Environ Monit Assess (2010) 168:277–283
DOI 10.1007/s10661-009-1111-6

Determination of some organochlorine pesticide residues in honeys from Konya, Turkey

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Received: 26 February 2009 / Accepted: 27 July 2009 / Published online: 14 August 2009

Abstract In this study, 24 organochlorine pesticide residues in 109 different honey samples collected from stores and open markets in Konya, Turkey were analyzed by gas chromatography-electron capture detection. Aldrin, *cis*-chlordane, *trans*-chlordane, *oxy*-chlordane, 2,4′-DDE, and 4,4′-DDE were found in all honey samples. The mean value was 0.0540 μg g^{-1} for *oxy*-chlordane. In the 55 samples of 109, levels of organochlorine pesticide residues of *oxy*-chlordane were determined as higher than those of Turkish Alimentarius Codex maximum residual limits (MRLs). Other organochlorine pesticide residues also exceeded MRLs except for *cis*-heptachlor epoxide and α-hexachlorocyclohexane. Since all of the honey samples are found contaminated and most of these samples exceeded MRLs, a control of organochlorine pesticide residues in honey is necessary for consumer health.

Keywords Organochlorine pesticides · Honey · Konya · Turkey

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Introduction

Pesticides are essential in modern agricultural practices, but due to their biocide activity and potential risk to consumers, the control of the presence of pesticide residues in foods is a growing source of concern for the general population (Torres et al. 1996). Slow degradation of pesticides, in environment, and extensive or inappropriate use by farmers can lead to environmental contamination of the water, soil, air, several types of crops, and, indirectly, humans (Hamilton and Crossley 2004; Olkowski 1991). For example, Yıldırım and Özcan (2007) observed decrease in species and number of migratory birds in Troia (Troy) National Park, Turkey, and they stated that it could be a result of the amount of pesticide residues in both water and soil resources, originating from intensive agricultural application.

Honey is usually consumed by most of people, especially by children and ill persons due to its various health effects. Organochlorine pesticide residues can be present in honey because of the plant treatment or by migration from wax

to honey. Since honeybees travel long distances and come close to many plants, honey may be an easily accessible environmental pollution indicator (Fernandez et al. 2002; Morzycka 2002). Pesticide determination in bee products is necessary to monitor contamination and guarantee consumer health (Fernandez et al. 2002). According to the European Commission regulations, honey as a natural product, must be free of any chemical contaminants and safe for human consumption (EEC Directive 1974).

A limit for the maximum concentration of pesticide residues in honey is not included in the Codex Alimentarius (Codex Alimentarius 1998). Up to now, European Union (EU) legislation has only regulated the maximum residual limit (MRL) for three acaricides (amitraz, coumaphos, and cymiazole) in honey (Commission Regulation EC 1990), but neither the Codex Alimentarius nor the EU has established MRLs for organochlorine pesticide residues. However, Turkish Alimentarius Codex has established MRLs of pesticide residues in honey (Turkish Alimentarius Codex 1997, 2005).

Turkey has an important place among the honey producing countries since it is suitable for apiculture in terms of the flowers (Erdoğrul 2007). Erdoğrul (2007) has determined levels of pesticides in nine honey samples from Kahramanmaraş, and Kolonkaya et al. (2001) analyzed organochlorine pesticide residues in 16 honeys and eight pollen samples from Turkey. Various researchers have reported pesticide residues in honey from different countries (Al-Rıfai and Akeel 1997; Blasco et al. 2003, 2004; Driss et al. 1994; Herrera et al. 2005; Jan and Cerne 1993; Muino and Lozano 1991), but reported investigations concerning the determination of residues of various pesticides in honey are very limited in Turkey. The purpose of this study is to determine the levels of some organochlorine pesticides residues in 109 honey samples from Konya, an important apiculture center in Turkey. For this purpose, these honey samples were extracted with liquid–liquid extraction method followed by gas chromatography-electron capture detection (GC-ECD).

Materials and methods

Sample collection

In this study, 109 item honey samples were used. The samples were obtained from stores and open markets in Konya, Turkey.

Extraction and cleanup

Four grams of honey was dissolved with 25 ml of deionized water and extracted with three portions of 15 ml light petroleum by mechanical shaking at 55 rpm for 15 min. When emulsion is formed, it was quickly broken, centrifuging at 3,000 rpm for 10 min. The organic phase was filtered by anhydrous sodium sulfate (1 g) and concentrated to 1 ml for analysis. The concentrated extract was loaded into a minicolumn filled with Florisil (2 g) and anhydrous sodium sulfate (1 g) and prerinsed with 10 ml light petroleum. The elution was performed with 25 ml of 5% diethyl ether in petroleum ether. The eluate was concentrated to dryness in graduated centrifuge tube and redissolved in 500 μl of *n*-hexane (Blasco et al. 2004).

Gas chromatographic analysis of organochlorine residues

The pesticides were analyzed on a Hewlett Packard (HP) Agilent 6890N model gas chromatograph (GC), equipped with an electron capture detector, and fitted with a DB-5ms capillary column (30 m, 0.25 mm i.d., and 0.25 μm). Injector and detector temperatures were 270°C and 320°C, respectively. Column temperature program was 80°C for 1 min, then increasing at 30°C/min up to 180°C, then increasing at 3°C/min up to 205°C where it was maintained for 4 min, and then increasing at 20°C/min up to 290°C where it was maintained for 2 min. Carrier gas was helium (2 ml/min). The injection was carried out in splitless, and the injection volume was 1 μl. Identification of pesticide was carried out by comparing sample peak relative retention times with those obtained for standards from

Dr. Ehrenstorfer (Augsburg, Germany). The area of the corresponding peak in the sample was compared and quantified with that of the standard.

GC confirmatory analysis was carried out on HP 6890 Series GC with HP 5973 mass detector. GC analysis was conducted on a DB-35ms (Agilent, Folsom, CA, USA) capillary column of 30 m, 0.25 mm i.d., 0.25 mm film thickness, and the following conditions described in Anastassiades et al. (2003) were used: helium constant flow 1 ml/min, inlet temperature 250°C, injection volume 1.5 ml (splitless), Mass Spectrometry transfer line temperature 290°C, temperature program 95°C for 1.5 min; then 20°C/min ramp to 190°C, followed by 5°C/min ramp to 230°C and 25°C/min ramp to 290°C (held for 20 min). Total run time was 36.67 min. Full-scan analysis (50–450 m/z) was used to determine cleanup effects, and selected ion monitoring mode was used for recovery experiment.

Results and discussion

Various techniques were used to obtain suitable extracts for analysis of pesticide residues in honey

Table 1 Levels of multiresidue of pesticides in a total of 109 honey samples and the conformity of Turkish Alimentarius Codex for honey rescript (2005/49)

Pesticide	Minimum–maximum ($\mu g\ g^{-1}$)	Mean concentration ($\mu g\ g^{-1}$)	Samples contaminated	Samples that exceeded limit	Frequency of detection (%)
Aldrin and dieldrin	0.0001–0.1143	0.0066			
Aldrin	0.0001–0.0401	0.0032	109	1	100
Dieldrin	0.0000–0.1072	0.0036	105	4	96
Σ Chlordane	0.0052–0.2384	0.0715			
C*is*-chlordane	0.0001–0.0200	0.0043	109	6	100
trans-chlordane	0.0005–0.0698	0.0146	109	39	100
o*xy*-chlordane	0.0033–0.1898	0.0540	109	55	100
Σ DDT	0.0004–5.0245	0.1079			
2,4′-DDE	0.0002–0.0186	0.0072	109	17	100
4,4′-DDE	0.0002–0.0230	0.0077	109	18	100
2-4′-DDD	0.0000–5.0113	0.0862	108	11	99
4-4′-DDD	0.0000–0.0130	0.0035	108	2	99
2,4′-DDT	0.0000–0.0146	0.0012	107	1	98
4,4′-DDT	0.0000–0.0129	0.0025	108	3	99
Σ Endosulfan	0.0000–0.0410	0.0033			
Alpha endosulfan	0.0000–0.0209	0.0016	108	1	99
Beta endosulfan	0.0000–0.0201	0.0018	108	2	99
Σ HCH (except γ-HCH)	0.0000–1.4432	0.1157			
Lindane (γ-HCH)	0.0000–0.0146	0.0037	108	4	96
α-HCH	0.0000–0.0038	0.0008	106	–	97
β-HCH	0.0000–0.0140	0.0017	107	2	98
δ-HCH	0.0000–1.4144	0.1180	105	39	96
ε-HCH	0.0000–0.0563	0.0049	107	7	98
Σ Heptachlor and heptachlor epoxide	0.0000–0.2621	0.0379			
Heptachlor	0.0000–0.1301	0.0116	107	23	98
trans-heptachlor epoxide	0.0000–0.1276	0.0149	89	38	82
c*is*-heptachlor epoxide	0.0000–0.0090	0.0024	105	–	96
Hexachlorobenzene	0.0000–0.0443	0.0116	92	29	84
Methoxychlor	0.0005–0.2267	0.0183	88	34	81
Endrin	0.0000–0.0682	0.0036	105	4	96

HCH hexachlorocyclohexane

(Al-Rıfai and Akeel 1997; Blasco et al. 2004; Driss et al. 1994; Jimenez et al. 2002; Rissato et al. 2004; Volante et al. 2001). In our study, we used liquid–liquid extraction with light petroleum and cleanup with Florisil (Blasco et al. 2004).

Levels of multiresidue of pesticides in honey samples were determined, and their conformity to the limits set by Turkish Alimentarius Codex (Turkish Alimentarius Codex 1997, 2005), shown in Table 1, was evaluated. The limits of detection, limits of quantification, and recovery values obtained for pesticides studied are given in Table 2. The recoveries ranged from 77.31% to 105.22%. Good linearity ($r^2 > 0.997$) was obtained for organochlorine pesticides by using standards (Fig. 1).

Erdoğrul (2007) has also analyzed nine honey samples from Kahramanmaraş, Turkey. In these samples, 32 pesticide residues and eight polybrominated diphenyl ether congeners were measured, and γ-hexachlorocyclohexane (HCH) was determined in all samples. However, Erdoğrul (2007) found that the pesticide levels were generally low in honey samples, and none of these samples exceeded the limits of Turkish Alimentarius Codex (Turkish Alimentarius Codex 1997, 2005). In our study, 24 pesticide residues were measured in 109 honey samples, and all of the honey samples contained detectable levels of residues of various chlorinated pesticides. Aldrin, chlordane isomers, 2,4′-DDE, and 4,4′-DDE residues were determined in all samples. Some of the organochlorine residue levels exceeded the Turkish Alimentarius Codex maximum residual limits. These samples that exceeded the limit have been shown in Table 1. Mean concentration of aldrin and dieldrin, Σ chlordane, Σ DDT, Σ endosulfan, Σ HCH (except γ-HCH), and Σ heptachlor and heptachlor epoxide were 0.0066, 0.0715, 0.1079, 0.0033, 0.1157, and 0.0379, respectively.

Kolonkaya et al. (2001) determined 13 organochlorine pesticide residues including α- and β-BHC, lindane, aldrin, dieldrin, endrin, DDT and its derivatives *pp′*-DDT, *op′*-DDD, *op′*-DDT, *op′*-DDE, and *pp′*-DDE, heptachlor, and heptachlor epoxide in 16 honey samples, and residues of

Table 2 Limit of detection, limit of quantification, and recoveries of organochlorine pesticides

Pesticide	Recovery (%)	LOD (μg g^{-1})	LOQ (μg g^{-1})
Aldrin	94.63 ± 0.99	0.002	0.007
Dieldrin	95.70 ± 1.07	0.0004	0.001
cis-chlordane	92.20 ± 0.85	0.002	0.007
Trans-chlordane	89.95 ± 0.91	0.002	0.007
Oxy-chlordane	88.75 ± 0.73	0.002	0.007
2,4′-DDE	86.43 ± 0.65	0.001	0.003
4,4′-DDE	89.89 ± 1.05	0.0005	0.002
2-4′-DDD	87.39 ± 1.20	0.002	0.007
4-4′-DDD	84.99 ± 0.82	0.001	0.003
2,4′-DDT	83.71 ± 1.13	0.001	0.003
4,4′-DDT	84.2 ± 1.38	0.002	0.007
Alpha endosulfan	77.31 ± 1.5	0.002	0.007
Beta endosulfan	95.99 ± 1.2	0.001	0.003
Lindane (γ-HCH)	105.22 ± 1.34	0.002	0.007
α-HCH	93.42 ± 0.51	0.002	0.007
β-HCH	82.71 ± 0.70	0.001	0.003
δ-HCH	88.12 ± 1.37	0.001	0.003
ε-HCH	86.17 ± 1.05	0.001	0.003
Heptachlor	86.99 ± 0.76	0.002	0.007
trans-heptachlor epoxide	80.14 ± 0.72	0.0008	0.003
cis-heptachlor epoxide	83.28 ± 0.76	0.001	0.003
Hexachlorobenzene	91.65 ± 1.12	0.002	0.007
Methoxychlor	90.82 ± 1.07	0.001	0.003
Endrin	92.71 ± 0.94	0.001	0.003

LOD limit of detection, *LOQ* limit of quantification, *HCH* hexachlorocyclohexane

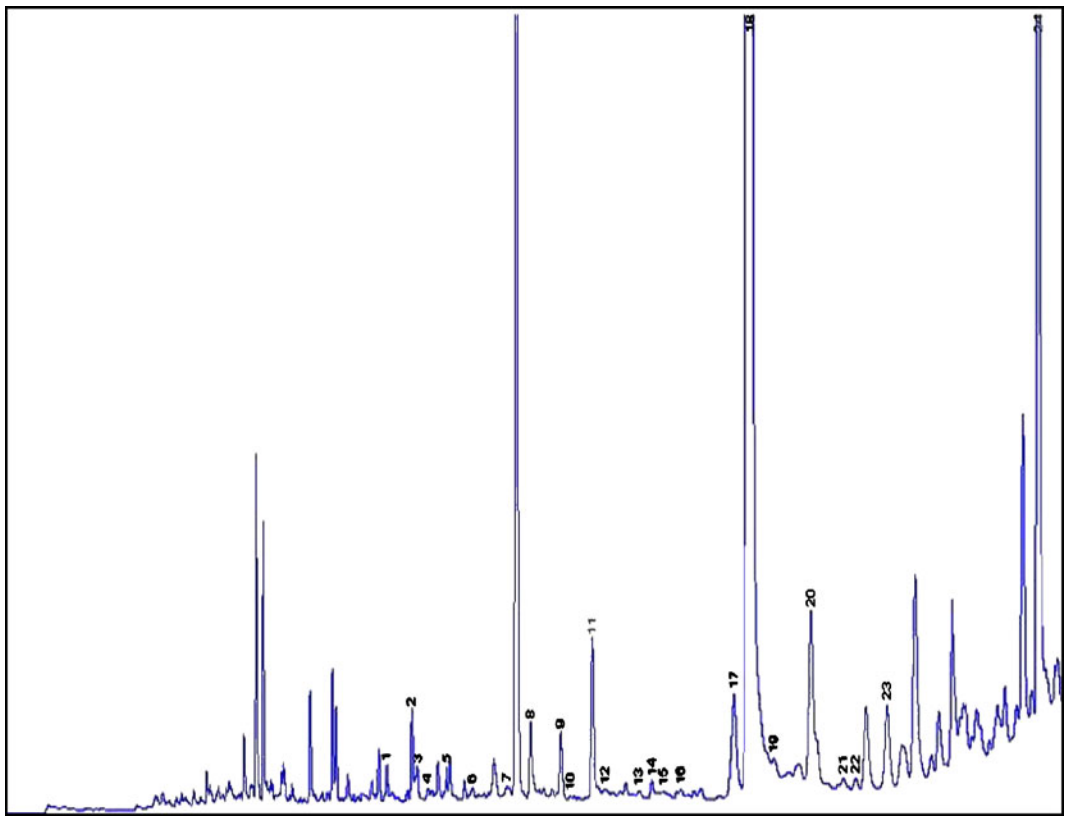

Fig. 1 GC-ECD chromatogram of a contaminated honey sample. *1* aldrin, *2 cis*-chlordane, *3 trans*-chlordane, *4 oxy*-chlordane, *5* 2,4′-DDD, *6* 4,4′-DDD, *7* 2,4′-DDE, *8* 4,4′-DDE, *9* 2,4′-DDT, *10* 4,4′-DDT, *11* dieldrin, *12* alpha endosulfan, *13* beta endosulfan, *14* endrin, *15*α-HCH, *16* β-HCH, *17* lindane (γ-HCH), *18* δ-HCH, *19* ε-HCH, *20* heptachlor, *21 cis*-heptachlor epoxide, *22 trans*-heptachlor epoxide, *23* hexachlorobenzene, *24* methoxychlor

DDT and its derivatives, as well as residues of aldrin and its metabolites endrin and dieldrin, were detected in six out of 16 honey samples. It was determined that the detected residue levels were below the level of toxicity. They found that these pesticides and their metabolites were present in honey because of their persistence, although they were no longer used. Similarly, in our study, residues of DDT and its isomers, aldrin, endrin and dieldrin, were detected in most of the honey samples, and most of these residues exceeded Turkish Alimentarius Codex maximum residual limits (Turkish Alimentarius Codex 1997, 2005).

Herrera et al. (2005) determinated 15 organochlorine pesticides, six polychlorinated biphenyls, and seven organophosphorus pesticides in honey from Spain. They have analyzed 111 honey samples from Aragon (Spain), and they found a low level of contamination. On the contrary, we found that most of the samples were contaminated with various organochlorine pesticide residues (Table 1).

Al-Rıfai and Akeel (1997) investigated pesticide residues in imported and locally produced honey in Jordan. They determined residue levels of 50 pesticides in 26 samples of honey. Results indicated that most pesticides found in the samples belonged to the organochlorine group. Residues of α-HCH, β-HCH, and lindane were detected in most samples. Residues of DDT, heptachlor, heptachlor epoxide, dieldrin, and aldrin were detected in some of the samples.

Blasco et al. (2003) investigated 42 organo chlorine, carbamate, and organophosphorus pesticide residues in 50 samples of honey from local

markets of Portugal and Spain during year 2002. Most of the pesticides found in honey were organochlorines. Among them, γ-HCH was the most frequently detected in 50% of the samples, followed by HCB in 32% of the samples, and the other isomers of HCH (α-HCH and β-HCH) in 28% and 26% of the samples, respectively. Residues of DDT and their metabolites were detected in 20% of the samples. These authors observed that honey consumers of both countries should not be concerned about the amounts of pesticide residues. On the contrary, in our study, most of the honey samples were contaminated with various organochlorine pesticide residues, and most of them exceeded Turkish Alimentarius Codex maximum residual limits (Turkish Alimentarius Codex 1997, 2005), so some honeys can be dangerous for consumer in Turkey.

Driss et al. (1994) investigated organochlorine pesticides residue in 28 samples of honey from many countries (Tunisia, Venezuela, Siberia, Canada, Italy, France, Egypt, Madagascar, and South China). They found that *p*,*p*′-DDE, one of the metabolites of *p*,*p*′-DDT, was the only compound detected in 24 samples (85.7%), and its mean concentration was 0.58 ng g^{-1}. In the present study, 4,4′-DDE was determined in all of the honey samples, and the mean concentration value of that residue was 0.0077 μg g^{-1}.

Blasco et al. (2004) determined nine organochlorine pesticide residues (α-, β-, and γ-HCH, hexachlorobenzene, aldrin, *p*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDT, and *p*,*p*′-DDT) in 49 samples of honey collected from markets of Portugal and Spain. They found that only 12 samples were acceptable under EU regulations.

Muino and Lozano (1991) determined organochlorine pesticides (lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, *p*,*p*′-DDT, and methoxychlor) in honey samples from Spain. Authors found that quantification limits for different pesticides were ranged from 0.56 to 2.78 μg kg^{-1}. In the present study, the mean concentrations of pesticide residues were ranged from 0.0008 to 0.1180 μg g^{-1}.

Organochlorine pesticides have been restricted or banned in agriculture because of their persistence and bioaccumulation in the environment. However, these pesticides are still frequently found in soil (Meijer 2003a, b). Biotransportation of organochlorine from contaminated soil to air and from root to tissues of different plants (Gonzalez et al. 2003) and to organisms were demonstrated in different studies (Hamers et al. 2003; Hoshi et al. 1998).

In conclusion, it was shown that contamination levels of these residues could be considered a serious public health problem according to Turkish Alimentarius Codex maximum residual limits for all residues except for α-HCH and *cis*-heptachlor epoxide. Therefore, a control of organochlorine pesticide residues in honey is necessary.

Acknowledgements This study was financed by Selcuk University, Scientific Research Foundation (BAP - Bilimsel Araştırma Projeleri) under project FBE - Fen Bilimleri Enstitüsü 06201048. The authors wish to acknowledge their support of this project.

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