

## Residues of Organochlorine Pesticides in Polish Honeys.

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### Abstract

This study was carried out in order to estimate organochlorine insecticides residues from HCH, DDT, aldrin, endrin, dieldrin, and methoxychlor in 178 samples of Polish honey by gas chromatography. In general the pesticide residues varied from trace levels to 60 µg/kg. Aldrin was found in 38 samples at levels between 1.0 and 14.27 µg/kg of honey, endrin in 13 samples at levels between trace and 65.3 µg/kg, dieldrin in 32 samples at levels between 1.0 and 5.93 µg/kg, o,p-DDT in 34 samples at levels between trace and 18.66 µg/kg, p,p-DDT in 108 samples at levels between trace and 227.85 µg/kg, HCH in 113 samples at levels between trace and 284.96 µg/kg, o,p-methoxychlor in 17 samples at levels between trace and 7.12 µg/kg, and p,p-methoxychlor in 51 samples at levels between trace and 38.67 µg/kg.

Key words: honey, pesticides residues, organochlorine insecticides

### 1. Introduction

According to the definition honey is the substance made of nectar and sweet deposits from plants, gathered, modified and stored in honeycombs by honey bees. No additions to honey are allowed. However, honey can be contaminated involuntarily from the environment and from beekeeping practice [1]. There are different types of pesticides such as insecticides, herbicides, fungicides and antibiotics. Pesticides are used in agriculture and beekeeping. In a recent review it was shown that honey could be contaminated from different pesticides [1]. It was found that the contamination of honey and other bee products was by pesticides (varroacides) used in beekeeping more so than ones originating from the environment. Other important honey contaminants are the fungicides.

Organochlorine pesticides belong to the group of insecticides. Organochlorine insecticides have been banned worldwide for many years. Because of their high

degree of stability and bioaccumulation, they persist in the environment and act indiscriminately in a harmful way on different forms of life. Organochlorine insecticides (DDT, HCH) have been forbidden in Poland since 1972. Despite this ban the residues of these pesticides are still detected in all environmental sectors. Residues of organochlorine insecticides in honey can possibly be dangerous for people, because of the insecticides mutagenic, teratogenic and embryotoxic activities.

In Poland honey has never been included in regular pesticide residues controls. Because no maximum residues levels (MRLs) have been established for pesticides in honey it is very hard to evaluate the contamination of honey with pesticides and the possible damage to human health. The aim of our study was to estimate the amounts of residues from organochlorine insecticides in Polish honey

## 2. Materials and methods

### 2.1. Material and reagents

The samples were 178 raw, liquid honeys collected in 2001-2002 from different regions of Poland. Honey samples were provided by beekeepers associations. The samples were from following origins: Pomeranian region (north-west Poland) 49 samples, Masuria (north-east Poland) 24 samples, Wielkopolska and Warsaw province (central Poland) 31 samples, Silesia (south-west Poland) 45 samples, Malopolska region (south-east Poland) 27 samples.

The honeys included acacia (*Robinia pseudoacacia*) honeys (11 samples), lime tree (*Tilia Europoea*) honeys (16), rape (*Brassica napus var. Arvensis*) honeys (28), multifloral honeys (49), buckwheat (*Fagopyrum*) honeys (23), heather (*Calluna vulgaris*) honeys (10), blossom-honeydew honeys (12) and honeydew honeys (29). The types of honey were classified to their botanical origin using the method of Gebala et al [2] and additionally confirmed by pollen analysis.

Pesticide standards (99,9% minimum purity) were obtained from IPO (Poland). Residue analysis grade methanol, n-hexane, dichloromethane were supplied by POCh (Poland). Florisil of 60-100 mesh was purchased from Baker (Netherlands). Pesticide stock solutions were made in n-hexane.

## 2.2. Analysis

The analysis of pesticides in honey was made using method recommended by Jimenez et al [3]. The procedure involves loading the honey sample on a Florisil packed-column, which is eluted with an n-hexane and dichloromethane (1:1, v/v) mixture. For the analysis of pesticides a GC procedure with electron-capture (ECD) detection was used.

Florisil was conditioned by heating at 120<sup>0</sup>C for 4 h before use. A glass column 300x25 mm I.D. was prepared with Florosil slurry in n-hexane-dichloromethane (1:1, v/v) and compacted with a rod. Care was taken to prevent the column from drying.

Honey (1g) was mixed with methanol (2 ml) and homogenized by shaking to reduce its viscosity and facilitate handling. Then the sample was poured onto Florisil column and percolated. The column was eluted by gravity with 75 of n-hexane-dichloromethane (1:1, v/v). The eluate was evaporated in a rotary evaporator from IKA (Germany) at 30<sup>0</sup>C and the residues dissolved in n-hexane for GC analysis.

A Varian CP-3800 gas chromatograph equipped with electron-capture detector and 30m x 0,25 mm capillary column from SGE was used. The oven temperature program was as follows: initial temperature 60<sup>0</sup>C, held for 1 min, 20<sup>0</sup>C/min ramp to 200<sup>0</sup>C, then 4<sup>0</sup>C/min ramp to 240<sup>0</sup>C and finally a 4<sup>0</sup>C/min ramp to 260<sup>0</sup>C, held for 4 min. The carrier-gas (nitrogen) flow-rate was 1ml/min. The detector temperature was 300<sup>0</sup>C.

Following limits of determination for all compounds were determined at level 1µg/kg.

## Statistics

Statistical evaluation of the residue data given in Table 2 and 3 was carried out by using Statistica 5.0 (Statsoft, 1999). To determine the differences among the means the  $\chi^2$  test was used (significance level = 0,05).

## 3. Results and discussion

GC allowed the separation and quantification of number pesticides and other substances in the analyzed honeys. Figure 1 presents a GC chromatogram obtained after analyzing a mix of standard compounds and Figures 2 and 3 present GC chromatograms of selected honey samples.

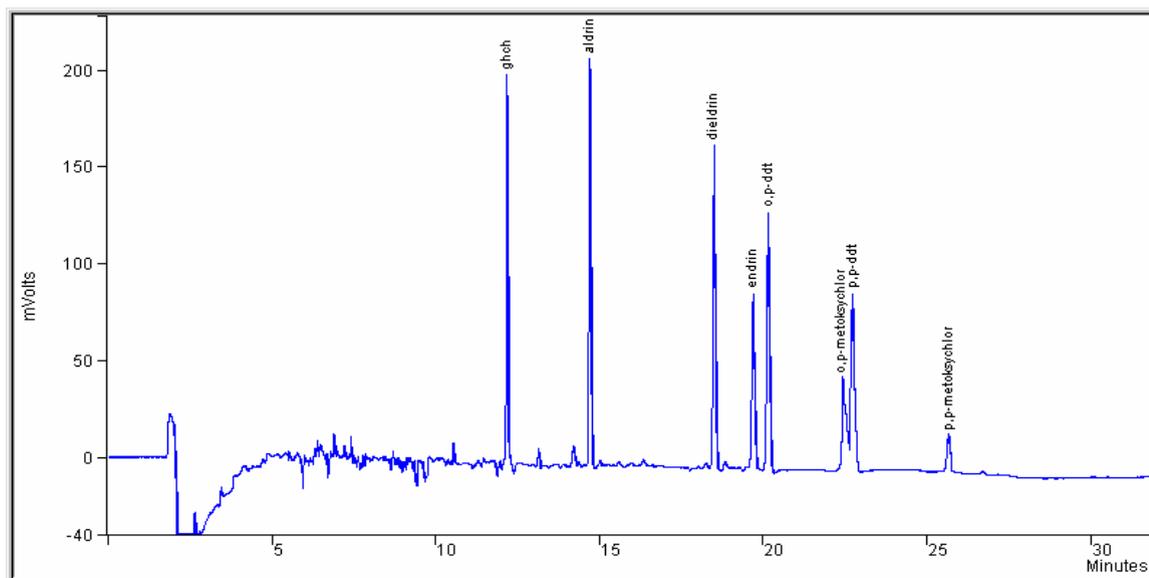


Figure 1. GC Chromatogram Of Standard Compounds. Retention Times:  $\gamma$ -HCH 12,160 min, aldrin 14,691 min, dieldrin 18,487 min, endrin 20,138 min, o,p-DDT 22,506 min, o,p-methoxychlor 22,710 min, p,p-methoxychlor 25,783 min.

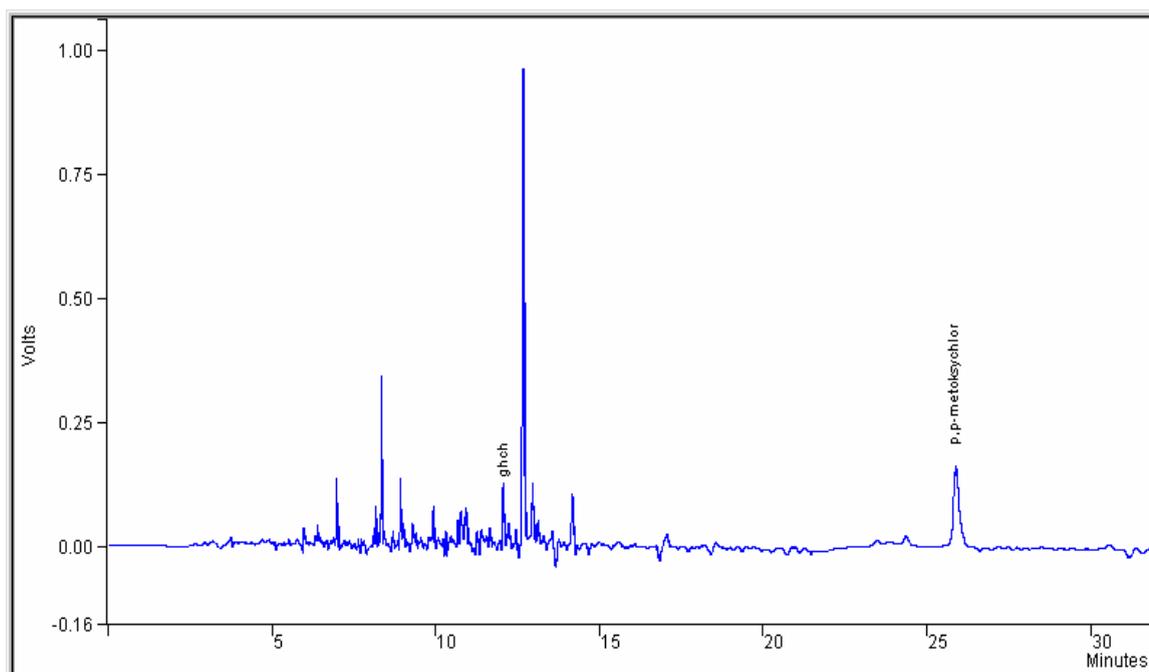


Figure 2. Chromatogram Of Buckwheat Honey. Retention Times:  $\gamma$ -HCH 12,160 min, aldrin 14,691 min, dieldrin 18,487 min, endrin 20,138 min, o,p-DDT 22,506 min, o,p-methoxychlor 22,710 min, p,p-methoxychlor 25,783 min.

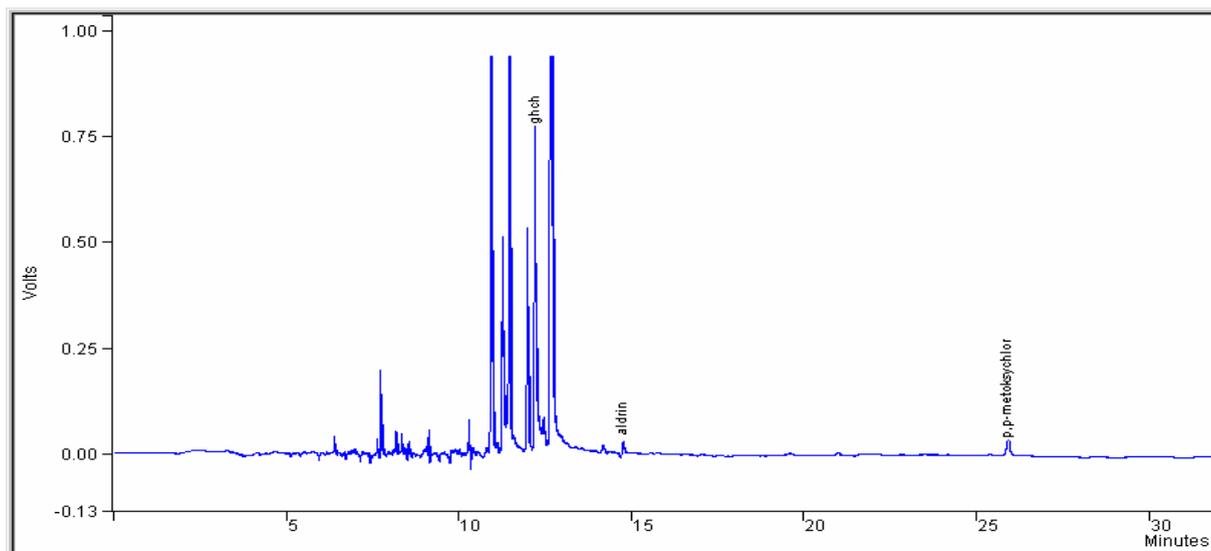


Figure 3. Chromatogram Of Heather Honey. Retention Times:  $\gamma$ -HCH 12,160 min, aldrin 14,691 min, dieldrin 18,487 min, endrin 20,138 min, o,p-DDT 22,506 min, o,p-methoxychlor 22,710 min, p,p-methoxychlor 25,783 min.

In Table 1 the results for the organochlorine insecticides residues in honey are shown. They are given average, standard deviation, range, median and 90th percentile value.

Table 1

The Content of Orgnochlorine Insecticides in Polish Honey (ppb).

Insecticide	average SD	range	Median	90th percentile	Number of samples with residues found
Aldrin	3,39 2,93	1,1-14,27	2,45	5,80	38 (21%)
Endrin	14,54 22,89	1,1-65,3	3,55	46,07	13 (7%)
Dieldrin	2,45 1,63	1,2-5,93	1,95	4,08	32 (18%)
o,p-DDT	6,14 5,44	1,5-18,66	3,97	14,75	34 (17%)
p,p-DDT	11,85 32,74	1,1-277,85	4,43	17,22	108 (60%)
$\gamma$ -HCH	13,69 32,87	1,1-284,96	4,48	25,28	113 (63%)
o,p-methoxychlor	3,73 1,83	1,1-7,12	3,87	6,26	17 (10%)
p,p-methoxychlor	9,64 9,03	1,2-37,45	5,84	25,08	51 (29%)

The aldrin was found in 38 samples, at levels between trace and 14,27  $\mu\text{g}/\text{kg}$ , endrin in 13 samples, at levels between trace and 65,3  $\mu\text{g}/\text{kg}$ , dieldrin in 32 samples, at

levels between trace and 5,93 µg/kg, o,p-DDT in 34 samples, at levels between trace and 18,66 µg/kg, p,p-DDT in 108 samples, at levels between trace and 227,85 µg/kg,  $\gamma$ -HCH in 113 samples, at levels between trace and 284,96 µg/kg, o,p-methoxychlor in 17 samples, at levels between trace and 7,12 µg/kg, and p,p-methoxychlor in 51 samples, at levels between trace and 38,67 µg/kg. Nine honey samples were free from detectable residues.

In general the determined values were comparable to the results obtained by other authors. In earlier studies carried out in Poland the residues of DDT and lindane were found. Dzilinski and Szymanowska [4] detected the residues of o,p-DDT, p,p-DDE and p,p-DDD in all analyzed honey samples. The total levels of DDT did not exceeded 10 µg/kg of honey. They also detected residues of lindane in 34 samples. In 1994 Szczesna et al [5] determined the amount of residues in 30 samples of Polish honey. The pesticides detected were organochlorine insecticides DDT and  $\gamma$ -HCH in 28 samples at levels between trace and 0,38µg/kg. A new study of the levels of pesticides was carried out by Romaniuk and Witkiewicz [6,7] on entomophilic plants and bee products. The pesticides detected in honey were  $\gamma$ -HCH at average level 1,7 µg/kg, DDT at average level 0,7 µg/kg and DDE 0,5 µg/kg. They did not find DDD in honey samples. The study carried out on 101 Spanish honeys by Fernandez Muino et al [8] showed that in honeys have been residues of many organochlorine insecticides. They detected aldrin in 36 samples in amounts ranging from 1 to 150 µg/kg, dieldrin in 9 samples in amounts ranging from 1 to 13 µg/kg, endrin in 1 sample at 7 µg/kg,  $\gamma$ -HCH in 47 samples in amounts ranging from traces to 161 µg/kg, heptachlor in 29 samples in amounts ranging from traces to 57 µg/kg, lindane in 57 samples in amounts ranging from traces to 59 µg/kg, methoxychlor in 11 samples in amounts ranging from 19 to 593 µg/kg, o,p-DDT in 7 samples in amounts ranging from 1 to 12 µg/kg and p,p-DDT in 18 samples in amounts ranging from 1 to 61 µg/kg. They did not find pesticides residues in 13 honey samples. Bogdanov (2006) reviewed different studies carried out on organochlorine pesticides residues in honey. The levels found in different countries differed considerably but were mostly below 0.5 mg/kg.

In our study differences have been noted between the pesticides concentrations in honey samples from different locations and between different honey varieties. The greatest tendency for accumulation of pesticides was in heather honeys. The highest average concentration of endrin, dieldrin, p,p-DDT, p,p-methoxychlor and  $\gamma$ -HCH

were found in these honeys. High concentrations of organochlorine pesticides were detected more often in honey samples coming from north-west and south-west regions of Poland than from eastern regions of Poland. But these differences were not significant at  $\alpha=0,05$  (Table 2).

Table 2

Value of  $\chi^2$  Depending on Location and Honey Types.

Pesticide	Value of $\chi^2$ depending on location	$\chi^2_{0,05}$	Value of $\chi^2$ depending on honey types	$\chi^2_{0,05}$
Aldrin	19,342	24,996	12,732	36,415
Endrin	12,031		12,631	
Dieldrin	12,565		6,546	
o,p-DDT	15,153		19,202	
p,p-DDT	15,014		13,546	
?-HCH	18,049		26,632	
o,p-methoxichlor	10,178		26,451	
p,p-methoxichlor	11,394		32,979	

It is worthy to note that a significant positive correlation was observed between the dieldrin and endrin contents ( $R=0,78$ ,  $p =0,05$ ), also between p,p-methoxichlor and o,p-methoxichlor contents ( $R=0,35$ ,  $p =0,05$ ).

Table 3

Value of Correlation Coefficients (R).

	Aldrin	Endrin	Dieldrin	o,p-DDT	p,p-DDT	?-HCH	o,p-methoxichlor	p,p-methoxichlor
Aldrin	1							
Endrin	-0,01	1						
Dieldrin	0,1	0,78	1					
o,p-DDT	-0,03	-0,01	0,16	1				
p,p-DDT	-0,04	0,01	0,01	-0,02	1			
?-HCH	-0,02	0,02	0,05	-0,02	-0,05	1		
o,p-methoxichlor	0,04	-0,04	-0,04	-0,08	-0,04	0,04	1	
p,p-methoxichlor	-0,05	0,01	0,02	0,04	-0,01	0,29	0,35	1

These correlations can be partly explained by the similarities in chemical structures and properties of these insecticides. Probably they are combined and applied together. The correlation coefficient between contents of another pesticides was very low and varied between  $R=-0,08$  for o,p-methoxichlor and o,p-DDT and  $R=0,29$  for  $\gamma$ -HCH and p,p-methoxichlor.

#### 4. Conclusions

1. This study showed that residues of organochlorine insecticides existed in Polish honeys in important amounts. In honey samples the dominate residues were DDT isomers and  $\gamma$ -HCH.
2. More pesticides residues were found in honeys coming from western regions of Poland; this differential content revealed the possibility that honey may be a monitor of the environmental contamination.

#### 5. References

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