

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

Verena Christen<sup>1</sup>, Lukas Jeker<sup>2</sup>

<sup>1</sup>University of Applied Sciences Northwestern Switzerland, Hofackerstrasse 30, 4132 Muttenz, Switzerland,

<sup>2</sup>Agroscope, Swiss Bee Research, 3003 Bern, Switzerland

E-Mail: verena.christen@fhnw.ch, lukas.jeker@agroscope.admin.ch

DOI 10.5073/jka.2020.465.003

#### Abstract

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 future 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<sup>1</sup>, Verena Christen<sup>1</sup>, Petra Kunz<sup>2</sup>

<sup>1</sup>University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Langackerstrasse 30, CH-4132 Muttenz, Switzerland, <sup>2</sup>Federal Office for the Environment, CH-3003 Bern,

E-Mail: karl.fent@bluewin.ch

DOI 10.5073/jka.2020.465.004

#### Abstract

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

**Abstracts: Oral Presentation**

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 MRL guideline SANTE11956/2016 rev.9 (2018)**

**Yotam Cohen, Christian Berg, Gundula Gonsior, Silvio Knäbe**

Eurofins Agrosience Services Ecotox GmbH

Eutingen Strasse 24, 75223 Niefern-Öschelbronn

E-Mail: GundulaGonsior@eurofins.com

DOI 10.5073/jka.2020.465.005

### **Abstract**

A new study design, according to the guideline SANTE11956/2016 rev:9 (2018), was established to determine the maximum residue level (MRL) of plant protection products in honey. The guideline describes a syrup bee feeding study designed as a worst-case scenario for transferring plant protection products into honey. Previously, field and semi-field studies designs were used. The objectives of this study were to validate the suitability of this feeding semi-field studies according to the new guideline.

Maximum Residue Levels, MRL, Honey, Honey Bees, Consumer Safety

### **Introduction**

Feeding studies could be a cost-effective and standardized way to determine residue levels of plant protection products in honey. The basic idea of the feeding study described in the SANTE11956/2016 rev:9 (2018) guideline, is to feed a solution containing the highest amount of pesticide residue that has been found in "aerial parts of plants" that were applied/sprayed with a pesticide. Usually, the maximum residue that has been found in nectar samples is used. Since practical experiences with this study design are to a large extent missing, different materials and different methods concerning the creation of the artificial honeybee swarms were compared.

### **Materials and Methods**

To examine different methods, four swarms (10,000 bees each) have been prepared with the artificial swarm technique (also known as "shook swarm method"). The colonies, two containing wax foundations and two containing drawn-out combs, were held in a dark cool (<15°C) in the basement for 48 hours and were fed with commercial sugar solution. The bees were then transferred into empty hive bodies in tunnels without any crop.

In addition to the four swarms kept in the dark and cool place, a colony containing brood and food storages was placed in a tunnel under field conditions. Once the swarms have been transferred into the hive bodies containing wax foundations or drawn-out combs in the tunnels, the fifth colony was also transferred into a hive body containing drawn-out combs.

After the set-up of the hives, the five colonies were fed with sugar solution containing a blue additive. During the first two feeding occasions, a 5 % dye sugar solution was provided. For the following two feedings, a 2.5 % dye sugar solution was provided. Subsequent feedings with uncolored sugar solution were done until honey stores (capped honey or honey containing less than 20 % water) were available.

### **Preparation of the Colonies**

Four artificial swarms of honeybees (*Apis mellifera*) with at least 10,000 bees each, were prepared by using the "shook swarm" method (Waite *et al.* 2013). Before the start of the study, each colony was visually inspected for a healthy egg-laying queen, healthy brood nest and no visible symptoms of viruses or diseases. The swarms, along with their caged queen, were placed in a dark and cool