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ORIGINAL ARTICLE

ANALYSIS OF SELECTED ORGANOCHLORINE PESTICIDES IN HONEY SAMPLES FROM POLAND: A PILOT STUDY

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ABSTRACT

Background. Organochlorine pesticides (OCPs) were widely used in crop protection in the past. Due to their high chemical persistence and widespread presence in the environment, they have been classified as persistent organic pollutants (POPs). Given their toxicological properties, dietary exposure to OCPs may lead to adverse health effects in humans.

Objective. The aim of this pilot study was to analyse selected obsolete OCPs (DDT, its metabolites and isomers, as well as dieldrin and heptachlor) in honey samples and to assess the associated health risks resulting from the intake of these compounds for children and adults.

Material and Methods. The study included 79 honey samples collected from various regions of Poland. The samples were prepared using the modified QuEChERS method. The tested substances were determined in honey using gas chromatography with an electron capture detector (GC-ECD). Health risk was characterized using a deterministic method by comparing the intake of the residues from a large portion of honey with toxicological reference values. A conservative approach was used to estimate short-term exposure using 0.5 × LOQ (limit of quantification) values for substances detected at levels below the LOQ.

Results. None of the OCPs analysed were detected above their LOQs. Only in two samples, *p,p'*-DDE and dieldrin, were detected at levels above the method's limit of detection (LOD). The results indicate a negligible health risk for consumers associated with the intake of these substances from honey.

Conclusions. The results indicate that levels of tested organochlorine pesticides in honey are low. The risk associated with exposure to the analysed OCPs, at the assumed levels, through the consumption of honey available in Poland can be considered negligible.

Keywords: *honey, organochlorine pesticides, persistent organic pollutants, QuEChERS, GC-ECD*

INTRODUCTION

Honey is valued not only as a culinary ingredient but also as a natural food product with health-promoting properties [1]. It is widely regarded as a product rich in nutrients, antioxidants and enzymes, which contributes to its popularity [2, 3]. Given that honey is often consumed by children, the elderly and health-conscious individuals, it is particularly important that it remains free from contaminants. Owing to its perceived health benefits and relatively easy accessibility for the average consumer, honey enjoys great popularity and is widely consumed across most European countries [4-7]. However, honey may contain residues of active substances used in plant protection products. This results from the potential for both direct and indirect exposure of honeybees (*Apis mellifera*) to these substances, for example during the collection of pollen and nectar. Due to the possible presence of pesticide residues in honey, Regulation (EC) No 396/2005 of the European Parliament and of the Council sets maximum residue levels (MRLs) for honey to ensure an adequate level of consumer safety [8].

Among the possible contaminants in honey are organochlorine pesticides, such as DDT, dieldrin and heptachlor. These are chemical compounds that were once widely used in agriculture and their intensive use became a major source of environmental pollution [9]. Improper storage and disposal of these substances in the past further contributed to environmental contamination [10-12]. Despite being banned in the

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European Union for several decades, they are still detected in plant- and animal-derived samples [13, 14]. Organochlorine pesticides have also been identified in bodily fluids (e.g., blood) and human breast milk [15, 16]. Monitoring studies make it possible to determine current levels of these legacy contaminants in food and if necessary, revise existing MRLs. Under EU law, the maximum residue level for DDT (sum of *p,p'*-DDT, *o,p'*-DDT, *p-p'*-DDE and *p,p'*-TDE (DDD), expressed as DDT), dieldrin and heptachlor in honey and other apicultural products is set at 0.05 mg kg^{-1} (DDT) and 0.01 mg kg^{-1} (heptachlor and dieldrin) [17-19]. The use of DDT is currently restricted to malaria control, specifically for targeting *Anopheles* mosquitoes in certain African and Asian countries, where effective vector management remains essential [20].

The presence of dieldrin, DDT or heptachlor in food may adversely affect human health. The toxic effects of these compounds are associated with an increased risk of various health issues, including endocrine disruption, cancer and the nervous system disorder [21-23]. Exposure to DDT (both prenatal and postnatal) may lead to developmental disorders of the adrenal gland. Dysfunction of this hormone-producing gland can lead to disturbances in the physiological regulation of other organs and systems. This may cause various pathological processes such as abnormal functioning of the immune, reproductive and cardiovascular systems [24]. Because of their ability to biomagnify in food chains and considering their classification as human carcinogens (IARC, International Agency for Research on Cancer: dieldrin and DDT – Group 2A, heptachlor – Group 2B; Regulation (EC) No 1272/2008: all analysed substances – Carc. 2), even low-level but chronic exposure may lead to adverse health outcomes, such as increased cancer risk [25-28]. There is also growing evidence that prenatal exposure to DDT and its metabolites may increase the risk of obesity later in life [29].

The aim of this study was to quantify the levels of selected persistent organic pollutants (*p,p*'-DDE, *p,p*'-DDT, *p,p*'-DDD, *o,p*'-DDE, *o,p*'-DDD, *o,p*'-DDT, heptachlor, dieldrin) in honey and to estimate potential exposure among consumers (children and adults), as well as to characterise the associated health risk arising from the intake of above compounds with honey.

MATERIAL AND METHODS

Details of the analytical method including its validation are described elsewhere [30].

Honey

A total of 79 honey samples from various regions of Poland were used in this study (Figure 1). The samples were submitted anonymously for analysis by volunteers interested in the research. Each sample was accompanied by information on its declared botanical and geographical origin. Until analysis, all samples were stored at room temperature, away from light.

Most of the samples were nectar honeys (N = 73). In addition, three samples each of honeydew and flavoured multifloral honey were included. More than half of the analysed samples consisted of multifloral

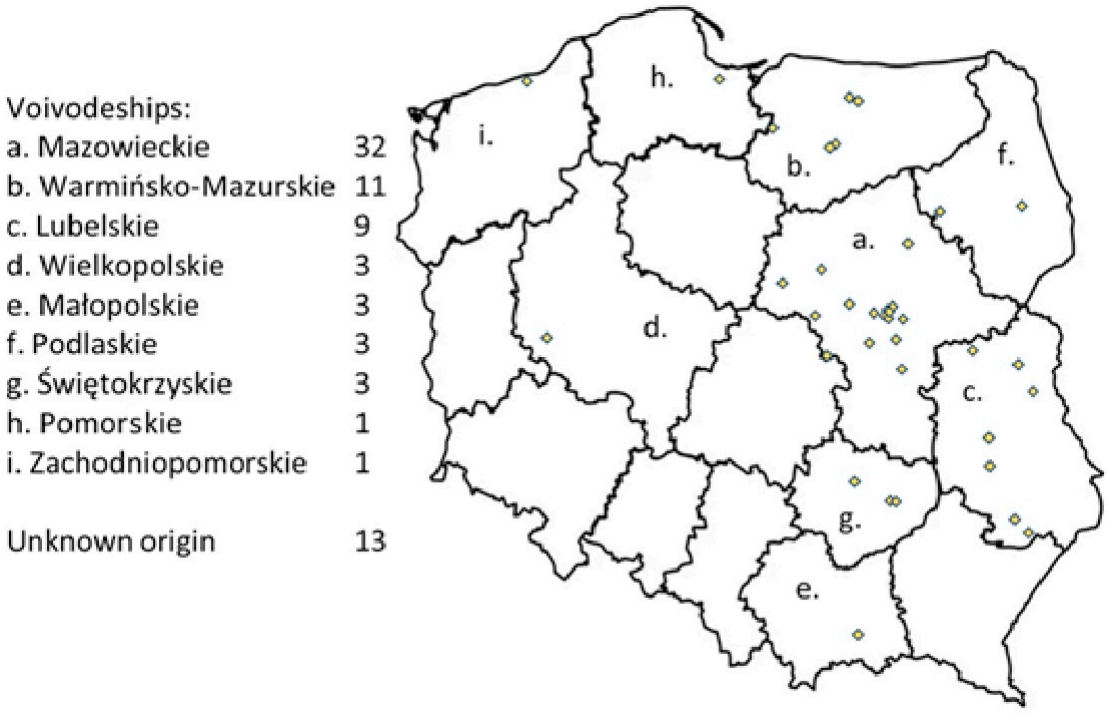

Figure 1. Declared geographical origin of the honey samples

and linden honey. The breakdown of honey types by declared botanical origin is presented in Figure 2.

Reagents

The following reagents were used in the study: acetonitrile for GC (POCH, Gliwice, Poland), *n*-hexane for pesticide residue analysis (POCH, Gliwice, Poland), QuEChERS extraction kits (Agilent, Warsaw, Poland), glacial acetic acid (BDH, Poole, UK) and *n*-dodecane (Merck, Warsaw, Poland). The following certified standards were applied: *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDD, *p,p*'-DDD, *o,p*'-DDT, *p,p*'-DDT, dieldrin and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) as an internal standard (Institute of Industrial Organic Chemistry, Warsaw, Poland) and heptachlor (Dr. Ehrenstorfer GmbH, Augsburg, Germany). All standards had a purity of > 99%.

Sample preparation

In brief, 5 g of honey was dissolved in 10 mL of water, followed by extraction with 10 mL of 1% acetic acid in acetonitrile using a commercial QuEChERS extraction kit (containing 0.5 g sodium hydrogencitrate sesquihydrate, 1 g sodium citrate dihydrate, 1 g NaCl, 4 g $MgSO_4$), vigorously shaken for 1 min. The resulting extract was centrifuged at 4000 RPM for 3 min and the supernatant was collected. A QuEChERS clean-up mixture (0.9 g $MgSO_4$ and 0.15 g PSA) was then added, vigorously shaken for 1 min and then the sample was centrifuged again at 4000 RPM for 1 min. From the resulting supernatant, 1 mL was taken and 70 µL of n-dodecane (keeper) was added, along with 10 µL of the internal standard (PCB 153 at a concentration of 1 µg mL^{-1}). The solution was then evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 1 mL of n-hexane and subjected to GC-ECD analysis.

Chromatography

Analysis of the OCPs in honey was performed using an Agilent 6890N gas chromatograph (Wilmington, NC, USA) equipped with a micro electron capture detector (µECD) and an Agilent 7683B autosampler (Shanghai, China). A HP-5 capillary column ((5%-Phenyl)-methylpolysiloxane) with dimensions 30 m × 250 µm × 0.25 µm was used. The oven temperature programme was as follows: 100°C (1.7 min) – 30°C min^{-1} – 210°C (0 min) – 5°C min^{-1} – 300°C (5 min). The injector temperature was set at 260°C and the detector temperature at 330°C. Helium was used as the carrier gas at a flow rate of 3.2 mL min^{-1} and nitrogen was used as the make-up gas at 60 mL min^{-1}. The injection volume was 5 µL. The limit of quantification (LOQ) was defined as the lowest validated concentration used to construct calibration curves (2.9 ng g^{-1} of honey for most analytes, except for heptachlor, for which a higher LOQ of 5.6 ng g^{-1} of honey was applied). The limit of detection (LOD) was determined to be 0.8 ng g^{-1} of honey.

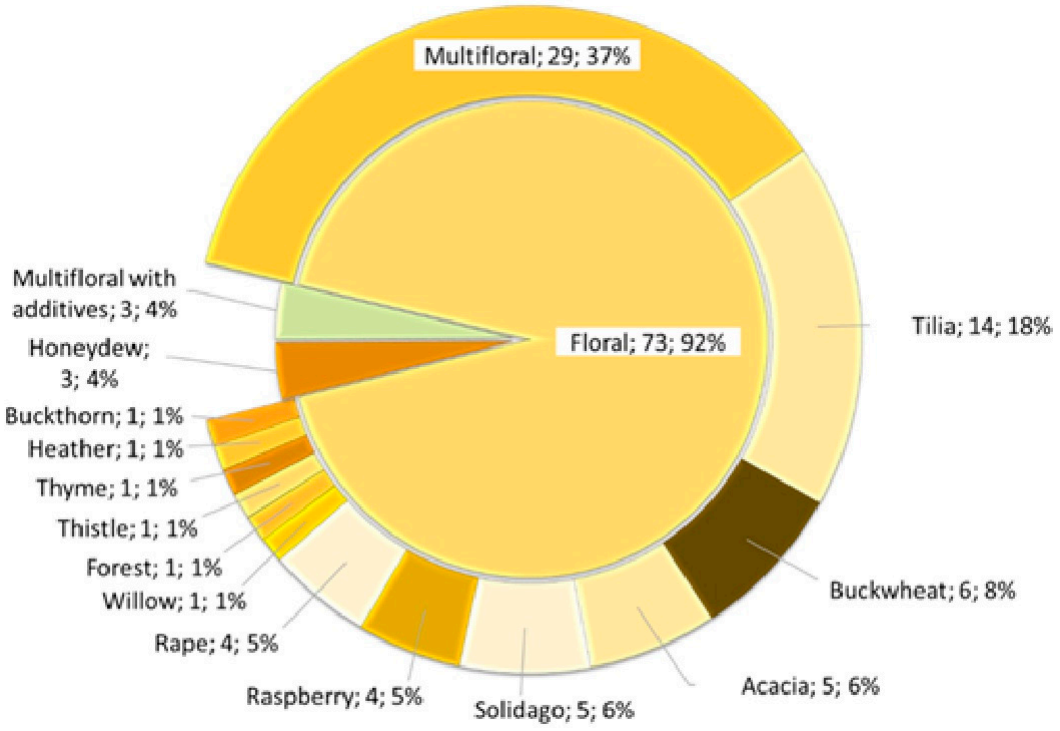

Figure 2. Declared botanical origin of the honey samples

Matrix effect

To reduce the matrix effect and improve the repeatability and reproducibility of the results, an internal standard (PCB 153) and a keeper (*n*-dodecane) were added. Additionally, due to the considerable diversity among the honey samples tested, calibration curves were prepared individually for each honey sample. These curves were obtained similarly to the method validation process by fortifying extracts with appropriate amounts of standard solution prior to solvent exchange and were determined for all the analytes studied [30].

Consumer exposure assessment

Consumer exposure and associated risk from intake of the analysed substances with honey were estimated using the EFSA PRIMo rev 3.1 model (Pesticide Residue Intake Model) [31]. The International Estimated Short-Term Intake (IESTI) was calculated, incorporating a variability factor for honey of $v = 1$ (case 1) and using the highest so-called 'large portion' of honey consumed by children and adults among all EU countries reported (the critical intake values used were respectively: 3.58 g kg^{-1} body weight for Dutch children and 1.38 g kg^{-1} body weight for Czech adults). Half of the limit of quantification value (1.45 ng g^{-1}) was assumed for detected substances in the calculations.

$$IESTI = HR \times LP \times BW^{-1}$$

where: HR – highest residue, LP – large portion and BW – body weight

Risk was then characterized by comparing the estimated intake to the toxicological reference value (TRV) specific to each substance. Intakes exceeding 100% of the TRV are considered potential health risks for consumers. For dieldrin, EFSA established an acute reference dose (ARfD) of 0.003 mg kg^{-1} bw [32]. For DDT (expressed as the sum of *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDD, *o,p*'-DDT, *p,p*'-DDD and *p,p*'-DDT), the provisional tolerable daily intake (PTDI) established by JMPR of 0.01 mg kg^{-1} bw was applied for risk characterization [33].

RESULTS

OCPs have not been found in 77 out of 79 honey samples analysed. In 2 samples, specifically a linden honey from the Warmian-Masurian Voivodeship and a multifloral honey from the Lublin Voivodeship, only dieldrin and *p,p'*-DDE were detected respectively. The levels identified were, however, below the method's limit of quantification (i.e. < 2.9 ng g^{-1}) (Table 1).

Risk assessment was conducted only for dieldrin and *p,p'*-DDE, which were detected in honey samples at levels below the method's limit of quantification. A conservative approach was applied, overestimating the potential risk. For calculations, a value of 0.5 × LOQ, i.e. 1.45 ng g^{-1} was used. The EFSA PRIMo revision 3.1 calculator was used to estimate short-term intake (ESTI) for both children and adults, based on honey consumption data for the most exposed population groups (Dutch children and Czech adults). $ESTI_{children}$ and $ESTI_{adults}$ were determined to be 5.2×10^{-6} and 2.0×10^{-6} mg kg^{-1} day^{-1}, respectively.

A negligible consumer health risk was identified in relation to a potential single (one-day) intake of dieldrin and *p,p*'-DDE with a large portion of honey. The estimated exposure for the first one did not exceed 0.2% of the ARfD for children and 0.07% of the ARfD for adults, while for the latter 0.05% PTDI for children and 0.02% PTDI for adults.

DISCUSSION

The results presented in Table 1 provide a partial overview of the contamination of honey produced and consumed in Poland with the studied organochlorine pesticide residues. Kędzierska-Matysek et al. [38], in their 2022 study based on 30 honey samples from the Lublin Voivodeship, also reported no presence of organochlorine pesticides. Similar findings regarding the presence of organochlorine pesticides in honey were obtained by Gaweł et al. [34] from research conducted between 2015 and 2017. They detected lindane and *p,p*'-DDT in only 1 out of 155 samples collected from 16 different municipalities. Likewise, Niewiadomska et al. [39], in their study on pesticide residues in animal-derived food products in 1997-2006 in Poland, including honey, did not detect any organochlorine pesticide residues in any of the 18 honey samples tested.

However, attention should be drawn to the results obtained by Kujawski et al. [35], who quantified *p,p*'-DDT in 15 out of 19 honey samples (with a maximum level of nearly 14 ng g^{-1} of honey). Other samples showed occasional presence of *o,p'*-DDD, lindane or aldrin. Similar findings were reported by Wilczyńska et. al [36], who found *p,p*'-DDT in as many as 60% of 178 honey samples collected from various regions of Poland in 2001-2002, with the highest concentration reaching 227.85 ng g^{-1}. Dieldrin and *o,p*'-DDT were detected in approximately 18% of the samples. Moreover, other OCPs were found in the tested samples, with lindane being the most frequent, detected in 63% of the samples. The presence of dieldrin, heptachlor and DDT in honey was also reported by Witczak and Ciemniak [37], who analysed OCPs in 6 types of honey from the Western Pomerania region of Poland sampled in 2008. The reported ∑DDT levels ranged from 0.05 to 0.18 ng g^{-1},

Table 1. Selected organochlorine pesticides in Polish honey

Compound	LOQ [ng g^{-1}]	Results [ng g^{-1}]	Method	No. of samples	Number (percentage) of samples with selected OCP	Ref. (year of publication)
o,p'-DDE	2.9	ND	GC-ECD	79	-	Our study
p,p'-DDE	2.9	< LOQ			1 (1.3%)	
o,p'-DDD	2.9	ND			-	
p,p'-DDD	2.9	ND			-	
o,p'-DDT	2.9	ND			-	
p,p'-DDT	2.9	ND			-	
Heptachlor	5.6	ND			-	
Dieldrin	2.9	< LOQ			1 (1.3%)	
p,p'-DDD	1	2	GC-MS/MS	155	1 (0.6%)	[34] (2019)
p,p'-DDE	1	ND			-	
o,p'-DDT	1	ND			-	
p,p'-DDT	1	ND			-	
Heptachlor	1	ND			-	
p,p'-DDT[a]	2.31	3.02-13.91	GC-MS	19	15 (78.9%)	[35] (2012)
Dieldrin	No data	1.2-5.93	GC-ECD	178	32 (18.0%)	[36] (2007)
o,p'-DDT	No data	1.5-18.66			34 (19.1%)	
p,p'-DDT	No data	1.1-227.85			108 (60.7%)	
Heptachlor	0.03	< LOQ-3.89	GC-MS	6	6 (100%)	[37] (2012)
p,p'-DDE	0.03	< LOQ-0.08			6 (100%)	
o,p'-DDD	0.03	< LOQ			6 (100%)	
p,p'-DDD	0.03	< LOQ-0.04			6 (100%)	
o,p'-DDT	0.03	< LOQ-0.06			6 (100%)	
p,p'-DDT	0.03	0.03-0.10			6 (100%)	
Dieldrin	0.03	0.06-0.10			6 (100%)	
p,p'-DDE	1	ND	GC-MS/MS	30	-	[38] (2022)
p,p'-DDD	1	ND			-	
o,p'-DDT	1	ND			-	
p,p'-DDT	1	ND			-	
Heptachlor	1	ND			-	
Dieldrin	5	ND			-	
p,p'-DDT[a]	No data	ND	GC-ECD	18	-	[39] 2008)

LOQ – limit of quantification; ND – not detected; [a] *p,p*'-DDT and metabolites expressed as *p,p*'-DDT

while dieldrin and heptachlor were found in the ranges of 0.06-0.10 ng g^{-1} and < LOQ-3.89 ng g^{-1}, respectively. The authors also measured the levels of the same compounds in rapeseed flowers and soil but did not find any correlation between the levels detected in these matrices and the OCP content in honey.

It is worth noting the findings of studies conducted in other countries, which indicate the presence of organochlorine pesticides in honey (Table 2). Yavuz et al. [40] reported the presence of dieldrin, heptachlor and DDT (including its metabolites) in nearly all of the 109 honey samples analysed from Turkey. In honey from Italy, Chiesa et al. [41] detected small amounts of DDT (and its metabolites), heptachlor and dieldrin, among others. The most frequently occurring OCP in Italian honey was endrin, which was also found at the highest concentrations. Blasco et al. [42, 43], in studies conducted on honey from Spain and Portugal, confirmed the presence of contaminants such as lindane and DDT. While lindane was found

Table 2. Selected organochlorine pesticides in honey from outside Poland

Compound	LOQ [ng g^{-1}]	Results [ng g^{-1}]	Method	Origin of honey	No. of samples	Number (percentage) of samples with selected OCP	Ref. (year of publication)
o,p'-DDE	3	< LOQ-18.6	GC-ECD	Turkey	109	109 (100%)	[40] (2010)
p,p'-DDE	2	< LOQ-23.0				109 (100%)	
o,p'-DDD	7	ND-5011.3				108 (99.1%)	
p,p'-DDD	3	ND-13.0				108 (99.1%)	
o,p'-DDT	3	ND-14.6				107 (98.2%)	
p,p'-DDT	7	ND-12.9				108 (99.1%)	
Heptachlor	7	ND-130.1				107 (98.2%)	
Dieldrin	1	ND-107.2				105 (96.3%)	
p,p'-DDE	2.55	5.4-8.8[b]	GC-MS/MS	Italy	72	24 (33.3%)	[41] (2016)
p,p'-DDD	2.74	1.9[b]				10 (13.9%)	
p,p'-DDT	2.83	5.8-15.4[b]				34 (47.2%)	
Heptachlor	2.84	4.2-6.5[b]				12 (16.7%)	
Dieldrin	3.02	3.9-4.3[b]				21 (29.2%)	
p,p'-DDT[a]	20	27-658	GC-MS	Portugal	24	10 (41.7%)	[42] (2003)
p,p'-DDE	40	186[b]	GC-ECD	Portugal	24	6 (25%)	[43] (2004)
p,p'-DDD	40	65[b]				2 (8.3%)	
o,p'-DDT	40	60[b]				1 (4.2%)	
p,p'-DDT	40	65[b]				2 (8.3%)	
p,p'-DDE	0.3	2.18[b]	GC-ECD	Spain	111	4 (3.6%)	[44] (2005)
p,p'-DDD	0.3	ND				-	
p,p'-DDT	0.6	0.45[b]				7 (6.3%)	
Heptachlor	0.1	0.17[b]				13 (11.7%)	
Dieldrin	0.3	0.34[b]				15 (13.5%)	
p,p'-DDT[a]	1	< LOQ-2.89	GC-ECD	Ukraine	104	No data	[45] 2020)
o,p'-DDE	0.05[c]	< LOD-14.3	GC-ECD	Uganda	20	No data	[10] (2021)
p,p'-DDE	0.05[c]	< LOD-0.45				No data	
o,p'-DDD	0.08[c]	< LOD-31.7				No data	
p,p'-DDD	0.07[c]	< LOD-33.9				No data	
p,p'-DDT	0.05[c]	< LOD-5.91				No data	
Dieldrin	0.1[c]	< LOD-11.0				No data	
p,p'-DDE	3.913	25.1-2697	GC-ECD	Mexico	36	6 (16.7%)	[46] (2018)
p,p'-DDD	3.913	ND				-	
p,p'-DDT	3.913	99-440.78				3 (8.3%)	
Heptachlor	3.913	24.35-2570.32				23 (63.9%)	
Dieldrin	3.913	15.72-47.06				2 (5.6%)	
DDT	0.6	1156	GC-MS	Armenia	30	No data	[47] (2020)
DDD	0.6	ND				-	
DDE	0.6	ND				-	
ΣDDT[d]	40	< LOQ	GC-ECD	Ghana	45	No data	[48] (2017)
Dieldrin	40	< LOQ				No data	
Heptachlor	40	< LOQ				No data	

LOQ – limit of quantification; ND – not detected. ND reflects how non-detection was reported in the original source (e.g. as ND, 0, – or similar). Ranges written as ND-X correspond to values in the cited studies, with ND representing non-detected or unspecified results; [a] *p,p*'-DDT and metabolites expressed as *p,p*'-DDT; [b] mean concentration; [c] Limit of detection (LOD), authors considered any result as quantifiable if it was above the LOD; [d] ΣDDT = sum of *p,p*'-DDT, *o,p*'-DDT and *p,p*'-DDE

in honey from both countries, DDT was detected only in samples from Portugal. In a later study of 111 Spanish honey samples, Herrera et al. [44] reported the presence of lindane, DDT, heptachlor and dieldrin. Some of these compounds were detected in up to 13.5% of the samples tested, with the highest levels observed for *p,p*'-DDE (93.57 ng g^{-1}). In a study of Ukrainian honey, Kasianchuk et al. [45] also found trace amounts of DDT (expressed as DDT and its derivatives) and HCH (hexachlorocyclohexane; expressed as HCH (α, β, γ isomers)) among 104 samples.

DDT, its metabolites and breakdown products are also present in honey from regions affected by malaria. In a 2021 study, Mukiibi et al. [10] focused on honey from apiaries located near a former pesticide store. They found high concentrations of organochlorine pesticides in the honey. *p,p'*-DDT, along with its metabolites and related isomers, was not only more frequently detected than other OCPs but also found at the highest concentrations, reaching up to 33.9 ng g^{-1} (for *p,p*'-DDD). Very high levels of *p,p*'-DDT and *p,p*'-DDE were also reported in honey from the Soconusco region of Mexico, known for its high malaria incidence, by Ruiz-Toledo et al. [46]. The concentrations of these compounds reached up to 440 ng g^{-1} and 2.697 ng g^{-1} of honey, respectively. This is attributed to the fact that nearly 70,000 tons of DDT were used in this region between 1957 and 2000 for malaria vector control.

An important complementary element in studies on the presence of xenobiotics in food is the assessment of the health risk resulting from their consumption. In this study, for the determined levels of dieldrin and *p,p'*-DDE, the risk associated with the intake of these substances through honey was considered negligible. Pipoyan et al. [47] focused in their study, among other things, on the risk assessment of DDT intake through the consumption of honey from Armenia. They estimated DDT intake at 0.262 mg kg^{-1} bw day^{-1}, which, according to the source they cited, does not exceed the tolerable daily intake (TDI) and therefore does not pose a non-carcinogenic risk. They also assessed the carcinogenic risk (CR), which exceeded the safe level but did not surpass the threshold of $CR > 10^{-4}$, the level considered unacceptable in risk assessment.

A different approach to risk assessment was adopted by Darko et al. [48] in their study of honey from Ghana. They determined ΣDDT, dieldrin and heptachlor at low levels, below the maximum residue limits (MRLs) established by the EU for these compounds. Consequently, they assumed that all levels below the MRL do not pose a health risk to consumers.

Honey, being a substance with low fat and wax content, should generally contain low levels of lipophilic substances. Their presence in honey is most often the result of bees bringing contaminated pollen or nectar into the hive [49]. The efficiency of various extraction techniques and the selection of appropriate analytical methods also influence the results obtained. Honey extracts often contain various compounds that interfere with the analysis and the need to use different purification techniques increases both the cost and the time required for the analysis.

One of the identified limitations of this study is the relatively high limit of quantification set during the method development. Future research should focus on improving the method presented here and include the determination of other substances from the group of persistent organic pollutants.

CONCLUSIONS

The results obtained in this study indicate that environmental levels of tested organochlorine pesticides are low. The risk associated with exposure to the analysed OCPs, specifically dieldrin and *p,p*'-DDE, at the assumed level of 0.5 LOQ, through the consumption of honey available in Poland can be considered negligible. The literature review also indicates no health threat related to the presence of trace levels of OCPs in Polish honey. At the same time, discrepancies in the results reported in the cited publications suggest the possibility of locally elevated levels of these contaminants. Verifying this assumption, however, would require more in-depth environmental studies.

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Conflicts of interest

The authors declare no conflict of interest.

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