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

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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 years (2011 - 2015) and was funded by BLE / BMELV. The main topic of this cooperative project was the comparison of different habitats and their influence on honeybee colonies (country site group, travel group, city site group) with six honeybee colonies per test group. To look at the different habitats in its entirety, residues from Plant Protection Products (PPPs), Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) were analysed in pollen and honey samples taken from the test colonies.

Materials and Methods

A total of 15 honey- und 340 pollen samples were analysed at LAVES food and veterinary institute in Oldenburg (LVIOL) for residues from Plant Protection Products (PPP). The pollen samples (beebread and pollen pellets) were taken per hive, the honey samples were pooled samples per test site. For analysis, it was used the QuEChERS – method (L 00.00-115 / CEN EN 15662, 2008) with a spectrum of about 375 active ingredients (a.i's). Glyphosat was not part of the spectrum. The limit of detection (LOD) laid between 0.0025 mg / kg and 0.01 mg / kg, the limit of quantification (LOQ) between 0.005 mg / kg and 0.02 mg / kg. Additionally 6 pollen und 6 honey samples per test site were analysed for Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) at the LAVES food and veterinary institute in Brunswick (LVIBS/H). The Heavy Metals were analysed by using Inductively Coupled Plasma-Mass Spectrometry and Atomic Fluorescence Spectrometry (ICP-MS/AFS). The LOQ laid between 0.005 mg / kg and 3.3 mg / kg. The PAHs were identified by using GC-MS/MS with Accelerated Solvent Extraction (ASE) and purification with Gel Permeation Chromatography (GPC). A total of 15 PAHs were part of the analysis which are classified by the European Scientific Committee on Food (SCF) as carcinogenic and Benzo(c)fluorine, additionally. The LOQ was 0.3 µg / kg for honey and 0.6 µg / kg for pollen.

Results

A total of 11 insecticidal, 18 herbicidal and 33 fungicidal substances plus one synergist were found in the analysed honeybee products in the years 2011 – 2014 (Fig. 2). Concentrations and numbers of PPP-residues in the samples taken at the country site were higher than in the samples taken at the city site (Fig. 1). In the country site group and the travel group over 80 % of the pollen samples had PPP-residues, in the city group 25 % (n = 80 / group, 2012 + 2013). In the country site group 15 active ingredients (a.i.) were parallel in one pollen sample and three in one honey sample, in the travel group 11 a.i's in pollen and 3 in honey and in the city site group 3 a.i's in pollen and 0 a.i's were analysed in honey samples (n honey = 15, n pollen = 340, 2011 - 2014). A total of four in this project analysed a.i's were also found in the honey samples (Tab. 1). Concentrations of PPP-residues were lower in honey samples than in pollen samples, the MRLs in pollen samples were frequently exceeded (Tab. 1). The findings of a.i's in samples differed between beebread and pollen pellets (Fig. 2). Out of 11 analysed insecticidal substances, five were without authorisation in Germany as well as four herbicidal and two fungicidal substances (Fig. 2). Insecticides classified harmful for bees (B1, B2, B3) were found in pollen samples (Fig. 2). Thiacloprid was analysed in every fourth sample on average and was therefore the most frequently found a.i. in this project.

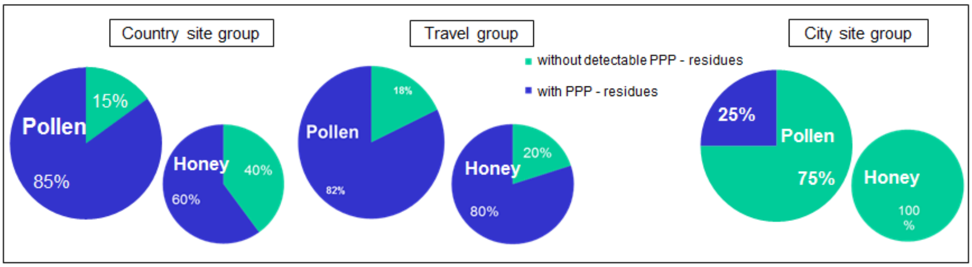

Fig. 1 Number of samples with residues from Plant Protection Products in honeybee products in % from different test groups (Pollen (beebread and pollen pellets): n = 80 / group, 2012 & 2013; Honey: n = 5 / group, 2012 - 2014)

Active ingredient analysed in honey	MRL Germany (mg / kg)	Honey (mg / kg)	Pollen (mg / kg)
Thiacloprid	0.2 / in 2016: 0.05	0.05	0.16
Boscalid	0.5	0.005	15.9
Dimoxystrobin	0.05	0.005	0.34
Carbendazim	1.0	0.04	0.15

Tab. 1 All a.i's analysed in honey 2011 – 2014
(n = 15) with maximum concentrations per sample found in honey and pollen (beebread + pollen pellets) plus Maximum Residue Levels (MRLs)

Discussion

Honeybee colonies are useful instrument for monitoring their nearer environment with its contaminants (foraging radius approx. 5 km). The contaminants solve better in lipophilic pollen than in hydrophilic honey and the bees act as a filter for the nectar / honey. Even between beebread and pollen pellets can be differences related to concentration and abundance of residues. Flusilazol has lost its authorisation in 2013 and carbendazim in 2014, which may reduce the findings of carbendazim in honey. In cities higher concentrations of PAHs and Heavy Metals occurred due to industry and traffic except for manganese. This element is part of fertilizers for soils with high sandy proportions as in the project area. On the countryside more residues from PPP were found. The frequent appearance shows the importance of agricultural landscape as nutrition source for honeybees. Because of unknown impacts on the fitness of the honeybee colonies, the PPP-residues have to be minimized in bee products by e.g. adjusting the period of spray application even for B4 and by using special application techniques.

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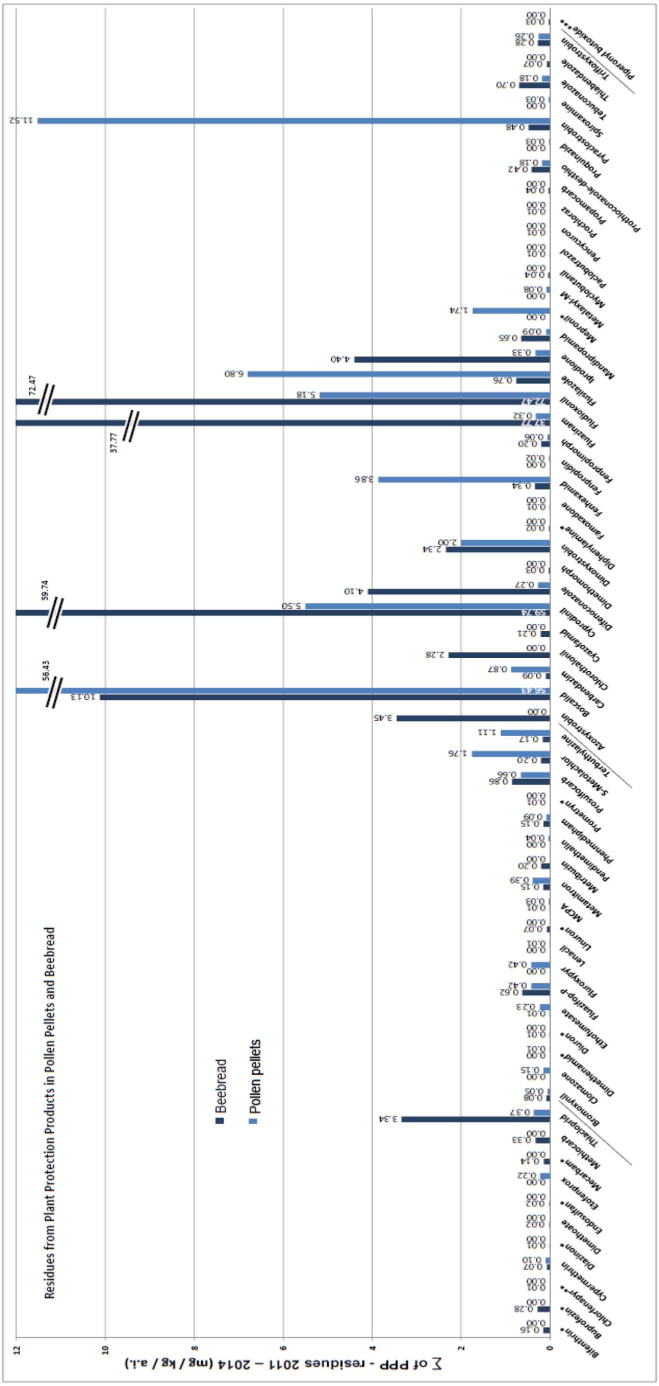

Fig. 2 Sum of concentrations from PPP-residues analysed in pollen pellets and in beebread 2011 - 2014, n pollen pellets = 102, n beebread = 238 (* = a.i. without authorisation in Germany as PPP, ** = a.i. authorisation as biocide except for PP, *** = synergist)

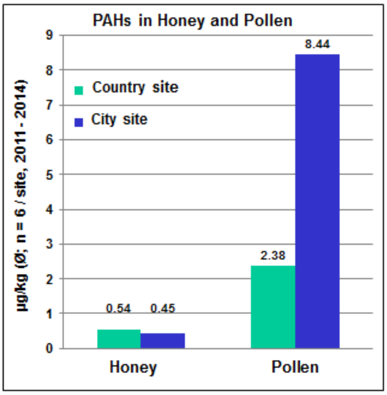

Fig. 3: Polycyclic Aromatic Hydrocarbons (PAH) in honey and pollen samples, e.g. Anthracene, Chrysene (n = 6 / test site)

PAHs and Heavy Metals were found more frequent in pollen samples than in honey samples (Fig. 3 + 4). MRL of PAHs (10 µg / kg) was not exceeded, the concentrations of PAHs analysed at the city site were higher than at the country site (Fig. 3). Manganese showed the greatest difference between country and city site regarding Heavy Metals (Fig. 4). MRL of lead (0.01 mg / kg) was exceeded in pollen at both sites, but higher at city site (Fig. 4).

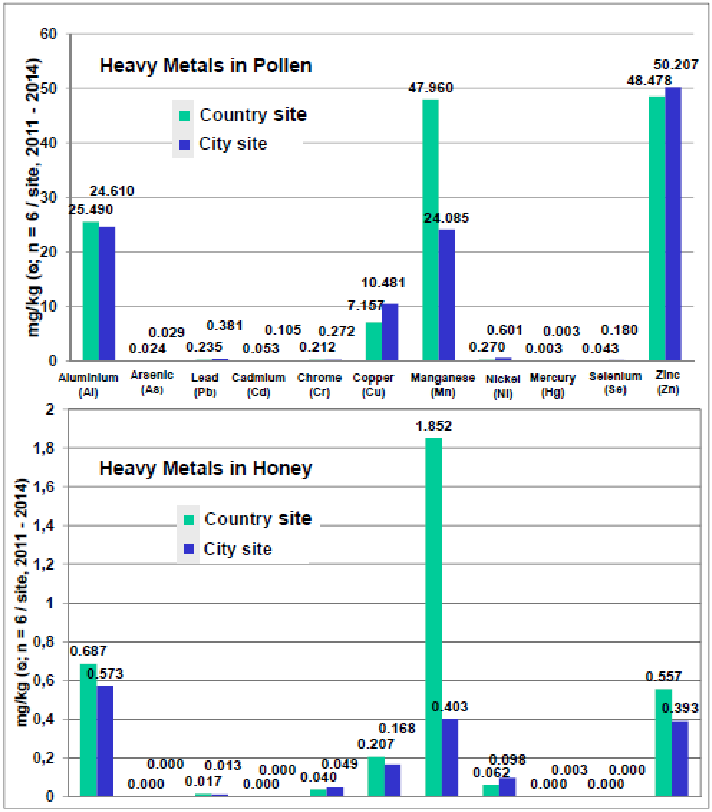

Fig. 4: Heavy metals in pollen and honey samples (n = 6 / site)