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Levels of Organochlorine Pesticide Residues in Butter Samples Collected from the Black Sea Region of Turkey

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Abstract The aim of the present study was to evaluate the levels of 9 organochlorine compounds (aldrin, hexachlorobenzene, 2,4-DDE, 4,4-DDE, 2,4-DDT, 4,4-DDT, and α-, β-, and γ-HCH) in butter samples collected in the Eastern, Middle and Western Black Sea Regions of Turkey between October 2009 and June 2010. The liquid–liquid extraction method was used to extract the organochlorine compounds from the samples and the measurements were performed by using a gas chromatograph-electron capture detector system. DDT metabolites, aldrin, hexachlorobenzene (HCB), and α-, and γ-HCH were not detected in the samples but β-HCH was detected in 3 of a total of 88 samples. In the first period, only one sample from the West Black Sea Region was β-HCH positive (0.014 mg kg^{-1}). The other β-HCH positive samples collected in Middle and West Black Sea Regions in the second period had a concentration of 0.066 and 0.019 mg kg^{-1}, respectively. All concentrations of the detected compounds exceeded the legal limits of 0.003 mg kg^{-1} for β-HCH, as prescribed by the Turkish Food Codex, and therefore pose a potential health risk for consumers. The contamination detected is most likely due to the past usage of β-HCH in agriculture and its long term persistence in the environment. These results strongly suggest that further research should be focused on the detection of pesticide residues in agricultural areas across the nation.

Keywords Organochlorine pesticide · Butter · Black Sea · Gas chromatography

Organochlorine pesticides (OCs) are important component of chemical pollutants (Sarkar et al. 2008). About 80 % of pesticides are used in agriculture and move in the environment by means of volatilization, runoff, infiltration and transport along the food chain. Although the application of OCs has been banned for a considerable period in many countries, the residues continue to have a significant impact on the environment and its ecosystems (El-Shahawi et al. 2010). In developing countries, OCs have been, or are still being used, for malaria control and against livestock ectoparasites and agricultural pests, as they are comparatively cheap and effective (Waliszewski et al. 1997; Nizamlioglu et al. 2005; Sarkar et al. 2008). In addition, OCs have slow degradation rates because of factors such as chemical stability, very low water solubility and resistance to microbial degradation (Darko and Acquaah 2008; Tiemann 2008). Therefore, the air, water and soil can be contaminated with the residues of these pesticides. They accumulate in milk producing animals, if they are fed with contaminated feed, including grass and hay, or inhale them (Pandit et al. 2002). Owing to their high lipid solubilities, the OCs and their residues are primarily stored in the fat-rich tissues of organisms. Consequently, the residues are

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translocated and excreted through milk fat (Waliszewski et al. 1997). Eventually, the consumers of milk and milk products can be exposed to the residues, which get concentrated in fat-rich products, such as butter and cheese (Erdogrul et al. 2005).

At present, due to their possible reproductive and carcinogenic effects and high accumulation levels in living organisms, the use of OCs is restricted or banned in the developed countries (Suarez et al. 1998; Badia-Vila et al. 2000). In Turkey, OCs were first used against pests in 1945, and large quantities were used in the 1960s and 1970s (Cok et al. 1997). Their usage was restricted in 1979 (Karakaya et al. 1987) and most of them have been banned since 1983 (Erdogrul et al. 2005). However, the residues of OCs are still circulating in various food chains. They were detected in milk and milk products because of their illegal usage in some parts of Turkey, albeit in very small amounts (Kolankaya 2006) and persistent and highly stable under most environmental conditions (Pardio et al. 2003).

Milk, principally cow's milk, and its derivative products, especially cheese and butter, have a special place in the human diet and constitute one of the most important media for OCs monitoring (Suarez et al. 1998). There have been many studies on OCs in dairy products in most regions of Turkey, except in the Black Sea Region, which is in the north of Turkey. In the past two decades, the Black Sea has experienced problems with organochlorine compounds, as well as other chemical pollutants, due to their possible illegal usage (Tuncer et al. 1998; Kurt and Ozkoc 2004). To develop a better understanding of the current situation, the aim of the present study was to determine the concentrations of some OC residues (aldrin, hexachlorobenzene, 2,4-DDE, 4,4-DDE, 2,4-DDT, 4,4-DDT, and α-, β-, and γ-HCH) in butter produced in different parts of the Black Sea region between October 2009 and June 2010.

Materials and Methods

A total of 88 butter samples produced by 44 different firms in the period of October–November 2009 (1st period) and May–June 2010 (2nd period) were obtained randomly from shops in the East, Middle, and West Black Sea Regions of Turkey (Fig. 1). Butter samples of 0.5–1 kg were transported to the laboratory in an insulated container at about 4°C and analyzed upon arrival.

The analytical grade solvents, *n*-hexane, acetonitrile, dichloromethane, petroleum ether, diethyl ether, acetone, methanol and dodecane, were purchased from Merck (Darmstadt, Germany). All the pesticide standards were purchased from Dr. Ehrenstorfer (GmbH, Germany). Butter samples were analyzed for nine organochlorine compounds (aldrin, hexachlorobenzene, 2,4-DDE, 4,4-DDE, 2,4-DDT, 4,4-DDT, and α-, β-, and γ-HCH).

The determination of OCs levels was performed according to a method described by Bordet et al. (2002), with some modifications. The pesticides were extracted from butter by cryogenic extraction. Firstly, the butter samples were heated to 40°C in an incubator and homogenized. Half a gram of the butter sample was then placed in a centrifuge tube. Three mL of acetonitrile (ACN) and one mL of dichloromethane (DCM) (Merck, Germany) (75 + 25, v/v) mixture were added to the butter sample. They were mixed vigorously with a vortex, centrifuged for 20 min at 1500 rpm and −9°C, and the supernatant was collected. For the lower phase, the procedure was repeated using the same solvent mixture. The sample extract was then concentrated to 2 mL under a nitrogen stream at 35°C (solution A). A C_{18} SPE cartridge (1 g/6 mL, Phenomenex, USA) was used for clean-up. The cartridge was twice conditioned with 5 mL each of petroleum ether, acetone, and methanol (Merck, Germany). After conditioning, solution A was loaded into the cartridge. The analyte was eluted from the cartridge with 10 mL of ACN. One hundred microliters of dodecane (Merck, Germany) was added to the final analyte. The mixture was evaporated under a nitrogen stream at 35°C. The analyte was dissolved in n-hexane (solution B). The second clean-up was carried out with a florisil cartridge (1 g/6 mL, Phenomenex, USA) which was conditioned with 10 mL of n-hexane. Solution B was then loaded into the cartridge. The sample was eluted with 10 mL of petroleum ether and diethyl ether (Merck, Germany) (98 + 2, v/v; 1 drop/s) and following that with 12 mL of petroleum ether-diethyl ether (85 + 15, v/v; 1 drop/3 s). The two extracts were mixed and dried under a nitrogen stream at 35°C. The final extract was dissolved with 2 mL of n-hexane to produce Solution C.

The OC pesticide residues were analyzed using Gas Chromatography (Shimadzu, GC-17A, Japan) with an Electro Chemical Detector and a TRB-5 fused silica capillary column (30 m length × 0.32 mm i.d. × 0.25 μm film thickness, Teknokroma, Spain). The column oven temperature program was as follows: 100°C (3 min) at 10°C min^{-1} to 200°C (3 min), at 3°C min^{-1} to 225°C (3 min), at 2°C min^{-1} to 270°C (3 min), at 1°C min^{-1} to 275°C (10 min). The temperatures of the injector and detector were 260°C and 280°C, respectively. Samples (1 μL) were injected using a splitless injection mode. The concentrations of OCs were calculated from the peak heights. The correlation coefficients of the OC calibration curves were 0.989. Recovery percentages were determined to establish the efficiency of the method. The recoveries were determined by adding known amounts of OC standards (0.125, 0.250 and 0.500 mg kg^{-1}) to the blank samples before extraction. The recovery percentages were

in the range of 60–136 % for the OC compounds. The limits of detection (LODs) of the OCs were defined as three times that of the signal-to-noise ratio (S/N). The LODs for the OCs were 0.26–2.59 ng g^{-1}.

Results and Discussion

The chromatograms of organochlorine pesticides for the mix standards at 0.25 mg kg^{-1} and β-HCH positive sample are shown in Figs. 2 and 3, respectively. The recovery rates and maximum residue limits (MRLs) accepted by the Turkish Food Codex for OCs are presented in Table 1.

DDT metabolites, aldrin, hexachlorobenzene, α-, and γ-HCH were not detected in any butter samples. However, β-HCH was found in 3 (3.41 %) of a total of 88 samples. In the first period in 2009, only one sample from the West Black Sea Region was β-HCH positive (0.014 mg kg^{-1}). The other β-HCH positive samples that were collected in Middle and West Black Sea Regions in 2010 (second period) had concentrations of 0.066 and 0.019 mg kg^{-1}, respectively. The β-HCH levels in the butter samples were found to be higher than the limit of 0.003 mg kg^{-1} prescribed by the Turkish Food Codex (2009). The results are similar to those of a study carried out in Ankara, Turkey (Yentur et al. 2001). On the other hand, Nizamlioglu et al. (2005) carried out a study of 18 butter samples collected from Konya and found 94 % incidence of OCs. In their study, 87 % and 78 % of the butter samples were contaminated by one or more HCH isomers or DDT and their metabolites, respectively. The high incidence of OCs in butter samples is a result of inappropriate use in many developing countries, including Turkey (Nizamlioglu et al. 2005). In Jordan, organochlorine pesticide residues (aldrin, dieldrin, endrin, heptachlor, hexachlorobenzene, HCH isomers and DDT metabolites) were determined in dairy products. β-HCH had the highest incidence among the various OCPs. The mean concentration of β-HCH in butter samples were found 0.019 mg kg^{-1} (Salem et al. 2009). In Mexico, Waliszewski et al. (2003) analyzed for the presence of HCB, β-HCH and DDT metabolites in 200 butter samples from Veracruz. The incidence percentages for HCB, β-HCH and DDT metabolites were 99 %, 99 % and 100 %, respectively. In another study, HCB residues were analyzed in many kind of food samples such as meat and meat products, fish and sea food and dairy products and the highest HCB levels were found in butter samples (0.860 ng g^{-1}) (Perelló et al. 2012). In research conducted by Guvenc and Aksoy (2010) in Samsun, a city of the Middle Black Sea Region of Turkey, no organochlorine and synthetic pyrethroid pesticides were detected in any of the 100 raw milk samples tested. Nag and Raikwar (2008) have reported that; 206 of 325 bovine milk samples (63.38 %) were found

Fig. 1 Study area and location of sampling points; East Black Sea ([1]Artvin, [2]Rize, [3]Trabzon, [4]Bayburt, [5]Gumushane, [6]Giresun), Middle Black Sea ([7]Ordu, [8]Tokat, [9]Samsun, [10]Amasya, [11]Sinop, [12]Corum) and West Black Sea Region ([13]Kastamonu, [14]Bartin, [15]Karabuk, [16]Zonguldak, [17]Bolu, [18]Duzce)

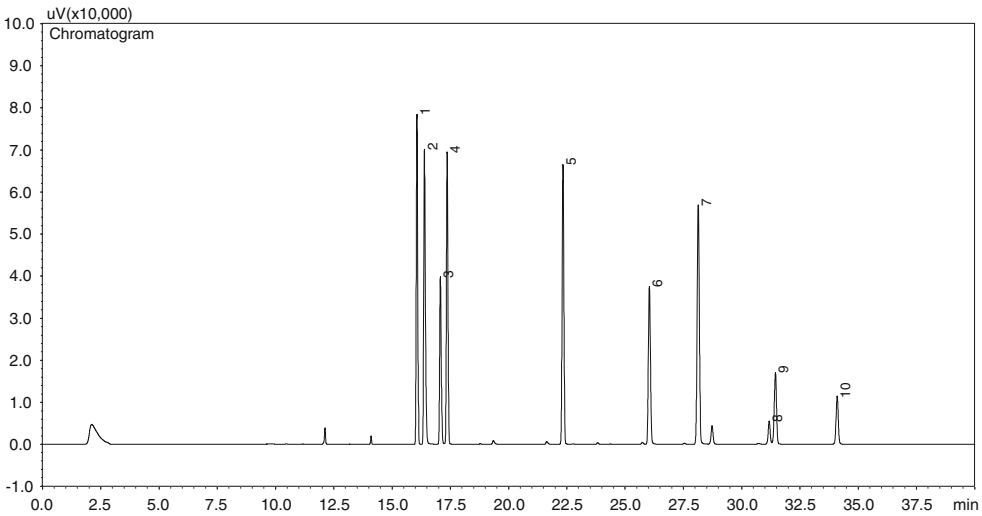

Fig. 2 Chromatogram of organochlorine pesticide mix standards at 0.25 mg kg^{-1}, 1, α-HCH; 2, HCB; 3, β-HCH; 4, γ-HCH; 5, Aldrin; 6, 2,4′ DDE; 7, 4,4′ DDE; 8, 4,4′ DDT; 9, 2,4′ DDT; 10, 4,4′ DDT

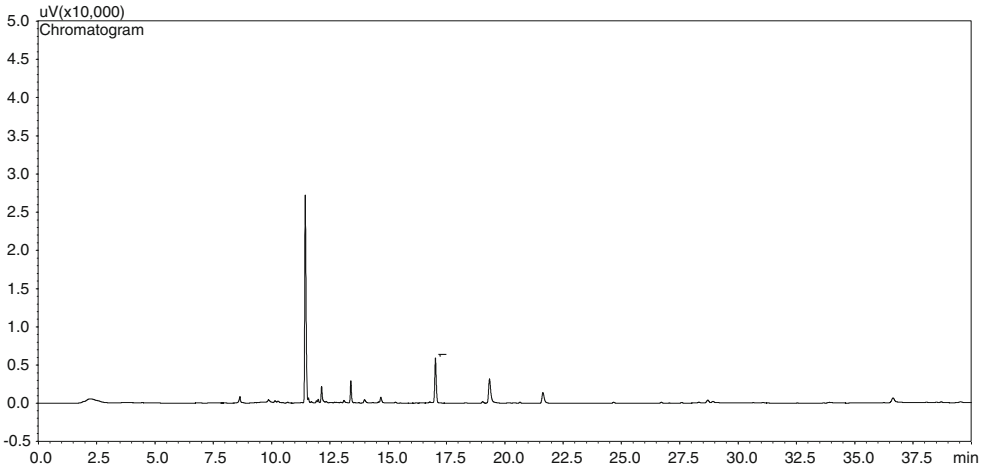

Fig. 3 Chromatogram of β-HCH positive sample; [1]β-HCH

Table 1 Mean recovery percentages for OCs, their mean concentrations in butter samples from the Black Sea region of Turkey, and their MRLs in the Turkish Food Codex

Compound	Mean recovery (%)	Mean values of OC residues detected (mg kg^{-1})	MRLs (mg kg^{-1})
Alpha-HCH	87 ± 8.01	bd	0.004
HCB	60 ± 10.80	bd	0.01
Beta-HCH	81 ± 3.86	0.033 ± 0.023	0.003
Gama-HCH	90 ± 8.06	bd	0.008
Aldrin	70 ± 11.58	bd	0.006
2,4-DDE	76 ± 9.53	bd	Σ0.04
4,4-DDE	71 ± 8.60	bd	
2,4-DDT	115 ± 10.96	bd	
4,4-DDT	136 ± 16.99	bd	

bd Below detection

contaminated with residues of different OCPs in Bundelkhand region of India. Technical-grade HCH principally consists of five isomers of α-HCH (60–70 %), β-HCH (5–12 %), γ-HCH (10–15 %), δ-HCH (6–10 %), and ε-HCH (3–4 %). α-, β-, and γ-isomers are the most common isomers in the environment. Amongst the HCH isomers, β-HCH is the most resistant to hydrolysis and environmental degradation (Doong et al. 2002). Its presence is probably associated with the past use of HCH on farms or conversion from γ-HCH in the environment by bioisomerization. Furthermore, the isomerized β-HCH is a more stable form than γ-HCH in the environment in the presence of sunshine and fungus. Therefore, this isomer is characterized by high persistence and lipophilicity, accumulating in the lipid parts of tissues (Waliszewski et al. 2003).

In Turkey, some studies related to OCs in milk and milk products (Karakaya et al. 1987; Cok et al. 1997; Cok et al. 2004; Erdogrul et al. 2004; Nizamlioglu et al. 2005) reported that OCs, especially HCH, are inclined to disperse and tend to accumulate in food chains, in spite of the restrictions and bans on their use. However, the levels of pesticides found in the present study indicate that the concentrations of OCs in the butter samples have dropped

markedly in Turkey since their restricted or ban in 1983 (Erdogrul et al. 2005).

The present study in the Black Sea Region detected notable amounts of HCH in butter samples. Furthermore, HCH isomers were the pesticides most frequently reported in recent studies from surface waters and sediments in the Black Sea Region (Bakan and Ariman 2004; Geyikci and Buyukgungor 2011). High concentrations of OCs in water and sediment samples are attributable to high rates of influx of contaminants into estuaries through rivers from drainage of contaminated water from surrounding agricultural fields; their abiotic degradation is influenced by various physicochemical characteristics of the sediments, including texture, pH and salinity, as well as microbial activity (Sarkar et al. 1997). Therefore, there may be associated health risks for the public in the Black Sea Region.

Pesticide residues detected in the present study are at similar or lower concentrations compared to recent studies in this region (Yentur et al. 2001; Bakan and Ariman 2004; Geyikci and Buyukgungor 2011). However, all the detected concentrations in the present study still exceeded the legal limits of the Turkish Food Codex. Given the detection of OCs in butter samples, more milk and milk product monitoring programs for organochlorine pesticides and other persistent contaminants are required in the Black Sea Region, for both environmental monitoring and human health-related reasons.

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