INTERNATIONAL COMMISSION FOR PLANT-POLLINATOR RELATIONSHIPS

Bee Protection Group

16th INTERNATIONAL SYMPOSIUM

Sevilla, Spain.

October 15 – 17, 2024

HAZARDS OF PESTICIDES TO BEES

Location: Caixa Forum

C. López Pintado, s/n, 41092 Sevilla, Spain

History ICPPR-Bee Protection Group conferences:

1st Symposium, Wageningen, the Netherlands, 1980
2nd Symposium, Hohenheim, Germany, 1982
3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
7th Symposium, Avignon, France, 1999
8th Symposium, Bologna, Italy, 2002
9th Symposium, York, UK, 2005
10th Symposium, Bucharest, Romania, 2008
11th Symposium, Wageningen, the Netherlands, 2011
12th Symposium, Ghent, Belgium, 2014
13th Symposium, València, Spain, 2017
14th Symposium, Bern, Switzerland, 2019
15th Symposium, York, UK, 2022

16th Symposium, Sevilla, Spain, 2024

Organizing Committee 16th Symposium

Teresa Martin	BioChem AGROLOGIA
Elisabeth Giddings	BioChem AGROLOGIA
Dr. Markus Barth	BioChem agrar
Dr. Anne Alix	Corteva
Dr. Tomas Steeger	US-EPA
Dr. Jens Pistorius	JKI

Supported and organized by

Streaming provided by the Pollinator Research Task Force”

3.3.	12:35	12:55	Anne Alix*	Precision application of plant protection products in agriculture: role of the European Precision Application Task Force EUPAF and implications for pollinators
	13:00	**14:15**	***Lunch***	
	14:15	15:00	Poster session	
	15:00	16:00	All/BOARD/WG	ICPPR organisational issues
	16:00	17:00	Working groups - work time & Possibility to visit exhibiton in Caixa Forum	
	18:00		Guided tour (Meeting at 17.50 at Plaza de España)	
	20:00		Social event (Casa Guardiola)	
END OF DAY 2				

Day 3 Thursday				**17th October 2024**
No.	**Start**	**End**	**Speaker**	**Title**
Monitoring, Others				
	9:00	9:05	A. Alix, J. Pistorius	Introduction and organisational information for the day
3.4.	9:05	9:25	Silvio Knäbe	Results of a Colony Feeding Test to Evaluate the Efficacy of dsRNA for Control of Varroa Mites in Honeybees (Apis mellifera L.)
3.5.	9:25	9:45	Silvina Niell	Pesticides monitoring with beehives data evaluation from a hazards perspective
4.1.	9:45	10:05	Daniel Schmehl	Strengthening the link between honey bee laboratory data and colony health outcomes
4.2.	10:05	10:25	Guido Sterk	Herbicidal treatment on R&D Bombus terrestris colonies under lab conditions
4.3.	10:25	10:45	Charlotte Elston	An interdisciplinary approach for designing selective insecticides for bee safety considering mechanistic processes
	10:45	**11:25**	***Coffee and Tea Break***	

Program - Week at a glance

Modelling, Microbials				
5.1.	11:25	11:45	Mark Miles	Integrating Higher Tier Studies with Mechanistic Models in Bee Risk Assessment
5.2.	11:45	12:05	Amelie Schmolke	Bee models in higher-tier risk assessments: overview and example of SolBeePop
5.3.	12:05	12:25	Jan Baas	BeeGUTS – Test Integration and optimization. Comparison of sensitivities of bee species
6.1.	12:25	12:45	Annelise Rosa-Fontana	Honeybee gut microbiota is an imperative endpoint for pesticide risk assessment
	12:45	**14:00**	***Lunch***	
Non-Apis				
7.1.	14:00	14:20	Ana Cabrera	Assessing acute oral toxicity to the alfalfa leafcutting bee, Megachile rotundata
7.2.	14:20	14:40	Susan Willis Chan	Understanding and comparing relative pesticide risk among North American wild bees from their association with agriculture
7.3.	14:40	15:00	Ève-Catherine Desjardins	Osmia tersula from the boreal forest, a Nordic indigenous bee for the pollination of indoor or outdoor fruit productions in northern communities
	15:00	16:00	Jens Pistorius/ Anne Alix	Discussion on lessons learnt, future directions, upcoming tasks, next ICPPR BPG meeting?
	16:00			**Closing of Symposium**
END OF DAY 3, End of Symposium				

* : online – oral presentation

6. Session – Microbials

6.1. Honeybee gut microbiota is an imperative endpoint for pesticide risk assessment

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A roadmap for the integration of environmental microbiota in risk assessments under the European Food Safety Authority (EFSA) remit has been published. Honeybees maintain a consistent core microbiota, though natural fluctuations can occur due to factors such as age, caste, and season. The primary challenge in ecotoxicological assays is establishing a standardized protocol to minimize fluctuations between control and test groups.

In nature, honeybee workers acquire their stable gut microbial community by the 7th day post-emergence, with older bees transmitting microorganisms to younger bees in the comb, thereby establishing natural microbial diversity. In contrast, younger caged bees sampled for laboratory trials (OECD Guideline No. 245) are in contact with older bees for only a few hours. Newly emerged bees harbor minimal to no bacteria, potentially resulting in lower diversity, richness, and bacterial loads in their gut. However, this method best simulates the natural state within a controlled environment.

Existing studies have modified standardized protocols to simulate the microbiota present in the honeybee digestive tract within the hive environment. A common approach involves diluting the gut contents of forager bees and incorporating this into the diet of caged bees.

In our trials, we strictly adhered to OECD Guideline No. 245 (Chronic Oral Toxicity Test; 10-Day Feeding), exposing newly emerged *Apis mellifera carnica* workers to a single concentration of the insecticide flupyradifurone (FPF, 36 ppm). The standard reference dimethoate (1 ppm) and control groups (pure food and food + acetone) were also included. DNA was extracted individually from the bee abdomens, and full-length 16S rRNA amplicon metagenomics were sequenced using PacBio Sequel II (HiFi/CCS mode).

The absolute abundance of four bacterial genera comprising the core honeybee microbiota revealed a *Lactobacillus*-dominated gut in both treated and untreated bees. Treated bees exhibited a twofold increase in the bacterial load of *Snodgrassella*, contrasting with a 50% reduction in *Bifidobacterium* and the complete absence of *Gilliamella* compared to untreated bees. Our findings demonstrate that FPF significantly disrupts the honeybee gut microbiota.

This study presents, for the first time, the composition of the gut microbiota in honeybees strictly subjected to the OECD guideline without modifications or adaptations. Results from OECD-based tests already meet reliability requirements for risk assessments. Therefore, following OECD standards strictly illuminate three distinct advantages: (1) streamlining the process leading to a ring test, (2) reducing variations introduced by external factors potentially brought into hives by foraging bees, and (3) reducing bacterial diversity in lab-tested bees, thereby facilitating the establishment of acceptable fluctuations in microbiota composition.

We have developed a new approach, overlooked in risk assessments studies so far, to assess the impact of pesticides on bee health. We propose adopting this approach as a new endpoint in pesticide risk assessments. Specifically, we advocate for the inclusion of honeybee gut microbiota dysbiosis as a sublethal effect in the first screening step of risk

assessments, and as a key parameter to assess pollinator's health. We will present a summary of the most relevant bacteria for bee health, alongside fluctuations in the microbiota and diversity indices. Additionally, we will provide recommendations on the most suitable indicators for assessing gut microbiota dysbiosis.

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