 8, 6120 Halle (Saale)	Germany	yehia.elnagar@sci-ence.tanta.edu.eg
Amankwah Opoku, Esther	Federation of Ghana Bee-keepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Amoah Gyami, Dennis	Federation of Ghana Bee-keepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Bartels, Anja	AGES Spargelfeldstraße 191 1220 Wien	Austria	anja.bartels@ages.at

List of participants

Name	Affiliation	Country	E-mail
Barth, Markus	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	markus.barth@ biochemagrar.de
Beqe, Hulaj	Food and Veterinary Agency - Kosovo, Rruga e Pejes 241, 10000 Prishtine	Kosovo	bhulaj@yahoo.com
Berg, Christian	Eurofins Agroscience Services Ecotox GmbH, Honey Bees Field Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	ChristianBerg@ eurofins.com
Blanco, Raul	Agrotox, Ramon y Cajal n5. 2B, Ramon y Cajal n5. 2B, 50280 Calatorao	Spain	raulblanco@agrotox.com
Borrek, Catherine	Bayer AG, Crop Science, Research & Development, Trial Management, Gut Höfchen, 51399 Burscheid	Germany	catherine.borrek@bayer.com
Cabrera, Ana	Bayer CropScience, 700 Chesterfield Parkway W, 63017 Chesterfield, MO	United States of America	ana.cabrera@bayer.com
Catae Antonio, Aline Fernanda	Friedrich-Bauer Strasse 7, 91058 Erlangen	Germany	aline_catae@hotmail.com
Charrière, Jean-Daniel	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161 3003 Bern	Switzerland	Jean-Daniel.Charrière @agroscope.admin.ch
Chon, Kyongmi	National Institute of Agricultural Sciences, Chemical safety division, Department of Agro-Food, 166, Nongsaegmyeong-ro 55365 Wanju	Korea	kmchon6939@korea.kr
Christen, Verena	University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Hofackerstrasse 30, 4132 Muttenz	Switzerland	verena.christen@fhnw.ch
Colli, Monica	BiotecnologieBT srl , Frazione Pantalla, 06059, Todi	Italy	mcolli@biotecnologiebt.it
Collison, Elizabeth	APC, Cromwell Office Park, York Road, LS22 7SU Wetherby	United Kingdom	elizabeth.collison @apc.eu.com
Cornement, Magdalena	IES Ltd, Benkenstrasse 260 4108 Witterswil	Switzerland	m.neale@ies-ltd.ch

Name	Affiliation	Country	E-mail
Coulson, Mike	ERM Regulatory Services Ltd, The Exchange, Station Parade, HG1 1TS Harrogate	United Kingdom	alice.almond@erm.com
Couture, Elodie	SynTech Research France, 613 route du bois de loyse, 71570 La Chapelle de Guinchay	France	
Crosland, Helena	HSE, Kings Pool, YO1 7PX York,	United Kingdom	Helena.Crosland @hse.gov.uk
Dainat, Benjamin	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	benjamin.dainat@ agroscope.admin.ch
De Waen, Berenice	ISK Biosciences Europe, 12B De Kleetlaan - Box 9, 1831 Diegem	Belgium	berenice1608@ hotmail.com
Deslandes, Line	SynTech Research France, 613 route du bois de loyse, 71570 La Chapelle de Guinchay	France	cloiselleux@syntechresearch.com
Dietemann, Vincent	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Vincent.dietemann@ agroscope.admin.ch
Dinter, Axel	FMC Agricultural Solutions, Westhafenplatz 1, 60327 Frankfurt am Main	Germany	axel.dinter@fmc.com
Dorigo, Adna	UNESP State University of São Paulo, 24A Avenue 1515, Rio Claro, SP	Brasil	adna_sd@yahoo.com.br
Droz, Benoit	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Benoit,droz@ agroscope.admin.ch
Duncan, Jennifer			jduncan@exponent.com
Eckert, Jakob H.	Julius Kuehn Institute, Institute for Bee Protection Messeweg 11-12, 38104 Braunschweig	Germany	jakob.eckert@ julius-kuehn.de
Elston, Charlotte	Syngenta, Jealott's Hill International Research Centre RG426EY Berkshire	United Kingdom	charlotteelston90@ hotmail.com
Erler, Silvio	Julius Kühn-Institut, Institut für Bienenschutz, Messeweg 11-12, 38104 Braunschweig	Germany	erler.silvio@gmail.com

Name	Affiliation	Country	E-mail
Estebaranz Yubero, Maria	ERM Regulatory Services Limited, The Exchange, Station Parade, HG1 1TS Harrogate	United Kingdom	alice.almond@erm.com
Exeler, Nina	Bayer AG Crop Science , Alfred-Nobel-Strasse 50, 40789, Monheim	Germany	nina.exeler@bayer.com
Eyer, Michael	Agroscope, Swiss Bee Research Center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	michael.eyer@agroscope.admin.ch
Feken, Max	Syngenta, 410 S. Swing Road 27419-8300 Greensboro, North Carolina	United States of America	Max.Feken@syngenta.com
Fent, Karl	University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Langackerstrasse 30, CH-4132 Muttenz	Switzerland	karl.fent@bluewin.ch
Forster Rolf	Federal Office of Consumer Protection and Food Safety (BVL), Messeweg 11/12, 38108 Braunschweig	Germany	rolf.forster@bvl.bund.de
Fourrier, Julie	ITSAP-Institut de l'Abeille, INRA Domaine Saint Paul, 228 route de l'Aérodrome, 84914 Avignon	France	julie.fourrier@itsap.asso.fr
Franke, Lea	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	LeaFranke@eurofins.com
Fusilero, Abegail	Rotam Agrochemical Europe, 75 cours Albert Thomas 6e avenue - Bâtiment D, 69003, Lyon 03	France	afusilero@rotam.com
Giffard , Hervé,	Testapi, 464, Sarré, 49350 Gennes-Val de Loire	France	herve.giffard@testapi.fr
Gimeno, Ignacio	Eurofins Trialcamp, Av Antic Regne Valencia, 25, 46290 Alcasser	Spain	soniarodriguez@trialcamp.es
Gimeno, Carmen	Eurofins Trialcamp, Av Antic Regne Valencia, 25, 46290 Alcasser	Spain	eugeniasoler@eurofins.com

Name	Affiliation	Country	E-mail
Giner Estarlich, Jose Francisco	GMW Bioscience Agricultural Servic.SL, CL/Jornalers 35, 46260 Alberic	Spain	maria.jose@gmwbioscience.com
Gonsior, Gundula	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	gundulagonsior@eurofins.com
Grossar, Daniela	Swiss Bee Research Center, Agroscope, Federal Department of Economic Affairs, Education and Research, Schwarzenburgstrasse 161, 3005 Bern	Switzerland	daniela.grossar@agroscope.admin.ch
Haas, Julian	Schürgespfad 19, 50259 Pulheim	Germany	haas_ju@web.de
Hanewald, Nicole	BASF SE, Speyerer Str. 2, 67117 Limburgerhof	Germany	nicole.hanewald@basf.com
Hart, Connie	2150 Maple Forest Drive, K0A2T0 Ottawa	Canada	connie.hart@canada.ca
Hecht-Rost, Sabine	Rifcon GmbH, Goldbeckstraße 13, 69493 Hirschberg	Germany	julia.walter@rifcon.de
Hein, Roman	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	RomanHein@Eurofins.com
Hellström, Sara	General Zoology, Martin Luther University, Ludwig-Wucherer-Strasse 42, 06120 Halle (Saale)	Germany	sara.hellstroem@zoologie.uni-halle.de
Hinarejos, Silvia	Sumitomo Chemical Co, Ltd, AgroSolutions Division International, 10A Rue de la Voie Lactée 69370 Saint Didier au Mont d'Or	France	silvia.hinarejos@sumitomo-chem.fr
Horacio, Heinzen	Universidad de la República Gral. Flores 2124, 11800 Montevideo	Uruguay	heinzen@fq.edu.uy
Hofkens, Sofie	European Commission Rue Froissart 101, 1049 Bruxelles	Belgium	sofie.hofkens@ec.europa.eu
Inada, Makoto	Nissan Chemical Corporation, 1470 Shiraoka, 3490294 Shiraoka	Japan	inadam@nissanchem.co.jp

Name	Affiliation	Country	E-mail
Jeker, Lukas	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	lukas.jeker@ agroscope.admin.ch
Jilesen, Claudia	Netherlands Food and Consumer Product Safety Authority, Catharijnesingel 59, 3511GG Utrecht	Netherlands	c.j.t.j.jilesen@nvwa.nl
Jones, Ben	Applied Insect Science Ltd, Ambrose House, Tanfield Lane, HG4 5JE Ripon	Great Britain	ben@ appliedinsectscience.com
Jütte, Tobias	Julius Kühn-Institut (JKI) - Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig	Germany	tobias.juette@ julius-kuehn.de
Kalbermatten, Isabelle	Agroscope Swiss Bee Research Center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Isabelle.kalbermatten@ agroscope.admin.ch
Kasiotis, Konstantinos	Benaki Phytopathological Institute/Laboratory of Pesticides& Toxicology, Stefanou Delta 8 street, 14561 Athens, Kifissia	Greece	K.Kasiotis@bpi.gr
Kast, Christina	Agroscope Swiss Bee Research Center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	christina.kast@ agroscope.admin.ch
Kilchenmann, Verena	Agroscope Swiss Bee Research Center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Verena.kilchenmann@ agroscope.admin.ch
Kimmel, Stefan	IES LTD, Ecotoxicology, Benkenstr. 260, 4108 Witterswil	Switzerland	s.kimmel@ies-ltd.ch
Kirsch, Karina	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	KarinaKirsch@ eurofins.com
Kleebaum, Katharina	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	katharina.kleebaum@ biochemagrar.de
Klein, Olaf	Eurofins Agroscience Services Ecotox GmbH, Ecotox Field, Eutingerstr. 24, Niefern - Öschelbron	Germany	olafklein@eurofins.com
Kling, Annette	Eurofins Agroscience Services Ecotox GmbH, Eutingerstr. 24, Niefern - Öschelbron	Germany	annettekling@ eurofins.com

Name	Affiliation	Country	E-mail
Knäbe, Silvio	Eurofins AgroScience Services Ecotox GmbH, Ecotox Field testing, Eutingerstr. 24, Niefern - Öschelbron	Germany	silvioknaebe@eurofins.com
Knauer, Katja	Federal office for agriculture, Schwarzenburgstrasse, 3003 Bern,	Schweiz	katja.knauer@blw.admin.ch
Kolkman, Jeff	Eurofins MITOX, LD PICHOY, 32250 Fources	France	jeffreykolkman@eurofins.com
Kolokytha, Paraskevi	IPM Impact, Gierkensstraat 21, 3511 Hasselt	Belgium	
Krahner, André	Julius Kühn-Institut (JKI) - Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig	Germany	andre.krahner@julius-kuehn.de
Krankah, Stephen	Federation of Ghana Beekeepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Kroder, Stefan	Adama Deutschland GmbH, Edmund-Rumpler-Strasse 6, 51149 Cologne	Germany	sarah.pisula@adama.com
Kunz, Petra	BAFU, Worblentalstrassse 68, 3063 Ittigen	Switzerland	petra.kunz@bafu.admin.ch
Laporte, Jean Michel	Syngenta, 1 avenue des Près, 78280 Guyancourt	France	jean-michel.laporte@syngenta.com
Lehto, Mikko	Agroscope, Müller-Thurgau-Strasse 29, 8820 Wädenswil	Switzerland	mikko.lehtohuerlimann@agroscope.admin.ch
Lenoir, Jean-Christophe	SynTech Research France, 613 route du bois de loyse, 71570 La Chapelle de Guinchay	France	
López, Felipe	Agrologia S.L.U., Finca La Dehesilla Ctra A362 Km 4.7, 41710 Utrera	Spain	felipe.lopez@agrologia.es
Lückmann, Johannes	Rifcon GmbH, Goldbeckstraße 13, 69493 Hirschberg	Germany	johannes.lueckmann@rifcon.de
Mair, Benjamin	Versuchszentrum Laimburg, Laimburg 6, 39040 Ora	Italy	benjamin.mair@laimburg.it

Name	Affiliation	Country	E-mail
Marin, Marcial	Eurofins Trialcamp, Av Antic Regne Valencia, 25 46290 Alcasser	Spain	
Martín, Teresa	Agrologia S.L.U., Finca La Dehesilla Ctra A362 Km 4.7, 41710 Utrera	Spain	teresa.martin@ agrologia.es
Maus, Christian	Bayer AG, Alfred-Nobel-Str. 50, 40789 Monheim a. Rhein	Germany	christian.maus @bayer.com
Merckelbach, Antoine	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Antoine.merchelbach@ agroscope.admin.ch
Mestres Lóbez, Isaac	Solvay Brussels Alumni - Université Libre de Bruxelles/Worldline Belgium, Chaussée de Wavre, 160, 1160 Brussels	Belgium	isaac.mestres@ solvaypostgrad.net
Miles, Mark	Bayer, 230 Cambridge Science Park, CB4 0WB Cambridge	United Kingdom	mark/miles@bayer.com
Mizoyama Yasunori	Nissan Chemical Corporation, Shiraoka 1470, 349-0294 Shiraoka	Japan	mizoyamay@ nissanchem.co.jp
Müther, Jutta	GAB Consulting GmbH, Ottenbecker Damm 10, 21684 Stade	Germany	annette.schilling@ gabconsulting.de
Noa, Simon Delso	CARI, Place Croix du Sud, 1348 Louvain-la-Neuve	Belgium	simon@cari.be
Nocelli, Roberta	DCNME - CCA – UFSCar, Rodovia Anhanguera, 13600-970 Araras	Brasilien	roberta@ufscar.br
Ntim, Dacosta	Federation of Ghana Beekeepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Oliveira Souza Doria, Hayda	OURO FINO QUÍMICA S.A, Avenida Coronel Fernando Ferreira Leite, nº 1520 - 15º Andar - Jd. Califórnia, 14026020 Ribeirão Preto	Brazil	hayda.doria@ ourofinoagro.com.br
Owusu Korankye, Isaac	Federation of Ghana Beekeepers Associations, P.O. Box 132, 0000 Odumase Krobo	Ghana	
Pamminge, Tobias	BASF SE, Speyererstraße 2, 67117 Limburgerhof	Germany	tobias.pamminger@ basf.com

Name	Affiliation	Country	E-mail
Passaseo, Aurelia	Bundesamt für Landwirtschaft, Schwarzenburgstrasse 165, 3003 Bern	Switzerland	aurelia.passaseo@blw.admin.ch
Pereira, Andrigo	Eurofins Agroscience Services Ltda, Hercules Mazzoni, 992, 13330-250 Indaiatuba, SP	Brazil	andrigopereira@eurofins.com
Peris, David	AGROTOX, Ramon y Cajal n5. 2B, 50280 Calatorao	Spain	
Perny, Agnes	ANADIAG, 16 Rue Ampere, 67500 Haguenau	France	agnes.perny@anadiag.fr
Peterek, Silke	Staphyt GmbH, Lierheim, 6, 86753 Möttingen	Germany	speterek@staphyt.com
Pierru, Cédric	SynTech Research France, 613 route du bois de loyse, 71570 La Chapelle de Guinchay	France	
Peris Felipo, Francisco Javier	Syngenta Crop Protection AG, Rossentalstrasse 67, 4058 Basel	Switzerland	peris.felipo@gmail.com javier.peris@syngenta.com
Pilling, Ed	Corteva Agiscience, Ecotoxicology, 3B Park Square, OX14 4RN Oxfordshire	United Kingdom	edward.pilling@corteva.com
Pistorius, Jens	Julius Kühn-Institut (JKI) - Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig	Germany	jens.pistorius@julius-kuehn.de
Poráczki, Krisztián	BioChem agrar GmbH, Kupferstraße 6, 04827Gerichshain	Germany	Krisztián. Poráczki @biochemagrar.de
Purdy, John	Abacus consulting Services Ltd., 8725 Twiss Rd. P.O. Box 323, L0P 1B0 Campbellville	Canada	johnrpurdy@gmail.com
Quambusch, Anja	Bayer AG, Alfred Nobel Str. 50, 40789 Monheim a. Rhein	Germany	anja.quambusch@bayer.com
Roat, Thaisa	UNESP-Rio Claro/Brazil, CEIS - Center of Study of Social Insects, Avenida 24-A, n 1515, 13506900 Rio Claro	Brazil	thaisaroat@yahoo.com.br
Roessink, Ivo	Wageningen Environmental Research, Droevendaalsesteeg 3, 6708PB Wageningen	Netherlands	ivo.roessink@wur.nl

Name	Affiliation	Country	E-mail
Roig, Josep	Eurofins MITOX, LD PICHOY, 32250 Fources	France	joseproig@ eurofins-mitox.com
Rosa-Fontana, Annelise	Paulista State University, 24A Avenue 1515, 13506900 Rio Claro	Brazil	annesouzar@gmail.com
Rossbach Andrea	tier3 solutions GmbH, Kolberger Str. 61-63, 51381 Leverkusen	Germany	andrea.rossbach@tier3.de
Ruddle, Natalie	Syngenta, Jealott's Hill International Reserach Centre, RG426EY Bracknell	United Kingdom	Natalie.Ruddle@ syngenta.com
Ruhland, Saskia	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	saskia.ruhland@ biochemagrar.de
Sabo, Rastislav	The University Of Veterinary Medicine And Pharmacy In Košice, Department Of Pharmacology And Toxicology, Komenského 73, 4181 Kosice	Slowaka	rastislav.sabo@uvlf.sk
Schade, Michael	Syngenta Crop Protection, Rosentalstraße, 67, 4058 Basel	Switzerland	michael.schade @syngenta.com
Scheiner, Ricarda	University of Wuerzburg, Biocenter, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Wuerzburg	Germany	ricarda.scheiner@ uni-wuerzburg.de
Scheller, Kathrin	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	kathrin.scheller@ biochemagrar.de
Schmidt, Katharina	apic.ai, Rintheimerstr. 33, 76131 Karlsruhe	Germany	katharina@apic.ai
Schneider Christof	BASF SE, Speyerer Str. 2 67117 Limburgerhof	Germany	christof.schneider@ basf.com
Schnurr, Alexander	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	alexander.schnurr@ biochemagrar.de
Schöbinger, Ulrike	GAB Consulting GmbH Ottenbecker Damm 10 21684 Stade	Germany	ulrike.schoebinger@ gabconsulting.de
Schwarz, Janine	Agroscope / ETH Zürich, Hofhölzliweg 21, 8046 Zürich	Switzerland	janine.schwarz@ agroscope.admin.ch
Soler, Eugenia	Eurofins Trialcamp, Av Antic Regne Valencia, 25, 46290 Alcasser	Spain	

Name	Affiliation	Country	E-mail
Sharples, Amanda	FMC, 8 Cardale Court, HG31RY Harrogate	United Kingdom	amanda.sharples@ fmc.com
Solomon, Lartey Wakemeh	Federation of Ghana Bee-keepers Associations P.O. Box 132, 0000 Odumase Krobo	Ghana	fbeekee@gmail.com
Steeger, Jan	U.S. Environmental Protection Agency, 2970 St Peters Chruch Rd, 20601 Waldorf	United States of America	steeger.jan@epa.gov
Steeger, Thomas	U.S. Environmental Protection Agency, 2970 St Peters Chruch Rd, 20601 Waldorf	United States of America	steeger.thomas@epa.gov
Sterk, Guido	IPM Impact, Gierkensstraat 21, 3511 Hasselt	Belgium	Guido.Sterk@skynet.be
Stiller, Manuela	BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain	Germany	Manuela.Stiller@ biochemagrar.de
Straub, Lars	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Lars.straub@ agroscope.admin.ch
Strobl, Verena	Agroscope - Swiss bee research center, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	Verena.strobl@ agroscope.admin.ch
Sultan, Maryam	Bayer AG, Alfred-Nobel-Strasse 50, 40789 Monheim a. Rhein	Germany	maryam.sultan@ bayer.com
Süßenbach,Dirk	German Environment Agency (UBA), Wörlitzer Platz 1, 06844 Dessau	Germany	dirk.suessenbach@uba.de
Szentes, Csaba	EFSA, Pesticide Unit (PREV), Via Carlo Magno 1/A 43126 Parma	Italy	csaba.szentes@ efsa.europa.eu
Tänzler, Verena	ibacon GmbH, Arheilger Weg 17, 64380 Roßdorf	Germany	verena.taenzler@ ibacon.com
Thompson, Linzi	University College Dublin, School of Agriculture and Food Science, 2A Beaumont Drive, Dublin 14 Dublin	Ireland	linzi.thompson@ ucdconnect.ie
Trodtfeld, Peter	Bayer AG, Devision Crop Science, Alfred-Nobel-Str. 50, 40789 Monheim a. Rhein	Germany	peter.trodtfeld@ bayer.com

List of participants

Name	Affiliation	Country	E-mail
Tschuy, Marianne	Bienengesundheitsdienst, Schwarzenburgstrasse 161, 3003 Bern	Switzerland	marianne.tschuy@apiservice.ch
van der Steen, Jozef	Alveus AB Consultancy, Kerkstraat 96, 5061EL Oisterwijk	Netherlands	alveusab@outlook.com
van der Wal, Leon	Environment, Health and Safety Division Environment Directorate, OECD, 2, rue André Pascal - 75775 Paris Cedex 16	France	Leon.vanderwal@oecd.org
Vergé, Emmanuelle	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	EmmanuelleVerge@eurofins.com
Villamar, Laura	EFSA Pesticides Evaluation (PREV) UNIT Via Carlo Magno 1/A, I-43126 Parma,	Italy	lauravillamar.ambiental@gmail.com
Wassenberg, Jacoba	Ctgb, Postbus, 6710 AA Ede	Netherlands	jacoba.wassenberg@ctgb.nl
Wendling, Kathrin	EFSA Pesticides Evaluation (PREV) UNIT Via Carlo Magno 1/A, I-43126 Parma,	Italy	
Wilkins, Selwyn	Fera Science Ltd., Sand Hutton, YO41 1LZ York	United Kingdom	Selwyn.Wilkins@fera.co.uk
Wolff, Carolina	BASF SA, Av Nações Unidas 14171, 04614001 São Paulo	Brazil	carolina.wolff@basf.com
Wu, Chi	Beijing ECO-SAF Technology Co., Liandong U middle district 60A, 010 Beijing	China	chi19890715@sina.com
Zafeiraki, Effrosyni	Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, St.Delta Str., Athens	Greece	e.zafeiraki@bpi.gr
Zaworra, Marion	Bayer AG, Crop Science Division, Alfred-Nobel-Straße 50, 40789 Monheim am Rhein	Germany	marion.zaworra@bayer.com
Zumkier	tier solutions, Kolberger Str. 61-63, 51381 Leverkusen	Germany	ulrich.zumkier@tier3.de

Participants not listed before:

Dictonary Swiss-german (Bärn Dütsch):

English	Swiss-german (Bärn Dütsch)
Hello	Hallo
Hello everybody!	Tschou zämme!
really	awuää
yes	Yu
Hello (formal)	Grüessech
Good morning	Guete Morge
Good evening	Guete Abe
My name is …	Mi Name isch … / I heisse …
How are you (Ihne is formal)?	Wie geit`s dir/Euch
I'm fine, thank you!	Mir geit's guet, danke!
I'm not too good!	Mir geiht's nid so guet!
What are you doing today?	Was machsch hüt?
Do you have anything planned yet?	Hesch scho öpis plant?
Where is Hotel …?	Wo isch ds'Hotel …?
What is the way to …?	Wo geit`s düre zum…?
At what time does the bus/train leave?	Wenn fahrt dr Zug?
Can you arrange a taxi for me?	Cheut Dir mir bitte äs Taxi bstelle?

English	Swiss-german (Bärn Dütsch)
How much will it cost to get to …?	Wie viu choschtet`s nach …?
I'm in a hurry!	Mir pressiert`ss!
Where is the police station?	Wo isch drPolizeiposchte?
Is there a hospital nearby?	Hets äs Spitau ir Nöchi?
I feel unwell.	Mir geits schlächt
I've lost my wallet!	I ha mis Portemonaie vrloore!
What time is it?	Was isch für Zyt?
Enjoy your meal!	Ä Guete!
Cheers! (a toast)	Proscht/Gsundheit
I would like a ….	I hätt gärn äs
A small beer (3dl)	Ä Stange, Bächer
A large beer (5dl)	Äs Groosses/Chübeli
A glass of wine	Äs Glas Wy
I would like to try a Swiss speciality.	I würd gärn ä Schwiizer Spezialität probiere.
What could you recommend?	Was choit Dir mir empfähle?
May I have the bill?	Chani bitte zahle?

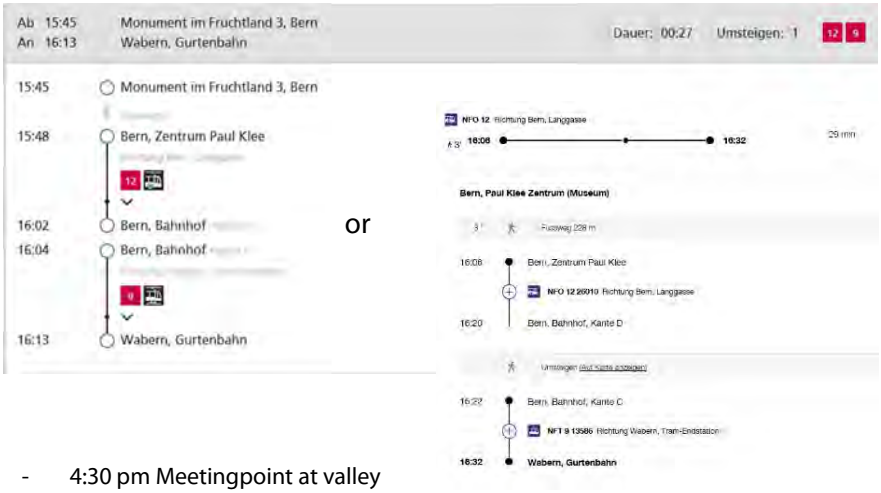
Public transport:

www.bernmobil.ch

Train station to Zentrum Paul Klee (www.zpk.org),
Address: Monument im Fruchtland 3, 3006 Bern, Switzerland
Take Bus No. 12 (direction Zentrum Paul Klee) and leave bus at end station "Zentrum Paul Klee". Walk approx. two minutes to congress location "Zentrum Paul Klee".

Social event:

Start latest at 4 pm from congress location "Zentrum Paul Klee" (Monument im Fruchtland 3) to Highland-Gurten
From station Zentrum Paul Klee take Bus No. 12 (direction Länggasse) and leave bus at station "Bern Bahnhof". Change to tram No. 9 ((direction Wabern) and exit at station "Gurtenbahn". Walk up the hill for approx. five minutes to our meeting point Gurtenbahn Talstation.

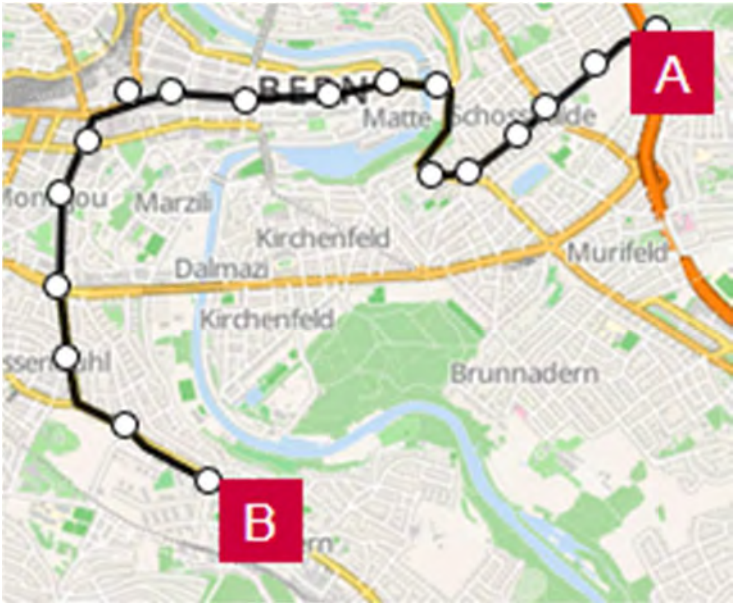


- 4:30 pm Meetingpoint at valley station Gurtenbahn
- Train to top of Gurten (5 min) or by foot (45 min)
- Overlook to Bern and Swiss Alps (when weather is fine)
- 5:30 pm easy walk to Highland-gurten (20 min)

- **6 pm – 11 pm: Social Diner at farm highland-gurten**

- Bus service from highland-gurten to tram station Wabern10 pm/10:30 pm/11 pm
- Late night hiking to tram station Wabern (duration 30 min) 11 pm
- Recommendation: Wear sturdy shoes and outdoor clothes.

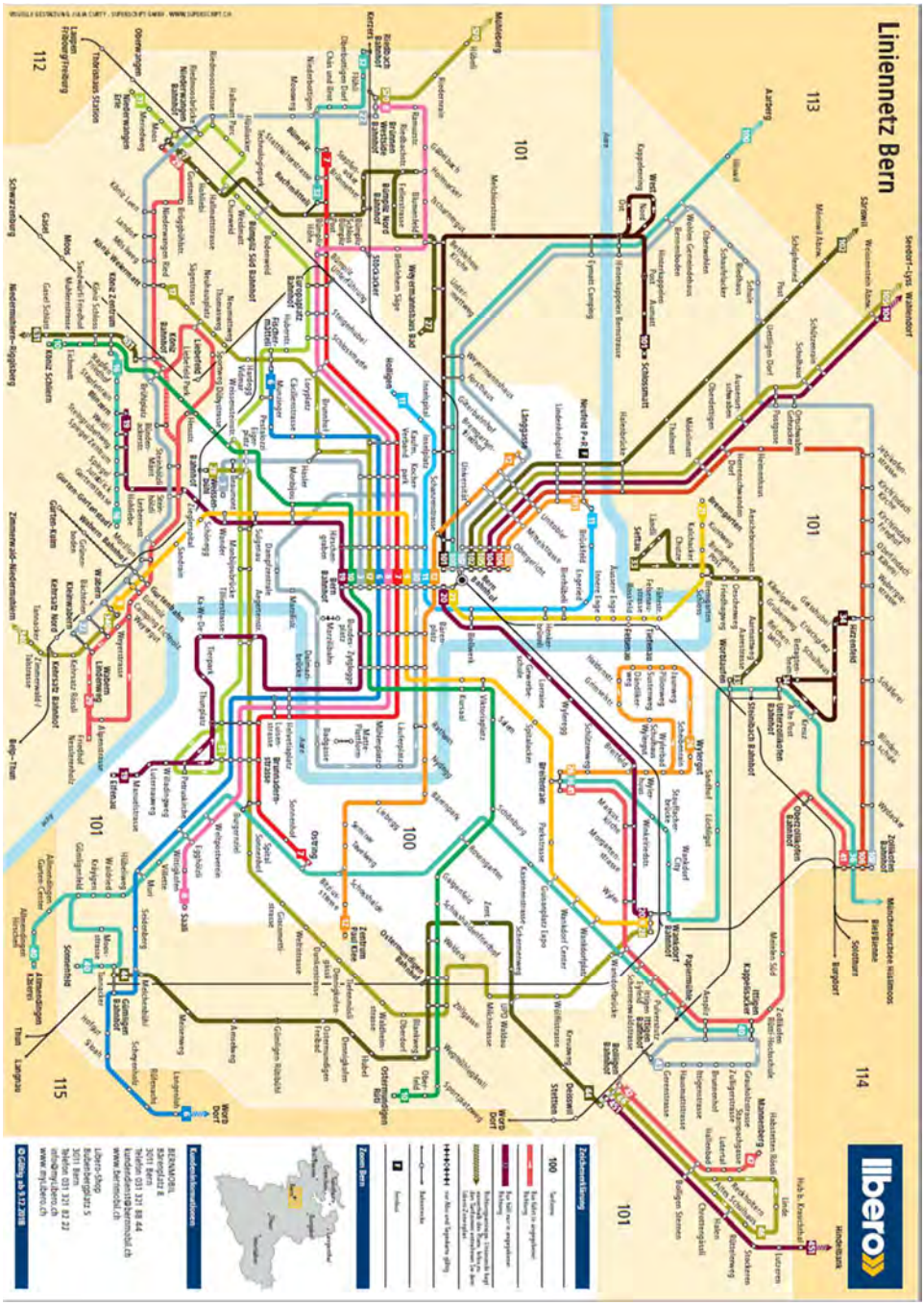
Public transport:



Public transport

https://www.gurtenpark.ch/

Public transport:



Notes:

Attachment to the 14 th ICPPR October 23-25, 2019 in Bern

List of additional participants and amendments:

Name	Affiliation	Country	E-mail
Aebi, Alex	Université de Neuchâtel, Emile-Argand 11, 2000 Neuchâtel	Switzerland	alexandre.aebi@ unine.ch
Assad, Ana Lucia	Brazilian Bees Study Association Av. Visconde do Rio Branco, 2955 apto 601, Torre 2 Fortaleza/CE, 60.055-172	Brazil	Assad@ufc.br
Blumenstein, Claudia	Eidgenössisches Departement für Wirtschaft, Bildung und Forschung WBF, Schwarzenburgstrasse 165 3003 Bern	Switzerland	claudia.blumenstein@ blw.admin.ch
Bocksch, Sigrun	Eurofins Agroscience Services Ecotox GmbH, Eutingerstr. 24, Niefern - Öschelbron	Germany	sigrunbocksch@ eurofins.com
Costa Junior, Helvio Campoy	OURO FINO QUÍMICA S.A, Avenida Coronel Fernando Ferreira Leite, nº 1520 - 15º Andar - Jd. Califórnia, 14026020 Ribeirão Preto	Brazil	
Danja Bättig	Agroscope, Agroecology and Environment, Reckenholzstrasse 191, 8049 Zürich	Switzerland	Danja.baettig@ agroscope.admin.ch
Fedrizzi, Davide	LKC Switzerland Ltd, Hauptstrasse 10, 4414 Füllinsdorf	Switzerland	lkc@lkc-ltd.com
Freitas, Breno	Brazilian Bees Study Association, Av. Visconde do Rio Branco, 2955 apto 601, Torre 2 Fortaleza/CE, 60.055-172	Brazil	freitas@ufc.br
Gätschenberger, Heike	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	KarinaKirsch@ eurofins.com
Hart, Connie	~~2150 Maple Forest Drive, K0A2T0 Ottawa~~ Health Canada Environmental Assessment Directorate Pest Management Regulatory Agency 2720 Riverside Drive, Ottawa, Ontario, Canada K1A0K9	Canada	connie.hart@canada.ca
Ingels, Brecht	Federal Public Service Health, Food Chain Safety and Environment, Victor Hortaplein 40/10, 1060 Brussel	Belgium	brecht.ingels@ health.fgov.be

Name	Affiliation	Country	E-mail
Janke, Martina	LAVES Institut für Bienenkunde Celle, Lindemannallee, 29221 Celle	Germany	martina.janke@ laves.niedersachsen.de
Kiemle, Annika	Eurofins Agroscience Services GmbH, Eutiner Straße 24, 75223 Niefern-Öschelbronn	Germany	annikakiemle@ eurofins.com
Martinovic Barrett, Barbara	Health Canada, 2720 Riverside Dr., K1A0K9 Ottawa	Canada	barbara. Martinovicbarrett @canada.ca
Thompson, Linzi	University College Dublin, School of Agriculture and Food Science, ~~2A Beaumont Drive,~~ ~~Dublin 14~~ Belfield, 4 Dublin	Ireland	linzi.thompson@ ucdconnect.ie
Sekine, Tatsuya	ibacon GmbH, Arheiliger Weg 17 64380 Roßdorf	Germany	tatsuya.sekine@ ibacon.com
Persigehl, Markus	tier3 solutions GmbH , Kolberger Str. 61-63, 51381 Leverkusen	Germany	markus.persigehl@tier3.de
Pisoni, Dennis	Federal Office for Agriculture, Schwarzenburgsstrasse 165, 3003 Bern	Switzerland	dennis.pisoni@ blw.admin.ch
Puy, Thomas	ANADIAG, 16 Rue Ampere, 67500 Haguenau	France	Thomas.puy@anadiag.fr
Schmidt, Katharina	apic.ai GmbH, Rintherimerstr. 33, 76131 Karlsruhe	Germany	katharina@apic.ai

Tausch , Frederic	apic.ai GmbH, Rintherimerstr. 33, 76131 Karlsruhe	Germany	
Rattis Motta, Rachel	OURO FINO QUÍMICA S.A, Avenida Coronel Fernando Ferreira Leite, nº 1520 - 15º Andar - Jd. Califórnia, 14026020 Ribeirão Preto	Brazil	
Ruhnke, Haike	SCC GmbH, Am Grenzgraben 11, 55545 Bad Kreuznach	Germany	adrian.willrich@ scc-gmbh.de
Wendling, Kathrin	~~EFSA~~ ~~Pesticides Evaluation (PREV)~~ ~~UNIT~~ ~~Via Carlo Magno 1/A, I-43126 Parma,~~ Eurofins Agroscience Services Ecotox GmbH, Eutingerstr. 24, Niefern - Öschelbron	~~Italy~~ Germany	kathrinwendling@ eurofins.com
Willis Chan, D. Susan	University of Guelph , 1922 Selwyn Rd, K0L2H0 Lakefield	Canada	peponapis@yahoo.com

Participation cancelled:

Name	Affiliation	Country	E-mail
Albercht, Matthias	Agroscope, Agroecology and Environment, Reckenholzstrasse 191, 8049 Zürich	Switzerland	matthias.albrecht@ agroscope.admin.ch
Fusilero, Abegail	Rotam Agrochemical Europe, 75 cours Albert Thomas 6e avenue - Bâtiment D, 69003, Lyon 03	France	afusilero@rotam.com
Horacio, Heinzen	Universidad de la República Gral. Flores 2124, 11800 Montevideo	Uruguay	heinzen@fq.edu.uy
Kirsch, Karina	EAS Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn	Germany	KarinaKirsch@ eurofins.com
Penketh, Sara	Covance, Woolley Road, Alconbury, PE28 4HS Huntingdon	United Kingdom	sara.penketh@ covance.com
Purdy, John	Abacus consulting Services Ltd., 8725 Twiss Rd. P.O. Box 323, L0P 1B0 Campbellville	Canada	johnrpurdy@gmail.com