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For the assessment of capped brood (pupae) cells the beekeepers did their assessment with low variation between each other and high correlation to the counted numbers. But there was a general overrating of capped brood cells by the beekeepers. As this is a stable trend it does not harm the informative value of honeybee studies but it may become troublesome for modeling of hive development and would have to correct for the overestimating done by the beekeepers.

Smaller hives increase the precision of the total estimate.

Assessments of number of honeybees from area on photos are a method comparable to counting individual honey bees.

Impact of weather conditions on the number of forager bees can be reduced by assessing replicate number by replicate number and not treatments as blocks after each other.

Determination of number of honeybees using weighing methods results in an overestimation of honeybees (load of nectar in honey stomach).

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

**Silke Peterek<sup>1</sup>, Elizabeth Collison<sup>2</sup>, Vincent Ortolì<sup>3</sup>, Alexia Faure<sup>3</sup>**

<sup>1</sup>Staphyt GmbH, Langenburger Str. 35, 74572 Blaufelden, Germany

<sup>2</sup>APC Wetherby (part of Staphyt Group), Cromwell Office Park, York Road, Wetherby LS22 7SU, United Kingdom

<sup>3</sup>Staphyt and Staphyt Regulatory, 23 rue de Moeuvres, 62860 Inchy en Artois, France

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#### Abstract

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.

#### Introduction

Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) were finalised in September 2018. These guidelines should be implemented by 1st January 2020 to fulfil EU data requirements concerning the placing of plant protection products on the market (Regulation (EU) No. 283/2013, Annex 6.10). Different study types are suggested in the guidelines, with the appropriate study type to be conducted dependent on the active substance mode of action, intended use and available data.

Furthermore, residue trials can provide valuable data to assess the potential environmental exposure of bees as part of the ecotoxicological risk assessment of bees to plant protection products (to be assessed under Annex 8.3.1 of Regulation (EU) No. 283/2013).

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

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subsequent residue analysis. Here, we present our tested field methods, with a focus on tunnel residue studies, to share our expertise.

#### **Choice of crop**

To date, we have conducted >100 bee trials on 10 different crops. Typically, we use highly melliferous crops, such as oilseed rape, phacelia, apple or sunflower.

Consideration should be given as to whether to use a surrogate crop or the intended target crop, as well as the chosen crop variety. Influencing factors may include the expected pollen and nectar production, crop height versus tunnel height, and other agronomic factors, such as sowing time, irrigation needs and pest pressures (for example, spring rape requires frequent insecticide application compared to winter rape varieties).

#### **Tunnel setup**

To date, we have conducted bee trials in 7 different European countries, covering Northern and Southern residue zones: Austria, France (N and S), Germany, Italy, Poland, Spain and United Kingdom.

As trial sites are distributed across different European countries, consideration should be given to the need for uniformity in study setup. This may include tunnel size, equipment and methods for recording climatic conditions, availability of water source for bees and honey bee colony size.

#### **Pesticide application**

Before application, honey bee colonies are assessed and then protected (to avoid overspray).

To date, we have conducted trials via spray application or seed treatment. Alternatively, we can perform syrup feeding studies.

The application regime will depend on the intended use.

#### **Residue sampling**

We have sampled various matrices from primary and/or succeeding crops, including nectar, pollen, anthers, mature honey, soil cores and guttation fluid. Here, we will present our tested field methods and some advantages and disadvantages of various sampling techniques, such as manual- versus honey bee-collected sampling:

##### Honey bee-collected sampling methods

Foraging bees can be collected directly from flowers across the plot or at the hive entrance, using different collection devices.

Pollen can then be scraped from the bees' pollen sacks, and if necessary, manually sorted by crop type.

Nectar can be sampled from the bees' honey crop (stomach) by squeezing the abdomen or dissection.

##### Manual sampling methods

Pollen can be collected from pollen traps fitted to the hive, and if necessary, manually sorted by crop type.

Pollen can also be collected by sampling aerial parts of the crop, e.g. anthers or whole flowers, which can be more time-consuming as many flowers have to be collected, but avoids the need for subsequent manual sampling for crop type.

Nectar can be sampled by capillary action, or centrifuging collected flowers, but this is highly time-consuming to collect sufficient sample for analysis.

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Honey can be sampled directly from the comb, by squeezing cells or using a vacuum pump. In a similar way, wax and royal jelly can also be collected.

Guttation fluid can be sampled directly from certain crops, which requires careful consideration of the crop irrigation and climatic conditions for guttation production (and usually some very early mornings!).

Soil cores can be sampled to inform on likely exposure to ground-dwelling bee species, and/or the potential for systemic residues in succeeding crops.

**Future work**

With the combined expertise of Staphyt's Bee Team, consisting of regulatory, scientific and field specialists, together we can provide both practical (field) and regulatory (consultancy) support on the conduct of pan-European field and tunnel residue studies for environmental and consumer risk assessments. In the coming seasons, we will continue to explore the following open questions:

Does the confinement of bees to a tunnel impact bee behaviour and are residues therefore still comparable to realistic field scenarios?

Is it possible to respect the intended interval time between applications if a surrogate crop is used?

Can the sampling methods be adapted to improve collection efficiency? i.e. to reduce the resources (manual time and cost) required, and increase the quantities of each matrix available for subsequent residue analysis?

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

**Kyongmi Chon<sup>1\*</sup>, Hwan Lee<sup>1</sup>, Bo-Seun Kim<sup>1</sup>, Yeon-Ki Park<sup>1</sup>, Are-Sun You<sup>1</sup>, Jin-A Oh<sup>2</sup>, Yong-Soo Choi<sup>3</sup>**

<sup>1</sup>Chemical Safety division, Department of Agro-food Safety and Crop Protection, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea;

<sup>2</sup>Agromaterial Assessment division, Department of Agro-food Safety and Crop Protection, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea

<sup>3</sup>Sericulture and Apiculture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea

E-Mail: kmchon6939@korea.kr

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**Abstract**

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<sup>2</sup> 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.