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.

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

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

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Abstract

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.

Keywords: honey bee, pollen, pesticides, citizen science, botanical origin, passive samplers

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 tube 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.

5.5 Report of the activities of the ICPPR Bee Brood Working Group

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ICP-PR Bee Brood Working Group (WG)

Co-Chairs: Verena Tänzler (Ibacon), Lukas Jeker (Agroscope) and Selwyn Wilkins (Fera)

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Abstract

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