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Determination of Pesticides in Bees and Pollen

by Liquid and Gas Chromatography coupled to Mass Spectrometry

Screening study of 14 honeybee communities in southern Sweden

Summary

- Analytical methods for 99 different pesticides developed
- Single-bee analysis possible if needed
- Typical detection limits were 0.01 - 2 ng/g bee or pollen
- Large variability in lipid content between samples
- 21 pesticides detected in bees and 26 pesticides in pollen
- Up to 13 different pesticides detected in a single bee sample and 16 pesticides in a single pollen sample
- The highest concentrations were found for two fungicides, azoxystrobin and prochloraz
- The neonicotinoid insecticides acetamiprid, clothianidin and thiacloprid were detected in both bee and pollen samples at low ng/g levels

Methods

Sample collection

Bees (foragers) and pollen (in pollen combs) were collected from 14 hives in 8 different sampling sites in southern Sweden, see map, on two occasions during 2012. From site 1, pollen was also collected from foraging bees by using a trap mounted on the hive entrance. All samples were stored at -20°C pending analysis.

Sample size

The bee method was based on four bees per sample. For better representativeness of average exposure to the bee community, a four-bee fraction from a homogenate of a larger number of bees may be used. To investigate individual exposure the method can be downscaled to a single bee. The pollen method used a sample size of approximately 0.25 gram.

Sample preparation

Each bee and pollen sample resulted in two final extracts, one for liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and one for gas chromatography (GC) coupled to single MS analysis.

The sample preparation included:

- weighing of 16 bees or 0.25 g pollen in 50 ml Falcon tubes
- homogenization with dry Na_2SO_4 (5x sample weight) using a glass rod
- extraction with ethyl acetate under strong ultra-sonication (Sonics VCX130 with 3 mm tip). Bees: fraction corresponding to four bees used
- dispersive solid phase extraction with C18 and PSA (primary secondary amine), split in two sub-extracts
- evaporation and re-dissolution with acetonitrile for LC-MS/MS analysis and cyclohexane:aceton 9:1 for GC-MS analysis

Mass spectrometric detection

All instrumentation was from Agilent technologies.

For each compound one ion was used for quantitation and one or two ions for identity verification (qualifier ions).

LC-MS/MS (ES+): 10 µl injected on a trap system with Strata C18 and Strata X in series. Back flush into an Eclipse C18 chromatography column. Methanol gradient in ammonium formate, pH 4.

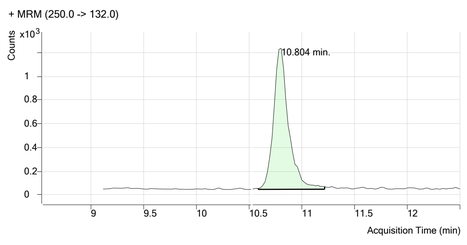
GC-MS (NCI): 2 µL injected on a temperature programmable injector with glass wool in the liner. Column: HP-5MS UI.

Calibration

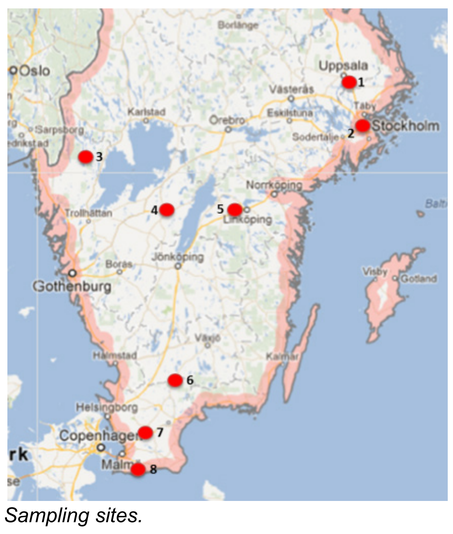
In this study calibration samples to produce calibration curves were prepared in the homogenized bee and pollen matrices and prepared in the same way as the study samples.

Example of LC-MS/MS chromatogram:

LC-MS/MS chromatogram showing 1.9 ng/g of clothianidin (a neonicotinoid insecticide) in a bee sample from location 1, collected in July.

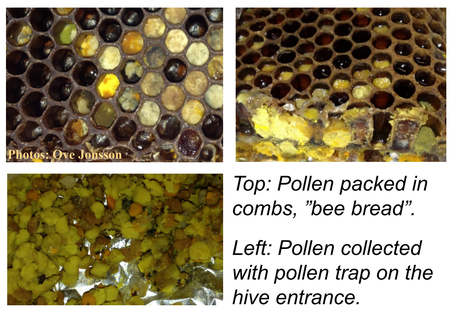


Bee on yellow melilot.



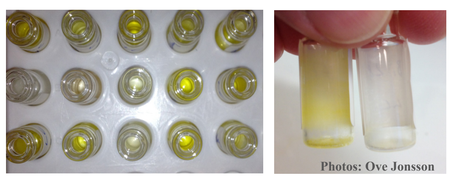
Sampling sites.

Foraging bees collected at the hive entrance. Each bee weigh approximately 0.1 gram. Pollen was gently removed before analysis.



Top: Pollen packed in combs, "bee bread".

Left: Pollen collected with pollen trap on the hive entrance.



Evaporation residues of bee extracts indicate large variation in lipid content.

Bees at work.

Results and Discussion

Identified pesticides in bees and pollen
In total 30 pesticides were detected, see Table 1 below.

Location and time point
Both the intensity in number of compounds and in concentration followed the intensity in farming, with clear dominance for the heavily farmed locations 7 and 8, as well as the early sampling occasion over the later one, see Table 1 and Table 2.

Analytical robustness
These very complex and lipophilic matrices were not easily analysed with GC and analytical runs had to be split in smaller sub-batches with cleaning in-between. The LC system was stable over hundreds of injections.

Matrix aspects
Lipids: Large differences in lipid content were observed in both bee and pollen samples, as shown in the photo at the bottom.
Pollen: Due to difficulties to isolate pollen from the combs, the so called bee bread, it is suggested to collect pollen with a trap mounted on the hive entrance. This is less invasive and gives a freshly collected pollen sample.

Table 1. Frequency and highest concentrations of pesticides in bee and pollen samples

Compound	Bees no. of detections	Highest conc. in bees (ng/g)	Pollen no. of detections	Highest conc. in pollen (ng/g)
acetamiprid (I)	8	1.4	3	2.1
atrazine (H)	-	-	1	<LOQ
azoxystrobin (F)	9	42	8	309
carbendazim (F, D)	15	1.8	6	0.71
chlorfenvinphos (I)	2	12	-	-
chloridazon (H)	4	2.2	5	1.4
chlorpyrifos (I)	-	-	1	2.1
clothianidin (I)	5	1.9	2	3.5
desmedipham (H)	4	5.1	5	15
ethofumesate (H)	-	-	1	<LOQ
fenpropidin (F)	1	0.082	-	-
fenpropimorph (F)	3	14	4	5.6
hexythiazox (I)	-	-	4	4.7
isoproturon (H)	3	0.067	-	-
metamitron (H)	3	1.9	5	4.7
metribuzin (H)	-	-	1	<LOQ
phenmedipham (H)	5	0.65	5	1.7
picoxystrobin (F)	2	0.17	6	0.83
pirimicarb (I)	-	-	1	<LOQ
prochloraz (F)	2	3.7	2	407
prosulfocarb (H)	-	-	8	2.9
prothioconazole-desthio (F, D)	4	1.8	4	<LOQ
pyraclostrobin (F)	7	1.4	4	<LOQ
tau-fluvalinate (I)	1	<LOQ	6	34
terbutryn (H)	1	3.8	-	-
terbuthylazine (H)	-	-	2	1.0
terbuthylazine desethyl (H, D)	5	0.28	2	<LOQ
thiacloprid (I)	10	0.6	6	10
tolylfluanid (F)	2	<LOQ	2	<LOQ
trifloxystrobin (F)	-	-	3	0.33

F=fungicide, H=herbicide, I=insecticide, D=degradation product,
- not detected, <LOQ=detected but under stated limit of quantification

Table 2. Frequency and total exposure in bees from different locations

Sampling site no. and hive ID	No. of detections	Total exposure (ng/g)	No. of detections	Total exposure (ng/g)
	May/June sampling		July sampling	
1 A	2	5.7	6	7.7
1 B	1	0.2	2	1.4
2	1	12	1	0.04
3 A	0	0	1	0.01
3 B	1	0.2	1	0.08
4 A	1	0.07	2	1.1
4 B	1	3.8	2	0.4
5 A	2	2.9	1	0.04
5 B	1	1.4	2	0.4
6	1	0.01	2	0.1
7 A	13	12	5	2.3
7 B	13	48	7	3.7
8 A	13	22	2	0.08
8 B	9	9.8	3	1.4

Analytical challenges to address

- Investigate compound stability in the frozen bee matrix
- Investigate variability between individual bees from the same hive
- Investigate variability within a homogenate of a large number of bees
- Enhance GC-MS robustness
- Develop method for other compound classes, e.g. acids
- Increase sample throughput