5.10 How do Regulatory Requirements and Assumptions Correlate to Practical Experience in Residue Studies with Nectar and Pollen?

Silvio Knaebe*, Pierre Mack, Andreas Appeltauer, Annette Kling

Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
* corresponding author: SilvioKnaebe@eurofins.com

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Abstract

Residues of pesticides detected in pollen and nectar (bee relevant matrices) represent a realistic research approach to estimate pollinator exposure. Therefore, a robust and reliable method to sample and measure these residues is part of risk assessment schemes in several parts of the world. EFSA guidance for pollinators was the first risk assessment to allow for the refinements of the expected residue values during exposure. EPA as well as IBAMA followed suite and proposed in vivo refinements for residue values. To achieve this goal nectar and pollen from plant species have to be collected in sufficient amounts to allow for residue analysis. Several methods are available for the collection of bee matrices. We list general methods developed to sample pollen and nectar, focus on some common issues encountered during the conduct of these studies and place the measurements derived from these studies into a risk assessment context. With all the information available now it would be a useful task to compare residue levels in matrices collected manually and with the help of pollinators to give advice for guidance document refinements and help to approve the design of studies in the future.

Methodology and Guidance Documents (GD)

Hand collection of nectar and pollen was refined over the last five seasons. In manual nectar sampling two main methods are established: capillary collection and micro-centrifugation, both depending on the crop. In manual pollen sampling, collection with sieves but also vacuum collection, sonic vibration and tumble drying of anthers are now standard methods. But it can be difficult to collect pure pollen, so for some crops anthers or pollen together with anthers and pistils are collected. It is often only clear during the sampling that pure pollen is not available in high enough amounts. Unfortunately, there is no real guidance how to proceed in such cases.

For pollinator collection a rule of thumb is to collect at least 200 honey bees to be able to have at least 100 µl of nectar (Knaebe et al. 2015). An easier way to collect nectar in Europe are commercially available bumble bees (*Bombus terrestris*). With this species about 20 specimen are sufficient to obtain 100 µl nectar. Residue levels are comparable to those observed in nectar from honey bees. In a lab study residues in honey stomach of bumble bees were about 10% higher than residues of honey bees (Kling et al. 2017). To work with bumble bees is easier since no permit is needed when bees are moved and additional colonies can be ordered on short notice. Additionally bumble bees can sample in colder temperatures than bees improving the time periods where sampling can take place. With bumblebees also broader variety of crops can be sampled.

Likewise the knowledge of varieties of the crop of concern and timing for samplings has improved over time. For instance not all varieties of oilseed rape or sunflower are good for collecting nectar. Also soil type, irrigation and timing of seeding can have a strong influence on the availability of nectar and pollen and on residue results. Another example are potatoes where not all varieties of plants produce flowers.

Table 1 Main requirements and the usage of data in three different regulatory frameworks.
* Manual of Environmental Risk Assessment of Pesticides to Bees, Brasília: Ibama/Diqua (2017); ** Guidance Document on the Risk Assessment of Plant Protection Products on Bees (*Apis mellifera, Bombus* sp. and solitary bees), EFSA (2013); ***Guidance on Exposure and Effects Testing For Assessing Risks to Bees, USEPA (2016)

Region	Number of studies requested for refinement	Crops and regions	Collection method	Usage of data in risk asssessment
Brazil *	1 study site in each zone where crop is important, study with 3 replicates for each relevant application method.	Crops are classed in 12 groups, minimum requirement trials in crop with highest ranking in guidance.	Hand collection and from pollinators (honey bees) and in hive collection. If nectar/pollen needs to be collected is given for each crop in GD. Additional plants, flower, stored nectar and pollen and royal jelly.	The maximum values found in each matrix should be used in calculating the acute risk and the highest daily average for calculating the chronic risk (BeeRex used).
EU **	5 study sites in each zone (3 zones) with 3 replicates for each relevant application method.	Each crop and surrogate off-crop.	Hand collection or from pollinators (honey bees). If nectar/pollen needs to be collected is given for each crop in GD.	The purpose of the five studies is to assess the 90th percentile case (i.e. the residues in the study that shows the highest values of the
USA ***	3 study sites with 3 replicates for each relevant application method.	Select number of crops that adequately represent the diversity of pollinator-attractive crops and registered uses is typically considered sufficient.	Hand collection or from pollinators (honey bees). If nectar/pollen needs to be collected is given for each crop in GD. Additional plant material, flower and royal jelly. If pollen is not possible, anthers to be sampled.	The relevant values found in each matrix should be used in calculating the acute risk and the highest daily average for calculating the chronic risk (BeeRex used).

Many important details are not provided in the present guidelines. The details are not only needed for the sampling part but also the residue analysis (i.e. which material to use for method validation) and most importantly the usage of the data in the risk assessment. The latter point is very important since available data already show there is a high variability in residue data across matrices, between years but also across plant species of the same family (Sappington et al. 2016). In the data presented by Sappington et al. 2016 medians are relatively similar for nectar but there are less so for pollen. If 90th percentiles are used, even higher variation is observed with values up to 10-fold higher for pollen and up to 4.5-fold for nectar. Individual values in single events are even up to 40-fold higher. There are outliers in similar ranges for the studies we have run in the past.

Summary and Discussion

Even after as much as 5 seasons of experience there are still basic questions to be considered to improve the design of bee exposure studies. From the applied side there are needs for guidance – which crop, how many replicates, what spatial scale and how many samples over what time. For the sampling: which type of sampling (manual or pollinators) and what matrices. For data: how to present and use the data in the risk assessment.

One solution is to prepare a guidance document based on the quite extensive data already available for several substances as shown by Sappington et al. 2016. An OECD guidance would also make it possible to compare residue values across temporal zones and if possible normalize data across temperature zones. Furthermore the usage of the geomean could be a possibility to derive one value where all data is included. The large amount of trials would make it possible, since this is also used in the risk assessment of birds and mammals or soil studies. Furthermore, a common design could also include additional matrices that would make it easier to calculate residue levels within the bee hive. A design should also include some flexibility for difficult crops so other pollinator species (e.g. bumble bees) can be used, too. For main crops tested with honey bees as standard worker jelly or royal jelly should be included as proposed by the Brazilian and US guidance document. This would give a more precise estimate of the possible exposure of honey bees during their development. For the risk assessment purpose it would make sense to implement also considerations of degradation behavior of the relevant substances in the bee food matrices.

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5.11 A research about different residues in pollen and honey samples

Dorothee J. Lüken, Werner von der Ohe

LAVES Institut für Bienenkunde Celle, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany

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Abstract

Within the cooperative project "Reference system for a healthy honey bee colony – FIT BEE" the subproject "Multifactorial influences on honey bee colonies and establishment of a GIS-based expert information system" was conducted by LAVES Institute for Apidology Celle. The project lasted for four years and was funded by BLE / BMELV.

In addition to research about influences of different habitats (city and country sites) on honey bee colonies, residues from Plant Protection Products (PPPs), Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) were analysed in pollen and honey samples.

During the project a total of 62 different residues from PPPs were analysed (11 insecticides, 18 herbicides and 33 fungicides) as well as one synergist. Thiacloprid was found in every fourth pollen sample on average with a maximum concentration of 0.16 mg / kg (bee bread). In the country site group and the travel group over 80 % of the pollen samples had PPP-residues, in the city site group 25 % (n = 80 / group, 2012 + 2013). In the country site group 15 active ingredients (a.i.) were parallel in one pollen sample, in the travel group 11 and in the city group 3 with maximum concentrations > 10 mg / kg in pollen samples from the country site. From the 15 pooled honey samples 7 had PPP-residues, especially the spring samples (oil seed rape honey). In all honey samples analysed, four a.i`s were found in the honey samples in total (Thiacloprid (max. 0.05 mg / kg)), Boscalid (0.005 mg / kg), Dimoxystrobin (0.005 mg / kg) and Carbendazim (max. 0.04 mg / kg)).

The PPP-data were comparable to the PAH- and the Heavy Metal data: In the pollen samples were more residues and in higher concentration than in the honey samples. Honey is a lipophobic matrix and pollen a lipophilic matrix. Most of the residues solve better in a lipophilic matrix and the bees act as a filter for the nectar / honey.

Introduction

LAVES Institute for Apidology Celle participated in the cooperative project "Reference system for a healthy honeybee colony – FIT BEE" with the subproject "Multifactorial influences on honeybee colonies and establishment of a GIS-based expert information system". The project lasted for four

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3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
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Foto
Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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