

THE PRESENCE OF LINDANE IN DIFFERENT TYPES OF HONEY IN THE PANNONIAN REGION

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Abstract

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Sixty samples of honey were collected in the Pannonian region of Serbia during the year 2013. Analytical procedure of sample preparation based on the Quechers method was used for determination of pesticide residue content, whereas the quantification of pesticide residue was performed on gas chromatography mass detector. The presence of organochloric pesticides was determined during the research. Lindane was the most frequent pesticide in all types of honey. 73% of lindane was found in sunflower honey of the total quantity of detected pesticides, 73% in meadow honey, 85% in linden honey, 58% in forest honey and 97% in acacia honey. The highest lindane concentrations were found in acacia honey in the quantity of 4.45 µg/kg. The content of other pesticide residues is insignificant. Due to the fact that honey is the indicator of environmental pollution and according to the study performed, it can be concluded that higher lindane concentration is present in the Pannonian region honey in comparison with the other organochloric pesticides. Although it was detected, lindane concentration in the Pannonian region honey does not exceed the maximum quantity allowed prescribed in the Rulebook of the Republic of Serbia.

Key words: honey, pesticide, lindane

Abbreviations: HCH – Hexachlorocyclohexane, DDE – dichlorodiphenyldichloroethylene, DDD – dichlorodiphenyldichloroethane, DDT – dichlorodiphenyltrichloroethane, OCP – organochlorine pesticides, PCB – polychlorinated biphenil, GCMSD – Gas Chromatography-Mass spectrometry

Introduction

A significant increase in the industrial production over the last few decades, frequent and uncontrolled pesticide and herbicide applications in agriculture as well as heavy international traffic have caused a significant increase in the environmental pollution by heavy metals, pesticides, radionuclides and antibiotics (Barišić and Associates, 1999; Mujić and Associates, 2011; Roman and Associates, 2011). Mass food production in large areas requires mass usage of different protective substances so that farmers could tackle diseases and pests. As it is widely known, bees fly in a radius of 6 km which is why their products are considered reliable indicators of environmental pollution (Lebedev

and Associates, 2004). Pesticides are certainly considered the most significant impurities that can be found in bee products.

Pesticides may enter honey:

1. During the treatment of blooming plants, bees frequently collect contaminated pollen, nectar, or plant juices, when they themselves can be poisoned.
2. Pesticides may enter the water consumed by bees.
3. Rarely, but still it is possible that bees and hives are directly splashed by insecticides.
4. Pesticides may be transferred to hives from the splashed plants (Aleksic and Associates, 2012).

Food contamination by organochloric pesticides is considered a serious threat to people's health due to their consistency

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in the environment, high accumulation and high level of toxicity for mammals (Bogdanov 2006). Organochloric pesticides (OCP) and polychlorinated biphenyls (PCB) are present in the environment as a consequence of their application in agriculture and industry (Djinovic-Stojanovic and Associates, 2013). Although the production and usage of OCP and PCB were banned or limited in some countries in the late 80s, a great deal of research indicates that these compounds are still present in different parts of the environment, such as water, air and soil (Barakat and Associates, 2013). As a result of their presence in the environment, these compounds can be found in food as well and have a negative effect on people's health (Djinovic-Stojanovic and Associates, 2013). Many studies show that OCPs bioaccumulate in plants through contaminated soil (Gonzales and Associates, 2003; Wang and Associates, 2010). Food contamination with pesticides, especially organochloric pesticides has been a problem to consumers and industrial leaders for a long time (Wang and Associates, 2010).

Lindane is organochloric pesticide known for its toxic characteristic and stability, frequently causing serious water and soil pollution. Hexachlorocyclohexane (HCH) represents a group of compounds that occur in eight isomeric forms of which we will select alpha, beta, gamma (lindane) and delta HCH.

Lindane is the most frequently used pesticide in agriculture. Lindane usage has been reduced in many countries due to its toxicity and persistency as well as possible cancerogenic effects it may cause (Girish and Associates, 2013). Some pesticides, such as lindane, accumulate in fat tissue which can cause cell membrane degradation and disbalance in the organism (Hosie and Associates, 2000). Uncontrolled and excessive use of pesticides, based on OCP, may cause lindane accumulation in so called "healthy products". The aim of this study is to determine lindane content in the Pannonian region honey.

Material and Methods

Sixty samples of honey were collected in the Pannonian region of Serbia during the year 2013. 10 samples of linden honey, 10 samples of forest honey, 10 samples of sunflower honey, 15 samples of acacia honey and 15 samples of meadow honey were collected. All honey samples were properly packed in glass or plastic bottles with lids. The collected samples were analysed on twenty organochloric pesticides as it is shown in Table 1.

Sample preparation was performed according to Quechers method, while gas chromatography with mass detector was used for the quantification of pesticide residues, Agilent Technology GCMSD 7890B/5977A with electron ionization and quadrupole detector. Pesticide standard with 99% of purity by Sigma-Aldrich manufacturer was used. High purity solvents,

Table 1
Examined organochloric pesticides and their retention times

No	Pesticid	Retentime (min)
1	α HCH	12.305
2	β HCH	12.918
3	γ HCH	13.007
4	δ HCH	13.563
5	heptahlor	14.46
6	aldrin	15.15
7	heptahlorepoksid	15.934
8	trans hlordan	16.393
9	alfa endosulfan	16.624
10	cis hlordan	16.667
11	pp'DDE	16.068
12	dieldrin	17.117
13	endrin	17.524
14	endosulfan	17.701
15	pp'DDD	17.851
16	endrin aldehid	18.061
17	endosulfansulfat	18.496
18	pp'DDT	18.55
19	metoksihlor	18.803
20	endrin keton	19.344

without pesticide, hexan, methanol and acetonitrile residues were used (Sigma-Aldrich). Regarding salts, anhydrous magnesium sulfate and sodium acetate were used for the extraction. Dispersive SPE fillings Bundesil C18, with a pore radius of 40 μm and Bundesil PSA, with a pore radius of 40 μm , by Merck manufacturer (Darmstadt, Germany) were used for sample purification. OCP concentration in basic solution of pesticide compound is 500 $\mu\text{g/ml}$. Working solution of pesticide was made by dilution of 0.1 ml of basic solution in a 10 ml vessel containing hexane, which resulted in the working solution concentration of 5 $\mu\text{g/ml}$.

Calibration solutions were obtained by dilution of working solution and resulted in five calibration solutions of concentration range from 0.005 to 0.5 $\mu\text{g/ml}$.

In order to eliminate the influence of matrix, calibration through honey matrix which does not contain pesticide was performed as well (SANCO, 2013). Measured samples are contaminated by pesticide working solution, therefore, pesticide content in them ranges from 0.005 to 0.01 $\mu\text{g/kg}$.

Basic and working solutions of pesticides are kept in a refrigerator at 4°C, basic solution not longer than three months and working solution not longer than one week. Matrix spike samples and honey samples are prepared in the same way, in accordance with Quechers method.

This method of preparation is based on the extraction with acetonitrile in the presence of salt. A sample (3 g) is measured and transferred into polyethylene tube of 50 ml, 3 ml of water is added and 3 ml of acetonitrile. It is intensely stirred on vortex after which 3 g of anhydrous magnesium sulphate and 1 g of anhydrous sodium acetate are added. Egzothermic reaction occurs within 1 min. after the intense stirring on vortex. The sample is then centrifuged for 5 min at 3000 rpm. 1 ml of upper acetonitrile extract is transferred into the 5 ml tube which contains 150 mg of anhydrous magnesium sulphate, 100 mg of PSA and 50 mg of C18 (Anastasiades and Associates, 2003). The tube content is centrifuged for 5 min at 3000 rpm. After centrifuging, purified and clear extract is obtained. 0.5 ml of extract is evaporated in nitrogen and reconstituted with hexane. A sample prepared in this way is ready for the analysis on gas chromatograph with mass detector.

Organochloric pesticides are separated on DB-5MS column (30 m·0.25 µm·0.25 mm). Sample volume of 4 µl (splitless mode) was injected at the constant pressure of 11.36 psi and flow through the column of the carrier gas of 1.2 ml/min. The carrier gas is helium with 99.999% of purity. The injector temperature is 250°C, the detector temperature is 280°C. The oven temperature programme is as follows: the initial temperature is 75°C and it remains there for 0.5 min, after that the temperature rises to 300°C with the velocity of 10°C/min and it remains there for 2 min. The analysis of organochloric pesticides, that is the temperature programme, lasts for 25 min. Retention time, molecule weight, ions important for the analysis of HCH isomer are shown in Table 2.

Table 2

Retention time (tR), molecule mass (MW), primary (target) ion (T) and secondary and tertiary ion (Qualifier Ions, Q1, Q2) for HCH isomers

Pesticid	tR(min)	MW	T	Q1	Q2
α HCH	12.305	290.8	183	181	219
β HCH	12.918	290.8	219	181	183
γ HCH (lindan)	13.007	290.8	181	183	109
δ HCH	15.536	290.8	109	219	183

The target and qualifier abundances were determined by injection mixture of pesticide standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from m/z 60 to 500. Standards were prepared in blank matrix extracts, to counteract the matrix effect (SANCO, 2013).

With the aim of obtaining more reliable results, further pesticide quantification is performed in SIM mode. Pesticide quantification is performed according to mass specters and

Table 3

SIM programme which was used for the analysis and confirmation of HCH isomer in honey (m/z , total dwell time)

Group	Time (min)	Pesticid	m/z	Total dwell time
1	10	α HCH	181. 219. 109	150
2	12.8	β HCH. γ HCH. δ HCH	181. 183. 219. 109	200

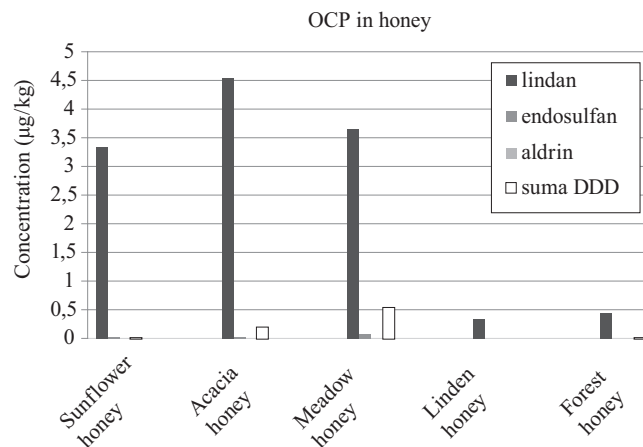
characteristic ions defined in SIM mode (Table 3.), as well as the retention time of exit components, pesticides (Selvi and Associates, 2012). The obtained data processing is performed through Mass Hunter Software.

The analysis of the method performance is performed in calibration range from 0.005 to 0.1 µg/g.

Results

The results of honey examination are shown graphically (Figure 1). It has been determined that in the examined samples of all detected pesticides (aldrine, endosulfan and suma DDD), lindane is present at a significantly higher degree (concentration). In sunflower honey, lindane was present in 73% of the total quantity of detected pesticides, in meadow honey 73%, in linden honey 85%, in forest honey 58% and in acacia honey 97%. The content of other pesticides is insignificant and it is shown graphically (Figure 2).

During the examination, it was determined that the content of lindane was the highest in acacia honey, where an average value of measurement is 4.45 µg/kg. Lindane concentrations in the samples of the examined honey are shown in Figure 2.

**Fig. 1. Content of OCP in different types of honey**

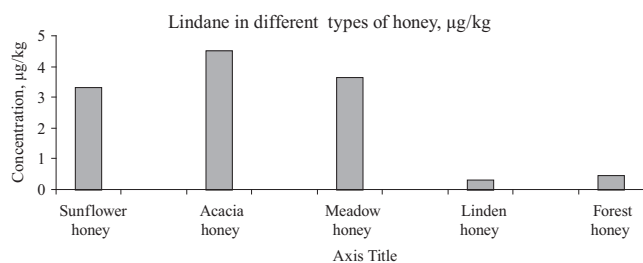


Fig. 2. Lindane content in different types of honey

Discussion

The obtained values of our examinations are in accordance with honey examinations in different geographic regions which were performed during the year 2010. During the examination it was determined that the concentration of HCH isomer, primarily lindane, significantly differs in developed and developing countries (Wang and Associates, 2010). An average value of lindane content in developed countries ranged from 1.6 to 8.7 ng/g, whereas in developing countries it ranged from 0.21 to 4.78 (Wang and Associates, 2010). In addition, the honey examination performed in Turkey confirmed the presence of lindane in the concentration of 3.7 ng/g (Yavuz and Associates, 2010). On the other hand, American studies from 2010 indicate the presence of lindane in honey in significantly greater quantities than those recorded in Europe, recorded quantities of lindane in honey samples are 4.3 ppm (Johnson and Associates, 2010). A research done by Mullin and Associates in 2010 confirmed the presence of 121 different pesticides and metabolites in bee samples and bee products on the territory of the East Coast of America.

The largest number of pesticide residues which were found belongs to the group of organochloric compounds. Pesticide residues α HCH, β HCH and lindane have been detected in 11 out of 26 samples, which were positive to the presence of pesticide (Al-Rifai and Associates, 1997). In the period from 1986 to 1996 in France, the presence of pesticide residues in honey was examined and it was determined that there is 17.5% of the examined samples (Aleksic and Associates, 2012). The examination results of the presence of pesticide residues in honey in the area of Pyrenees Peninsula indicate the presence of organochloric pesticides in 1 to even 57% (Fernandez-Muino and Associates, 1995), and in 2003, the presence of 42 pesticides, mainly organochloric, was detected in 50 samples. The presence of γ HCH was determined in 50% of samples, HCB in 32% of samples, and other HCH isomers such as α HCH and β HCH recorded the presence in 28%, that is, 26%. The obtained concentrations

ranged from 0.03 to 4.31 mg/kg, and in most cases they were below 0.05 mg/kg (Blasco and Associates, 2003). Lindane as the most frequent organochloric pesticide in honey was confirmed during the study in types of honey from Portugal, where detected lindane ranged from 0.01 to 4.3 $\mu\text{g/g}$ (Albero and Associates, 2004). The most frequently examined insecticides in European honey are organochloric pesticides such as lindane and its isomers hexachlorocyclohexan (HCH), aldrine, dieldrine, endrine, DDT isomers, heptachlor, endosulfan and the like.

Many organochloric pesticides are no longer used in agriculture, however, they are still present in the environment.

Honey and other bee products are considered natural, healthy and pure products. However, today bee products are produced in the environment polluted by different pollution sources. News about “contaminated honey” has been present in the media lately. Such messages may undermine a good image of honey and apiculture may become a very endangered activity (Bogdanov, 2006).

Conclusion

Bearing in mind the fact that honey is an indicator of environmental pollution, in accordance with this study it can be concluded that the content of lindane in the Pannonian region honey is higher in comparison with the other organochloric pesticides, as it has been highlighted in the studies of other authors in Western Europe.

Lindane quantity which was determined is not toxic for people and it is below maximum allowed concentration as prescribed by the Rulebook of the Republic of Serbia. However, it is a warning about the increased presence of lindane in the environment.

In order to determine pesticide traces, it is advisable to use gas chromatography with mass detector which provides us with a confirmation of the result reliability by comparing the obtained specters with the specters from the library. Quecher method for sample preparation enables quick and straightforward honey sample preparation to pesticide residues.

In view of the fact that acacia honey contained the highest percentage of gamma HCH, monitoring of this type of honey should be performed on certain localities across the Pannonian region of Serbia as well as to other contaminants in order to make a realistic assessment of this highly appreciated bee product.

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