Chemosphere 188 (2017) 389–396

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Are pesticide residues in honey related to oilseed rape treatments?

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HIGHLIGHTS

- The amount of pesticide residues in honey can vary largely between the years.
- The residues in honey tend to be connected to those used in oilseed rape fields.
- Clopyralid and glyphosate residues prevailed in honey samples.
- The concentrations found do not pose any health risk to consumers.
- The concentrations probably do not cause any acute toxicity to honey bees.

ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form
2 September 2017
Accepted 4 September 2017
Available online 4 September 2017

Handling Editor: I. Cousins

Keywords:
Honey
Pesticide residues
LC-MS/MS
GC-MS

ABSTRACT

Pesticide treatments before and during the flowering of honey bee forage crops may lead to residues in honey. In northern regions oilseed rape belongs to the main forage crops that is mostly cultivated by means of intensive agriculture, including several pesticide treatments. However, in addition to the focal forage crops, pesticides from non-forage crops can spread to wild flowers around fields, and thus the residues in honey would reflect the whole range of pesticides used in the agricultural landscape. The aim of our study was to clarify which currently used pesticides are present in honey gathered from heterogeneous agricultural landscapes after the end of flowering of oilseed crops.

Honey samples (N = 33) were collected from beehives of Estonia during 2013 and 2014, and analysed for residues of 47 currently used agricultural pesticides using the multiresidue method with HPLC-MS/MS and GC-MS and a single residue method for glyphosate, aminopyralid and clopyralid. Residues of eight different active ingredients with representatives from all three basic pesticide classes were determined. Although no correlation was detected between the cumulative amount of pesticide residues and percent of oilseed crops in the foraging territory, most of the residues are those allowed for oilseed rape treatments. Among all pesticides, herbicide residues prevailed in 2013 but not in 2014. Despite the relatively small agricultural impact of Estonia, the detected levels of pesticide residues sometimes exceeded maximum residue level; however, these concentrations do not pose a health risk to consumers, also acute toxicity to honey bees would be very unlikely.

1. Introduction

Using honey as natural food sets high demands on its quality. However, honey production occurs hand-in-hand with agricultural activities, and pesticide residues have been detected in honeys from several countries at varying levels, sometimes even exceeding the maximum residue levels (MRL) allowed (Souza Tette et al., 2016).

Pesticides can enter beehives via several routes. Hive treatments using medical products to combat honey bee parasites and

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http://dx.doi.org/10.1016/j.chemosphere.2017.09.013
0045-6535/

pathogens bring about residues in wax and other bee products (Kujawski and Namieśnik, 2011; Nakajima et al., 2015). Honey bees collect pollen and nectar from treated crops: they might not avoid freshly treated fields even if the product used has been labelled as being repellent to bees (Karise et al., 2007). Foraging outside the fields may also result in contaminated food resource through spray drift from fields to wild vegetation (Long and Krupke, 2016). It has been convincingly demonstrated that pesticides used on fields can drift a long way to neighbouring areas (Krupke et al., 2012; Hladik et al., 2016; Long and Krupke, 2016), thus contaminating the pollen and nectar of wild flowers, which in turn may lead to contaminated honey production even in organic apiaries, as described in Italy (Chiesa et al., 2016). In addition to currently used pesticides, field soil tends to retain many chemicals used throughout (Kumar et al., 2016; Lozowicka et al., 2016; Zhang et al., 2016), and moreover, traces may occur in every plant product including nectar and pollen (Malhat et al., 2015; Chiesa et al., 2016).

Analyses of pesticide residues in honey have been carried out in several countries (reviewed by Souza Tette et al., 2016). An important set of studies analysed honey for contamination by organochlorines, many of which are banned (Panseri et al., 2014; Al Naggar et al., 2015; Chiesa et al., 2016). However, although this data is interesting, it does not lead to any understanding of the consequences of those pesticides used nowadays when pyrethroids and neonicotinoids are becoming more and more popular. We know of no up-to-date survey on honey contamination by a broader spectrum of currently used pesticides. For Nordic areas, there is only one study which analyses honey and this considers the presence of four pesticide residues of neonicotinoid insecticides (Laaniste et al., 2016), where it is shown that the frequency of pesticide residues in honey was correlated with the year-wise increase in product importation.

As in other Nordic countries, the pesticide input into Estonian agriculture is relatively low, being less than 1 kg ha^{-1} of utilised agricultural area (Eurostat, 2015), whereas in most Central European countries like France, Germany, Belgium and the Netherlands, the amount of pesticides sold is over 2 kg ha^{-1}. In Estonia, more than half of the country's territory is covered with forests and other wooded lands (Eurostat, 2015). This, and the low pesticide input, makes people assume that the nectar from wild and presumably unpolluted flowers should dilute the nectar from cultivated plants to a level where residues are no longer detectable.

Estonian honey is polyfloral; however, Brassicaceae pollen belongs to the four most common plant species found from honey samples (Puusepp and Koff, 2014). Most of the Brassicaceae pollen probably belongs to cultivated oilseed crops, from which most are grown by means of conventional agricultural methods. The pesticide treatment suggested for oilseed rape starts with soil preparation using herbicides, followed by sowing dressed seed to protect the seedlings against fungal diseases and flea beetles. Later, several treatments against other insect pests and phythopathogens are suggested. As pre-harvest treatment, glyphosate is suggested to reduce harvesting losses. Due to the large content of Brassicacea pollen in Estonian honey (Puusepp and Koff, 2014), we hypothesize that the possible residues found in honey reflect those used in oilseed rape agrotechnology. However, there is evidence that different groups of pesticides are correlated differently with forage crops in foraging ranges of honey bees (McArt et al., 2017). Therefore, we aimed to clarify which of the currently used 50 pesticides are present in honey gathered from heterogeneous agricultural landscapes after the flowering of oilseed crops.

2. Material and methods

2.1. Study location

Honey samples were gathered from Eastern and Southern Estonia (Ida-Viru, Tartu, Põlva and Valga Counties) in 2013 (N = 14) and 2014 (N = 19). This area is representative of typical agricultural landscapes in Estonia with mostly intensively managed fields, forested areas and human settlements. Among other field crops, both winter and spring oilseed rape are often grown in Estonia, and both belong to the common forage crops of honey bees. Within a 2 km radius of each hive there is on average 34.6 ± 20.7% cultivated land (min. 0.81%, max. 70.2%), 48.1 ± 20.6% forest, 5.3 ± 7.6% waste and vacant land, 7.6 ± 5.0% grassland and 2.1 ± 3.6% garden. The average oilseed crop coverage within the foraging territory remained between 0 and 12.9%.

2.2. Pesticide selection

The 47 active ingredients analysed were selected for the survey as being the most commonly used in Estonian fields according to the pesticide ordering lists of the Tartu County Farmers Association for the year 2013–2014. These include the most commonly used contemporary herbicides (21), fungicides (15) and insecticides (10), and plant growth regulator and retardant (1). The active ingredients searched for were: 2,4D, alpha-cypermethrin, amidosulphuron, aminopyralid, azoxystrobin, clopyralid, cypermethrin, cyproconazole, deltamethrin, dicamba, dimethachlor, dimethoate, ethyl trinexapac, fenoxaprop-p-ethyl, fenpropidin, florasulam, fludioxonil, fluoxastrobin, flutriafol, fuberidazole, glyphosate, imazalil, imidacloprid, indoxacarb, iodosulfuron-methyl-sodium, lambda-cyhalotrin, MCPA, mefenpyr-diethyl, pencycuron, picloram, pinoxaden, prochloraz, propaquizafop, propiconazole, propoxycarbazone-sodium, prothioconazole, pymetrozine, pyroxsulam, quizalofop-p-ethyl, spiroxamine, sulfosulfuron, tau-fluvalinate, tebuconazole, thiacloprid, triadimenol, triasulfuron and tribenuron-methyl.

2.3. Sample collection and handling

A total of 33 honey samples were collected from beehives in the eastern and southern part of Estonia (Tartu County and its near vicinity) during 2013 and 2014 for analysis of pesticide residues. Each honey sample originated from a different apiary, each of which consisted of 10–20 honey bee hives. The sampled hive was selected randomly for testing. The distance between sampled apiaries was at least 4 km in 2013 and at least 8 km in 2014 to preclude overlapping of the main forage area. The samples were gathered from honeycombs within beehives during the honey harvest in mid-July after the end of oilseed rape flowering. Due to the funding allocated for this study, it was decided to concentrate only on honey samples, and in order to cover more apiaries from the largest possible territory, we sampled only one hive per apiary. The honey was extracted from the comb wax and thereafter kept at 5 °C until analysis.

2.4. Chemicals and materials

The reference standards of pesticides were purchased from AccuStandard (New Haven, USA) and Dr. Ehrenstorfer (Germany). HPLC grade acetonitrile and methanol were purchased from Merck-Millipore (Darmstadt, Germany). ACS grade formic acid (≥96.0%), acetic acid (glacial, ≥99.85%), and ammonium formate (99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure deionised water was generated by a Millipore Milli-Q™ system

(Billerica, MA, USA). A buffer-salt mixture (1 g trisodium citrate dihydrate, 1 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate and 4 g of anhydrous magnesium sulphate) and a mixture of dSPE (900 mg anhydrous magnesium sulphate, 150 mg PSA and 150 mg C18E) were obtained from Phenomenex (Torrance, CA, USA).

Stock solutions of approximately 1000 mg L^{-1} concentration were prepared by weighing 10 mg of standard in a 10 mL graduated flask and dissolving it in acetonitrile. The purity of the standard was taken into account in the preparation of standard solutions of final concentration. The mix of working standard solution with a concentration of 0.01 mg L^{-1} was prepared by diluting the appropriate volume of stock solution in acetonitrile. The stock and working standard solution were stored at −20 °C.

2.5. Sample preparation

Different sample extraction and detection procedures were used for analysis of the selected pesticides. Most compounds were analysed using QuEChERS extraction methodology followed by detection using GC-MS and UHPLC-MS/MS. Analysis of glyphosate, aminopyralid and clopyralid was performed as single analyses using extraction with methanol.

5.0 ± 0.1 g of the sample was weighed into a 50 mL polypropylene centrifuge tube. For calibration and quality control samples, the standard solutions were added at the appropriate spiking level. Deionised water (10 mL) and acetonitrile (10 mL) were both added and the tubes were shaken vigorously by hand for 1 min. Then a salt mixture of 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate was added, the tubes were closed and immediately shaken by hand for 1 min and centrifuged for 5 min at 4500 rpm. An aliquot of 8 mL of supernatant was transferred into a 15 mL PP centrifuge tube and frozen out at −80 °C for 30 min using a Heto Ultra freeze (Thermo Fisher Scientific, USA), followed by centrifugation of the resulting organic sample fraction for 5 min at 4500 rpm. For pesticides with acidic groups that interact with amino sorbents such as PSA, an aliquot of 250 μL of the raw extract was mixed with 500 μL of the mobile phase A (5 mM ammonium formate and 0.1% formic acid in water) and analysed by UHPLC-MS/MS using negative electrospray ionisation mode. For further clean-up procedure, 6 mL of extract was transferred into 15 mL PP tubes containing 900 mg anhydrous magnesium sulphate, 150 mg PSA and 150 mg C18E. The tubes were shaken vigorously for 30 s and centrifuged for 5 min at 4500 rpm. For analysis with GC-MS, 5 mL of cleaned extract were evaporated in a water bath (40 °C) under a gentle nitrogen stream. The samples were reconstituted in 100 μL of acetonitrile and transferred into screw cap vials with inserts. For UHPLC-MS/MS analysis using ESI in positive ionisation mode, an aliquot of 250 μL of cleaned extract was mixed with 500 μL of the mobile phase A. When final sample extracts were misty, they were filtered through 0.22 μm PVDF centrifuge filters before transferring them into autosampler vials for analysis.

For analysis of glyphosate, aminopyralid and clopyralid, 5.0 ± 0.1 g of samples were weighed into a 50 mL polypropylene centrifuge tube, then 10 mL of water and 10 mL of methanol were added for extraction. The samples were shaken for 20 min and centrifuged for 10 min at 4500 rpm. An aliquot of extract was transferred to an autosampler vial for analysis by UHPLC-MS/MS.

2.6. GC-MS analysis

The sample extracts in acetonitrile were analysed on an Agilent HP 6890 gas chromatograph coupled with an HP 5973 mass spectrometer (Agilent Technologies, CA, USA) operating in SIM mode (Table 1). The capillary column used was Agilent DB-5MS (30 m × 0.25 mm × 0.25 μm). Operating conditions: the carrier gas was helium at a constant flow rate of 1.2 mL min^{-1}, injector temperature of 250 °C and the interface temperature was 250 °C. The initial oven temperature was 60 °C (held for 2 min), then increased to 150 °C at a rate of 30 °C min^{-1} (held for 2 min), then increased to 240 °C at a rate of 3 °C and held for 2 min, afterwards increased to 270 °C at a rate of 10 °C and held for 30 min. The total analysis time was 72 min. Injection volume was 1 μL.

2.7. UHPLC-MS/MS analysis

An Acquity UHPLC system (Waters, USA) coupled to QTrap 5500 (AB SCIEX, USA) equipped with an electrospray ionisation source was used for the analysis of pesticides in honey. The parameters of the ion source were as follows: source temperature was set at 500 °C, ion spray voltage 5.00 kV for positive ionisation mode and −4.50 kV for negative, curtain gas nebulizer 45 psi, ion source gas 1 (GS_1) 40 psi, and ion source gas 2 (GS_2) 60 psi. The analysis was performed by multiple reaction monitoring (MRM) in the positive and negative ionisation modes. Table 2 lists the analyte dependent parameters − MRM transitions, collision energies (CE) and declustering potential (DP). The control of the instrument conditions and the data processing were performed using Analyst 1.6 software (AB SCIEX, USA).

Chromatographic separation for most pesticides (except glyphosate, aminopyralid and clopyralid) was performed on a Kinetex C18 analytical column (50 × 3.0 mm, 1.7 μm) from Phenomenex. The mobile phase (A) consisting of 5 mM ammonium formiate and 0.1% formic in water and (B) acetonitrile was delivered at the flow rate of 0.4 mL min^{-1}. A gradient program was used: 20% of mobile phase (B) was used from 0 to 1.0 min, 20% (B) to 90% (B) from 1.0 to 10.0 min, maintained at 90% (B) for 1 min, then decreased back to 20% (B) at 11.0 min and finally the column was re-equilibrated with 20% (B) from 11.0 to 15.0 min. An aliquot of 10 μL of the extract was injected. The column and autosampler were maintained at 30 °C and 10 °C, respectively.

Aminopyralid and clopyralid were analysed on a Luna SCX analytical column (50 × 4.6 mm, 5 μm) from Phenomenex. The mobile phase (A) consisted of 5 mM ammonium formiate and (B) methanol was delivered at the flow rate of 0.6 mL min-1with isocartic elution mode (40% of A and 60% of B). The time of analysis was 5 min, the injection volume was 10 μL and the column and autosampler were maintained at 30 °C and 10 °C, respectively.

Glyphosate was analysed on a Hypercarb analytical column (100 × 2.1 mm, 5 μm) from Thermo Scientific (MA, USA). The mobile phase, consisting of 1% acetic acid in water, was delivered at the flow rate of 0.3 mL min^{-1}. The time of analysis was 10 min, the injection volume was 10 μL and the column and autosampler were maintained at 40 °C and 10 °C, respectively.

3. Results and discussion

3.1. Performance of the method

The performance of the method was evaluated according to the EC guidance document SANCO/12571/2013. The method showed good linearity with the determination coefficients, higher than 0.990 for all compounds included in the study. The mean variation of coefficients for repeatability of the method ranged from 3.0% to 16%, and the recovery ranged from 78% to 115%.

The limit of quantification (LOQ) for which the S/N ratio exceeds 10 was assumed at a concentration level of 0.010 mg kg^{-1} for all pesticides with the exception of aminopyralid, clopyralid,

Table 1
Acquisition parameters for the selected pesticides analysed by GC-MS.

Analyte	Ions selected for monitoring (*m*/*z*)	Retention time (min)
Cypermethrin I	163, 181, 165, 91	42,02
Cypermethrin II	163, 181, 165, 91	42,28
Cypermethrin III (alpha)	163, 181, 165, 91	42,35
Cypermethrin IV	163, 181, 165, 91	42,49
Deltamethrin	181, 253, 251, 255	46,25
Indoxacarb	218, 150, 203, 264	46,04
Lambda-cyhalothrin	181, 197, 208, 141	37,15
tau-Fluvalinate I	250, 252, 181, 251	44,45
tau-Fluvalinate II	250, 252, 181, 251	44,69
Trinexapac-ethyl	151, 224, 251, 95	19,46

Table 2
Analyte depended parameters for the analysis of pesticide residues in honey with LC-MS/MS.

Analyte	Ionisation mode	Declustering potential (V)	Multiple reaction monitoring 1 (*m*/*z*)	Collision energy (V)	Multiple reaction monitoring 2 (*m*/*z*)	Collision energy (V)
2,4-D	ESI -	−50	219 > 161	−16	219 > 125	−36
Amidosulphuron	ESI +	50	370 > 218	20	370 > 261	35
Aminopyralid	ESI +	50	207 > 189	25	207 > 161	40
Azoxystrobin	ESI +	50	404 > 372	21	404 > 344	35
Clopyralid	ESI +	50	192 > 146	46	192 > 110	46
Cyproconazole	ESI +	50	292 > 70	33	292 > 125	37
Dicamba	ESI -	−50	221 > 177	−10	219 > 175	−8
Dimethachlor	ESI +	50	256 > 224	20	256 > 148	35
Dimethoate	ESI +	50	230 > 125	29	230 > 199	15
Fenoxaprop-p-ethyl	ESI +	50	362 > 288	28	362 > 121	40
Fenpropidin	ESI +	50	274 > 147	40	274 > 86	40
Florasulam	ESI +	50	360 > 129	30	377 > 129	30
Fludioxonil	ESI -	−50	247 > 180	−42	247 > 126	−50
Fluoxastrobin	ESI +	50	459 > 427	30	461 > 429	25
Flutriafol	ESI +	50	302 > 123	39	302 > 109	43
Fuberidazole	ESI +	50	185 > 157	40	185 > 65	50
Glyphosate	ESI -	−50	168 > 63	−20	168 > 150	−16
Imazalil	ESI +	50	297 > 159	31	299 > 161	29
Imidacloprid	ESI +	50	256 > 209	21	256 > 175	19
Iodosulfuron-methyl	ESI +	50	508 > 167	20	508 > 235	30
MCPA	ESI -	−50	199 > 141	−20	201 > 143	−20
Mefenpyr-diethyl	ESI +	50	390 > 373	10	390 > 327	20
Pencycuron	ESI +	50	329 > 125	26	329 > 218	24
Picloram	ESI -	−50	239 > 195	−15	241 > 197	−15
Pinoxaden	ESI +	50	401 > 317	32	401 > 57	45
Prochloraz	ESI +	50	376 > 308	20	378 > 310	20
Propaquizafop	ESI +	50	444 > 371	24	444 > 100	24
Propiconazole	ESI +	50	342 > 159	43	342 > 69	33
Propoxycarbazone	ESI +	50	416 > 399	15	416 > 199	25
Prothioconazole	ESI -	−50	342 > 100	−32	342 > 125	−38
Pymetrozine	ESI +	50	218 > 105	25	218 > 51	75
Pyroxsulam	ESI +	50	435 > 195	30	435 > 124	70
Quizalofop-p-ethyl	ESI +	50	373 > 299	30	373 > 271	40
Spiroxamine	ESI +	50	298 > 144	29	298 > 100	49
Sulfosulfuron	ESI +	50	471 > 211	20	471 > 261	30
Tebuconazole	ESI +	50	308 > 70	39	308 > 125	47
Thiacloprid	ESI +	50	253 > 126	29	253 > 99	57
Triadimenol	ESI +	50	296 > 70	15	298 > 70	15
Triasulfuron	ESI +	50	402 > 167	25	402 > 141	30
Tribenuron-methyl	ESI +	50	396 > 181	20	396 > 155	20

glyphosate, dicamba and picloram for which the LOQ was 0.050 mg kg^{-1}.

3.2. Analysis of the honey samples

The amounts and composition of the pesticide residues found in the honey samples differed between years (Table 3). The agricultural practices generally do not vary so much, but the need for different kinds of pesticides can vary widely from year to year. The proportions of samples with at least traces of any particular pesticide were comparable, being 78% in 2013 and 63% in 2014 ($Chi2 = 0.16$; $df = 1$; $p = 0.69$), but the composition and the average cumulative amount of chemicals per sample was significantly higher in 2013 than in 2014 ($KW{-}H(1; 23) = 5.9$; $p = 0.015$) (Fig. 1). In 2013, five different compounds were found in the honey samples; herbicides formed a major part: clopyralid was found in 64% of samples (twice above MRL) and glyphosate in 21% (twice above MRL), whereas all glyphosate was always accompanied by clopyralid. The other compounds found in the samples in 2013 were insecticides: dimethoate, thiacloprid and tau-fluvalinate, all amounts

Table 3
The concentrations (μg kg^{-1}) of pesticide residues found in honey samples in Estonia 2013–2014.

Honey sample	Year	% of oilseed rape in foraging range	Herbicide			Fungicide		Insecticide		Insecticide, acaricide	Gross amount	No. of different compounds	
			Clopyralid	2,4D	Glyphosate	Tebuconazole	Azoxystrobin	Dimethoate	Thiacloprid	Tau-fluvalinate		Total	>LOD
1	2013	3.4	n.d.	n.d.	n.d.	n.d.	n.d.	(4)	n.d.	n.d.	4	1	0
2	2013	5.7	**272**	n.d.	14	n.d.	n.d.	n.d.	n.d.	n.d.	286	2	2
3	2013	6.2	48	n.d.	**56**	n.d.	n.d.	n.d.	n.d.	n.d.	104	2	2
4	2013	12.1	29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29	1	1
5	2013	10	29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29	1	1
6	2013	9.2	30	n.d.	n.d.	n.d.	n.d.	n.d.	(1)	n.d.	31	2	1
7	2013	12.9	(6)	n.d.	**62**	n.d.	n.d.	n.d.	n.d.	n.d.	68	2	1
8	2013	9.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
9	2013	9.1	27	n.d.	n.d.	n.d.	n.d.	(5)	n.d.	(1)	33	3	1
10	2013	14	**91**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	91	1	1
11	2013	8.6	16	n.d.	n.d.	n.d.	n.d.	(1)	n.d.	n.d.	17	2	1
12	2013	5.1	n.d.	n.d.	n.d.	n.d.	n.d.	(1)	n.d.	n.d.	1	1	
13	2013	9.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
14	2013	9.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Average (μg kg^{-1})		**8.86%**	**60.9**		**44**			**2.8**	**1**	**1**	**21.2**		
15	2014	0	n.d.	n.d.	n.d.	(2)	n.d.	n.d.	n.d.	n.d.	2	1	
16	2014	8.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	14	n.d.	14	1	1
17	2014	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13	n.d.	13	1	1
18	2014	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
19	2014	0	n.d.	n.d.	n.d.	(5)	n.d.	n.d.	(9)	n.d.	14	2	
20	2014	2.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
21	2014	8.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
22	2014	2.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
23	2014	11.6	n.d.	n.d.	(9)	n.d.	n.d.	n.d.	n.d.	n.d.	9	1	
24	2014	12.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
25	2014	8.9	n.d.	(2)	n.d.	n.d.	n.d.	n.d.	(5)	(7)	14	3	
26	2014	13.3	n.d.	n.d.	n.d.	n.d.	n.d.	(1)	n.d.	n.d.	1	1	
27	2014	11.6	n.d.	n.d.	n.d.	n.d.	n.d.	(2)	n.d.	(6)	8	2	
28	2014	1.8	n.d.	n.d.	n.d.	n.d.	n.d.	(3)	n.d.	n.d.	3	1	
29	2014	5.9	n.d.	n.d.	n.d.	(5)	n.d.	n.d.	(7)	(6)	18	3	
30	2014	5.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
31	2014	8.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
32	2014	3.5	n.d.	n.d.	n.d.	n.d.	**31**	(3)	n.d.	n.d.	34	2	1
33	2014	4.9	n.d.	n.d.	n.d.	n.d.	n.d.	(3)	n.d.	(8)	11	2	
Average (μg kg^{-1})		**6.08%**		**2**	**9**	**4**	**31**	**2.4**	**9.6**	**6.8**	**11.8**		
% of samples	**2013**		**64**	**0**	**21**	**0**	**0**	**29**	**7**	**7**	**78**		
	2014		**0**	**5**	**5**	**16**	**5**	**26**	**26**	**21**	**63**		

() The numbers in parenthesis represent values under the limits of detection (LOD). The numbers in **bold** represent values above the maximum residue limits (MRL).

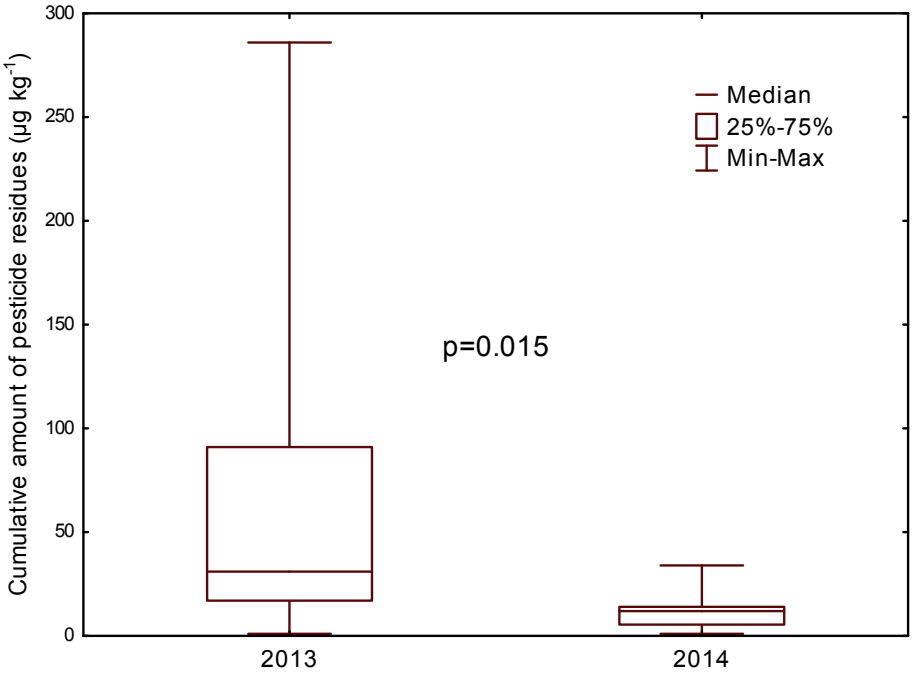

Fig. 1. Gross amount of pesticide residues calculated over all positive samples in 2013 and 2014. The Kruskal-Wallis test was used to compare median values.

remained below LOD. In 2014, however, seven different compounds were found, of which two were herbicides (2,4D and glyphosate), two were fungicides (tebuconazole and azoxystrobin) and the same three insecticides were present as in the previous year. In 2014, the amounts of pesticide residues found were much lower than in 2013, remaining between 1 and 9 µg kg^{-1} staying below the LOD, except in one sample where the fungicide azoxystrobin was found in a concentration of 31 µg kg^{-1} which is also above the MRL. In both years, the numbers of different compounds per sample stayed between 0 and 3. Although some detected pesticide residues exceeded the MRL set in Europe, these amounts still do not pose a health risk to honey consumers since the numbers remain far below the hazard index (Juan-Borrás et al., 2016). The MRLs of most pesticide residues in honey are fixed according to their lowest detection level, which means that the compounds are not allowed to contaminate the honey.

The Spearman rank order correlation did not reveal significant correlations between the cumulative amount of pesticide residues and the proportion of cultivated land ($p = 0.17$) or the proportion of oilseed rape ($p = 0.15$) in the territory within a 2 km foraging radius of honey bees. However, all except two of the pesticide residues found in the honey samples can be related to oilseed rape (Estonian Agricultural Board, 2017), which indicates the high spreading capability of pesticide residues from the fields as a potential source of contamination. The herbicide 2,4D is not allowed for weed control in oilseed rape fields, and in our study it was found only once in concentration below LOD. The herbicide clopyralid is allowed for spraying on rape plants until the formation of flower buds. Glyphosate is used before the germination of rape seed or as pre-harvest treatment against many weeds. These two herbicides, however, are commonly used in agrotechnology of many different crops. In addition, glyphosate is also sprayed to combat herbaceous grass during summer maintenance of larger roads, in greenery works in towns and cities, and also by the owners of private gardens (Estonian Agricultural Board, 2017). This means that there are several routes for glyphosate to end up in nectar collected by honey bees. The two fungicides (azoxyztrobin and tebuconazole) found in this survey are commonly used on oilseed rape against a complex of fungal diseases, and both are allowed to be sprayed during the whole flowering period. These preparations are only meant for professional pesticide users. The insecticide dimethoate is not allowed for controlling insect pests in oilseed rape cultivation in Estonia. However, this is a highly effective compound and is often used on other crops. Thiacloprid is a systemic insecticide, which is allowed to be sprayed until the full flowering of oilseed crops. The systemic nature of this compound allows it to persist in plant tissues for a long time. It is transmitted from leaves to nectar and pollen, and is thus easily attainable for foraging bees. Tau-fluvalinate, a contact insecticide, is also allowed to be sprayed against oilseed rape pest insects during flowering. Tau-fluvalinate is considered to be relatively safe for bees due to its high value of LD_{50}, which makes it possible to use the same active ingredient as varroacide inside honey bee hives. Therefore there are two different routes for how tau-fluvalinate can end up in honey (Tremolada et al., 2011), unfortunately we are not able to distinguish between them.

Honey as a product contains surprisingly few pesticide residues compared to bee bread or pollen (Thompson et al., 2014). Pesticide residues in different matrixes differ in their chemical composition and physical characteristics. Fat or lipid soluble compounds tend to contaminate wax, whereas water-soluble compounds are more readily found in nectar or honey. Besides contaminated nectar, honey contamination may also occur via translocation of the compounds from comb wax to honey (Kochansky et al., 2001; Tremolada et al., 2004).

The relatively large areas with natural vegetation, and the low amounts of pesticides used in Estonian agriculture (Eurostat, 2015) has shaped the notion that the bee forage environment should be unpolluted in Estonia and probably also in other Nordic countries. Our results, however, suggest the situation may be of concern. Despite the general low input of pesticides compared to the average usage over the European countries (Eurostat, 2015), some compounds found in honey samples exceeded the MRL. On the background of landscape characteristics, this might arise from relatively

homogeneous land cover type – in Estonia, as in Ireland and the United Kingdom, the landscape in 2015 is dominated by larger areas composed of the same land cover type, also the number of structural green elements in the landscape is small (Eurostat, 2015). Larger forest areas may serve as barriers for bees, for instance. Forests have been shown to negatively affect bumble bees with larger foraging territories (Diaz-Forero et al., 2011). Such barriers may concentrate bees on other land, thus increasing the risk of forage on polluted plants. Honey bees prefer to forage in larger open areas rich in flowers, and flowering crops make up an important part of the forage. Since it is one of the most profitable crops, oilseed rape crops are common in crop rotations: covering 15% and 11% of total cultivated land in 2010 and 2015 accordingly (Statistics Estonia, 2012).

In northern regions, the most common group of pesticides sold are herbicides: these comprise more than 70% of pesticides sold in Estonia (Eurostat, 2015). The higher amounts of herbicide active ingredients needed for effective treatments compared to insecticides, for instance, may also be one reason why herbicide residues in particular were higher in our samples. The amounts of herbicides used on fields may differ from year to year depending on the weather conditions throughout the spring and summer. The amounts of herbicides sold in Estonia were higher in 2013 compared to 2014 (Eurostat, 2015) and this appears to have been reflected in our honey samples. Although pesticide residues may be retained in soils from the previous year or even from treatments made decades ago (Hilber et al., 2008; Lozowicka et al., 2016), the authors believe this probably did not affect our results because the samples with higher concentrations in 2013 did not show higher residue level in 2014. Most of the locations sampled in 2013 were also sampled in 2014. We suppose that in those cases where we found herbicide residues higher than the MRL, the bees must have foraged on recently treated fields. For instance, glyphosate residues may remain very high in nectar for up to seven days after treatment, as demonstrated by Thompson et al. (2014). Glyphosate-based herbicides are the most common herbicides worldwide. Moreover, its usage nowadays has gone beyond pest control purposes – being more of an agricultural instead of a pest management tool (Steinmann et al., 2012). We believe that this is something to consider for reducing the levels of pesticide residue found in food: by excluding the routine spray applications and retaining the weed management purpose of glyphosate, one could facilitate a less polluted environment.

The concentrations of all residues found from honey samples in this study remained below the lethal dose to honey bees. LD_{50} is measured for 2,4D was 0.0115 mg bee^{-1} (Extension Toxicology Network, 1996), clopyralid >100 μg bee^{-1} (Dow AgroSciences, 2007) and glyphosate 100 μg bee^{-1} (Thompson et al., 2014), tebuconazole 83 μg bee^{-1}, azoxystrobin 200 μg bee^{-1}, dimethoate 0.11 μg bee^{-1}, thiacloprid 27.89 μg bee^{-1}, and tau-fluvalinlate 45 μg bee^{-1} (Sanchez-Bayo and Goka, 2014). This means that the concentrations found are definitely below acute lethal dosages, although sub-lethal effects cannot be excluded when considering that at least nurse bees consume the contaminated food until they produce the royal jelly, and also larger larvae are fed with nectar and pollen collected by foragers.

4. Conclusion

Our results demonstrate that intensively treated oilseed rape fields can be a source for pesticide residue contamination in honey, however no direct correlation was found. We believe that pesticides escape from fields over larger neighbouring areas with wild vegetation and contaminate the nectar of wild plants. Our study indicates that most of the agrochemical residues in Estonian honey can originate from oilseed treatments, however the same active ingredients are used for different crops, which is why no direct references can be made. The compounds that were represented in the highest amounts belonged to herbicides, the most frequently used pesticide group in Northern European climatic conditions. In the context of honey as human food, the concentrations of pesticide residues do not pose any health risk to consumers, although in some cases the levels detected exceeded the MRLs. Concerning the health of bees, the residues remained below acute lethality, however some sub-lethal effects cannot be excluded.

Author contribution

RK, RR, PP, IK, MM, HV conceived and designed the study, RK, RR, PP, IK collected data, VB, IP carried out pesticide analyses, RK; RR; IK; PP analysed the data, RK, RR, VB, IP, MM, HV, IHW wrote the paper, all authors read and approved the paper.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

This research was supported by the Institutional Research Funding (IUT36-2) of the Estonian Ministry of Education and contracts 8-2/T13055PKTK and 8-2/T13059VLLG of the Estonian Ministry of Rural Affairs. The authors are grateful also to Mr Peeter Tammisto for the language correction.

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