Organochlorine pesticide residues in dairy products in Jordan

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A B S T R A C T
The use of aldrin, dieldrin, endrin, heptachlor and hexachlorobenzene (HCB) has been banned in Jordan officially in 1981, and of dichlorodiphenyltrichloroethane (DDT) in 1995. However, residues of such compounds can still be found in the environment and in foodstuffs. Dairy products are an important exposure route for organochlorine pesticides (OCPs) to humans. For this reason, the presence of OCP residues in 233 dairy product samples; comprising milk, butter, cheese, labaneh and yoghurt collected from Jordan was determined. All samples were analyzed for their residual contents of aldrin, DDT and metabolites (DDTs), dieldrin, endosulfan isomers, endrin, hexachlorocyclohexane isomers (HCHs), heptachlor and HCB. Levels of these compounds were determined by gas chromatography with electron capture detector (GC-ECD). The results indicated that 9% (21/233), 8.5% (20/233), 6% (14/233) and 2.1% (5/233) of the examined samples were contaminated with β-HCH, pp'-DDE, α-HCH and γ-HCH, respectively. Heptachlor and α-endosulfan were only present in less than 2% of the analyzed samples. None of the samples revealed the presence of aldrin, op'-DDD, pp'-DDD, op'-DDE, op'-DDT, pp'-DDT, dieldrin, β-endosulfan, endrin and HCB at their detection limits. The order for the contamination in the analyzed dairy products was labaneh > cheese > yoghurt > butter > milk. This study has provided the preliminary information on the concentration of OCPs in dairy products for the first time in Jordan. The results will help in a scientific assessment of the implications of pesticide residues with regards to human risks in Jordan.

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1. Introduction

Organochlorine pesticides (OCPs) are compounds that, because of their pressures and partitioning behavior under ambient conditions, are persistent and highly stable under most environmental conditions. They are fat-soluble, thus leading to its bioaccumulation through food chain. Their residues have become a factor for the environmental pollution and their toxic effects have been observed in humans and animals. The acute health risks of OCPs, their long persistence and tendency to accumulate in body tissues have raised a great concern about possible human health impacts due to low but chronic exposure. Some of OCPs have been considered as “endocrine-disrupting chemicals” (Colborn et al., 1993; Kalpana, 1999) and carcinogenic substances (Surendranath et al., 1998). The International Agency for Research on Cancer (IARC) classified compounds such as dichlorodiphenyltrichloroethane (DDT), mirex, toxaphene, hexachlorobenzene (HCB) or hexachlorocyclohexane (HCH) as possibly carcinogenic to humans (group 2B) (IARC, 2006).

Jordan was one of the first countries in the Middle East to take prompt and appropriate decisions to protect human health and environment against the hazards caused from using OCPs. Therefore, Jordan has taken unilateral measures by holding the use and handling of OCPs (aldrin, chlordane, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene) since the early 1980s for control of disease vector till 1995. Furthermore, Jordan signed the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) on 18/1/2002 and ratified it on 8/11/2004. Jordan has a National Implementation Plan (NIP) for SC on POPs, to identify and reduce sources, and to monitor for effectiveness in reducing ambient levels and human/wildlife exposures. At present, there is no manufacturing formulation, import and legal use for any OCPs in Jordan.

Human exposure to OCPs is attributed mainly to food chain. The contamination of food, including dairy products, by OCPs is a worldwide phenomenon. OCPs get accumulated in fat-rich dairy products, such as butter, cheese, etc. and as such, consumers of dairy products are exposed to these residues (Bentabol and Jodral, 1995; Waliszewski et al., 1997; Kalantzi et al., 2001; Jafari et al., 2008). Although the studied OCPs use has been restricted or banned in many countries, several studies have documented their presence in dairy products (Kalra et al., 1983; Mukherjee and Gopal, 1993; Lozada et al., 1996; Wong and Lee, 1997; Kalra et al., 1999; Fontcuberta et al., 2008). This fact has caused concern since dairy products are an important exposure route for persistent pollutants in general. No data is however available on the levels of OCP residues in dairy products which constitute an important part of meal of many communities in Jordan. Since most Jordanians...
consume milk daily as a custom, either in the form of beverages or in its various forms such as butter, cheese, labaneh (strained yoghurt) and yoghurt, there is need for monitoring levels of OCP residues in dairy products. Therefore, this work was carried out to investigate the extent of contamination with aldrin, DDTs, dieldrin, endosulfan isomers, endrin, HCHs, heptachlor and HCB residues in dairy products, and to assess human exposure to OCPs through the consumption of these products.

2. Materials and methods

2.1. Sampling

A total of 233 samples of dairy products; comprising 70 of milk, 25 of butter, 46 of cheese, 23 of labaneh and 69 of yoghurt of different brands available in the local market a cross the country were selected randomly to determine the concentrations of OCPs. Labaneh was included in this study as it is very common in Middle Eastern diets. It is strained yoghurt made of full fat yoghurt that has been strained overnight. Samples were kept in cold ice during their transportation to the Industrial Chemistry Centre (ICC) testing laboratory where they were kept at 4 °C until analysis. Samples were subjected to analysis within 24 h from their arrival.

2.2. Chemicals and reagents

Acetone, acetonitrile, anhydrous sodium sulfate, dichloromethane, diethyl ether, n-hexane and petroleum ether of pesticide residue grade were purchased from Scharlau, Spain. Analytical standards of aldrin, DDE (op', pp'), DDD (op', pp'), DDT (op', pp'), dieldrin, endosulfan (α, β), endrin, HCH (α, β, γ), heptachlor and HCB were obtained from Dr. Ehrestorfer GmbH, Germany. Purities of pesticide standards were greater than 99%. Florisil, 60/100 mesh obtained from A&K Petrochem, USA, was activated at 550 °C for 12 h and kept in a closed container at room temperature. The needed amount was heated once again at 130 °C for 5 h prior to use.

2.3. Sample preparation, extraction and cleanup

Fat portions were extracted from each individual sample of milk, cheese, labaneh and yoghurt according to the method of the Association of Official Analytical Chemists (AOAC, 1995). Butter does not normally require extraction procedures. Partitioning of the extracted samples was carried out according to the method of AOAC. Briefly three grams or less of the fat was dissolved into 40 ml petroleum ether. This was partitioned three times into acetone/ether saturated with petroleum ether (3 × 30 ml). The acetone/ether fraction, after dilution with saline (600 ml), was again partitioned into petroleum ether (3 × 100 ml). This was dried over anhydrous sodium sulfate, and concentrated at 30 °C on a rotary vacuum evaporator to a volume less than 5 ml to be used for Florisil cleanup (Kodba and Voncina, 2007).

Cleanup of the extracted samples, to remove the residual fat, was performed by transferring the extract into a glass chromatographic column (25 mm i.d.) containing 25 g activated Florisil (60/100 mesh) topped with 1-cm layer of anhydrous sodium sulfate. The prepared column was rinsed with 100 ml petroleum ether, and then the extracted sample was transferred onto the column. The column was eluted with 300 ml eluent (20% dichloromethane + 80% petroleum ether). The collected eluate was concentrated to dryness on a rotary vacuum evaporator and dissolved in hexane to a volume of 5 ml (Alawi et al., 1992). An aliquot of each extract was transferred to 2-ml injection vials to be ready for the analysis with the electron capture gas chromatography.

2.4. Determination of organochlorine pesticide residual concentrations

The OCP residues were determined by analysis of samples using a Hewlett-Packard gas chromatograph (GC-HP 5890 Series II) equipped with a 63Ni electron capture detector (ECD), using a silica capillary column (HP-5 30 m × 0.32 mm i.d. with 0.25 µm film thickness). The carrier gas was helium at a flow rate of 2 ml min⁻¹ through column and 30 ml min⁻¹ make up. The gas chromatography oven temperature was initiated at 80 °C for 2.2 min, raised to 175 °C (at a rate of 30 °C min⁻¹), then raised to 225 °C (at a rate of 10 °C min⁻¹) and held for 2 min. Injection port temperature and detector temperature were maintained at 280 °C and 300 °C, respectively. The sample volume injected was 1 µl.

Calibration standard curves were created and OCP residues were quantitatively determined by comparison of the retention time and peak heights/areas of the sample chromatogram with those of standard solutions run under the same operating conditions. The concentrations of various residues in each sample were reported as mg kg⁻¹ on a fat basis.

2.5. Quality control and quality assurance

The method used for OCPs determination in dairy products was developed in ICC testing laboratory and has been validated, including control of blanks, spikes recovery and linearity. This method was accredited by the United Kingdom Accreditation Service (UKAS) in 2003, as part of the ISO/IEC 17025 accreditation process of the laboratory.

Recovery study was performed on dairy products spiked with pesticide standards. After extraction and solvent evaporation, the samples were analyzed according to the proposed method. The recovery values were calculated from calibration curves constructed from the concentration and peak area of the chromatograms obtained with standards of the OCP. Detection limits of the method were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC. Blank analyzes were also performed in order to check interference from the sample. Samples were analyzed in duplicate and represent the arithmetic mean. The detection limits, the average recoveries with their standard deviations (SDs) of OCPs are shown in Table 2. The average recoveries of OCPs in milk and milk products were from 89.4% to 102.3% and 79.4% to 103.6%, respectively, which indicates that the reproducibility of the method was satisfactory.

3. Results and discussion

3.1. Organochlorine pesticide residues in milk, butter, cheese, labaneh and yoghurt

The present study showed the presence of OCP residues in milk, butter, cheese, labaneh and yoghurt owing to their use in sanitary and agricultural purposes. A total of 233 dairy product samples obtained from different areas in Jordan during 2001–2007 were analyzed of which 48 (20.6%) samples were found to be contaminated with different OCP residues. The mean and range values of OCP residues in dairy products samples, on a fat basis (Table 2), are presented and discussed as below.

3.1.1. DDTs (dichlorodiphenyltrichloroethane and its metabolites)

The pp'-DDE was the only metabolite among the DDTs (op'-DDE, pp'-DDD, op'-DDE, pp'-DDE, op'-DDT, pp'-DDT) that was detected in levels higher than the detection limit. DDT was not detected, this due to the fact that there is no actual usage of DDT in recent times.
Out of the 233 samples analyzed, 5 (7.1%), 2 (8%), 8 (17.4%), 2 (8.7%) and 3 (4.3%) milk, butter, cheese, labaneh and yoghurt samples analyzed, respectively, were positive for pp'-DDE, with an overall detection of 8.6% (20/233) among the analyzed samples of the dairy products. The mean values of the residual concentrations of pp'-DDE in the examined milk, butter, cheese, labaneh and yoghurt samples, were 0.027, 0.009, 0.032, 0.004 and 0.014 mg kg⁻¹ fat, respectively. Cheese samples showed the highest concentration of pp'-DDE compound, compared to the other dairy products. Out of the 20 contaminated samples, concentration of pp'-DDE in two milk samples was higher than MRL for DDT (sum of pp'-DDE, op'-DDD, pp'-DDE and pp'-DDD) of 0.02 mg kg⁻¹ fat (FAO/WHO, 2006).

The results obtained in other monitoring studies in other countries of OCPs in milk are compared with the present study in Table 3. As shown in Table 3, the level of pp'-DDE in milk samples is lower than those recorded in China (Zhong et al., 2003), Bundelkhand, India (Nag and Raikwar, 2008), Medellin, Mexico (Pardio et al., 2003), Slovakia (Prachar et al., 1995), and higher than those reported in Ghana (Darko and Acquaah, 2008), Germany (Weigert et al., 1983), Maharashtra, India (Pandit et al., 2002), Paso San Juan and Tlalixcoyan, Mexico (Pardio et al., 2003) and Spain (Lozada et al., 1996).

Higher levels of pp'-DDE, were detected in butter from Mumbai and Punjab state, India (Pandit and Sahu, 2002; Battu et al., 2004), Mexico (Waliszewski et al., 1997) and Slovakia (Prachar et al., 1995). On the other hand, much lower pp'-DDE concentration had been detected in cheese analyzed in Maharashtra (Pandit et al., 2002) and Mumbai, India (Pandit and Sahu, 2002). In contrast, higher levels were detected in cheese in Ghana (Darko and Acquaah, 2008), India (Kalra et al., 1983) and Spain (Suarez et al., 1998) and in yoghurt from Ghana (Darko and Acquaah, 2008).

### 3.1.2. HCHs (hexachlorocyclohexane isomers)

Among three different isomers of HCH (α-isomer, β-isomer, γ-isomer), α-isomer was found to be present in 1 (1.4%), 3 (6.5%), 2 (8.6%) and 8 (11.6%) milk, cheese, labaneh and yoghurt samples analyzed, respectively. The mean values of the residual concentrations (mg kg⁻¹ fat) of α-HCH in the examined samples of milk, cheese, labaneh and yoghurt in the present study, were 0.060, 0.027, 0.014 and 0.044, respectively. The α-isomer of HCH was below detection in all the butter samples. The predominantly accumulating, most persistent and metabolically stable β-isomer of HCH could be identified in 6 (8.6%), 4 (16%), 2 (4.3%), 1 (4.3%) and 8 (11.6%), with the mean concentration of 0.073, 0.019, 0.015, 0.045 and 0.028 mg kg⁻¹ fat in milk, butter, cheese, labaneh and yoghurt, respectively.

Most active and important isomer of HCH, γ-isomer (lindane) was detected in three samples (13%) of labaneh with the concentration range varying between 0.01 to 0.05 mg kg⁻¹ fat and mean concentration being 0.027 mg kg⁻¹ fat. In addition, the γ-isomer of HCH was detected in one sample of cheese and yoghurt with mean concentration of 0.030 and 0.022 mg kg⁻¹ fat, respectively. The MRL of 0.010 mg kg⁻¹ fat, as designated for lindane (FAO/WHO, 2006), was violated in all labaneh, cheese and yoghurt contaminated samples. The presence of lindane above its MRL in all contaminated samples in the present study is viewed with serious concern, as it is carcinogenic in nature, and may affect the functioning of other vital organs of the body (Vettorazzi, 1975). Furthermore, in the fourth Conference of the Parties to the SC on POPs, the α-, β- and γ-isomer have been implemented in the list of SC on POPs (COP-4, 2009).

The mean total HCH (Σ-HCH) concentration was found to be 0.083, 0.019, 0.028, 0.031 and 0.043 mg kg⁻¹ fat in milk, butter, cheese, labaneh and yoghurt contaminated samples, respectively. The present results revealed that the frequency of different isomers of HCH residues in dairy products on fat basis was of the order of β > α > γ. In addition, with regard to mean concentration in the contaminated samples β-isomer was highest (0.039 mg kg⁻¹ fat) followed by α-isomer (0.037 mg kg⁻¹ fat) and γ-isomer (0.026 mg kg⁻¹ fat). The ratios and levels of HCH isomers are often used as evidence of the passed or current technical HCH application (Jafari et al., 2008). However, as the β-HCH is the most abundant isomer, this suggests rather older residues/past than current usage of technical mixtures in Jordan.

As shown in Table 3, the level of β-HCH detected in milk in this study is higher than those reported from other countries except those reported in Bundelkhand, India (Nag and Raikwar, 2008). Levels of HCHs in analyzed cheese samples of the present study are comparable with those reported in cheese from Mumbai (Pandit and Sahu, 2002) and Punjab state, India (Battu et al., 2004). Mean levels of β-HCH found in butter is lower than those reported in India (Kalra et al., 1983) and Mexico (Waliszewski et al., 1997) and higher than those detected in better in Maharashtra (Pandit et al., 2002) and Mumbai, India (Pandit and Sahu, 2002).

### 3.1.3. Endosulfan (α- and β-isomers)

The residues of endosulfan, an insecticide of the cyclodiene group of OCPs, was only detected in one milk sample in the form of its α-isomer, with mean concentration of 0.03 mg kg⁻¹ fat,
which was above the MRL (0.004 mg kg$^{-1}$ fat) of endosulfan in milk (FAO/WHO, 2006). As the detection limit (0.004 mg kg$^{-1}$ fat) of endosulfan was similar to MRL, consequently, this can have masked the occurrence of endosulfan in this study.

Endosulfan is generally assumed to be not passing into the milk. So, most of the monitoring studies of dairy products in respect of OCP residues have not reported about occurrence of residues of endosulfan excepting that of Darko and Acquaah (2008) and Nag and Raikwar (2008) who reported the presence of endosulfan in dairy products in Ghana and India, respectively.

3.1.4. Aldrin, dieldrin, endrin, heptachlor and HCB

Heptachlor was detected in two milk samples with a mean concentration of 0.026 mg kg$^{-1}$ fat and its MRL of 0.006 mg kg$^{-1}$ fat (FAO/WHO, 2006) was exceeded in both samples. Residues of aldrin, dieldrin, endrin and HCB have not been detected in any of the samples. Due to the limited use of these insecticides, their residues are less likely to occur in dairy products in Jordan as well as in other countries (Zhong et al., 2003). However, aldrin and dieldrin have been detected in dairy products from India (Suarez et al., 2003; Battu et al., 2004; Nag and Raikwar, 2008).

HCB is known as a by-product of some industrial chlorinated processes and as an impurity, and in combustion process too, which are probably the main sources of environmental contamination. Nasir et al. (1998) found high mean levels of HCB in human milk from Jordan, highest in industrial areas. Environmental levels in Jordan can have declined due to restrictions and ban of the chemical and this may explained our result where HCB was not found in any of the samples in the present study.

3.2. Incidence of contamination with the different organochlorines

Generally, the incidence of contamination of the examined samples by the OCPs followed the order of $\beta$-HCH > $\gamma$-HCH > $\alpha$-HCH > heptachlor > heptachlor + $\alpha$-endosulfan (Fig. 1). Among various OCPs examined in the present study, HCHs and DDTs are the most prominently noticed compounds, as they were detected at a high incidence. On the other hand, heptachlor and $\alpha$-endosulfan compounds were detected at a low incidence and they were only present in less than 2% of the analyzed samples. The high detection limits and the low quantities of heptachlor and $\alpha$-endosulfan could mask the occurrence of these OCPs in dairy products.

Likewise, HCHs and DDTs have been found to be the principal contaminants of dairy products in other countries (Gupta et al., 1997; Waliszewski et al., 1997; Pandit and Sahu, 2002; Pandit et al., 2002; Pardio et al., 2003; Waliszewski et al., 2003; Zhong et al., 2003; Battu et al., 2004; Nag and Raikwar, 2008).

3.3. Variations in organochlorine among the different dairy products

Amongst the five dairy products examined (milk, butter, cheese, labaneh, yoghurt) in the present study, the incidence of contamination of all OCPs detected in milk were lower than those detected in other products (Fig. 2). The order of contamination in the analyzed samples was labaneh > cheese > yoghurt > butter > milk. The main reason for the order of contamination is the fat content, which is the main site for organochlorine accumulation, in the milk is lower than that determined in other dairy products (Table 2). Although the fat content of the butter was the highest among dairy products, the levels of OCPs in butter were lower than other dairy products. This could be attributed to the source of the milk (cow, goat or sheep). Cow, goat and sheep are all ruminants, and the distribution, metabolism, and clearance of OCP residues may be considerably different. Further, the amount and composition of the milk from milk producing animals also may alter the distribution and
clearance of residues and consequently the levels of OCP residues in dairy products.

Similar results have been obtained in other studies where butter, cheese or yoghurt samples showed higher concentrations of OCPs compared with milk (Pandit et al., 2002; Pandit and Sahu, 2002; Darko and Acquaah, 2008). Interestingly, we have not found any references reporting OCP residues in Labaneh, which is considered as traditional food in the Middle East and South Asia. So far, this study could be the first one of its kind that provided the concentration of some OCPs in Labaneh.

The overall results from this study show that dairy products are an important route for OCPs, and consumption of these products contribute in high degree to human exposure to OCPs. Breast feeding women can transfer the OCPs to sucking infants in Jordan as showed by Alawi et al. (1992) and Nasir et al. (1998). The presence of OCP residues could be attributed to the extensive use of HCH and DDT from 1960s until their use in Jordan was officially banned in 1981 and 1995, respectively. The residues of such persistent compounds may exist for a long time in the environment and so, they would require much more time to be completely phased out from the system. Despite the low percentage 4.3% (10/233) of samples exceeding the MRLs in this study, these compounds represent a potential risk to human heath because of their accumulation properties in human fat tissue. Finding of possible local sources could also be a measurement for future studies.

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**References**


**Table 3**

Levels of organochlorine pesticides residues (mg kg⁻¹ on fat basis) detected in milk fat compared with results from different countries.

<table>
<thead>
<tr>
<th>Country/location</th>
<th>pp’-DDE</th>
<th>α-HCH</th>
<th>β-HCH</th>
<th>γ-HCH</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>China/Beijing</td>
<td>0.038</td>
<td>0.024</td>
<td>0.011</td>
<td>0.012</td>
<td>Zhong et al. (2003)</td>
</tr>
<tr>
<td>Ghana/Kumasi</td>
<td>0.001</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.d.</td>
<td>Darko and Acquaah (2008)</td>
</tr>
<tr>
<td>Germany</td>
<td>0.007</td>
<td>0.010</td>
<td>0.006</td>
<td>0.015</td>
<td>Weigert et al. (1983)</td>
</tr>
<tr>
<td>India/Bundelkhand</td>
<td>0.036</td>
<td>0.019</td>
<td>0.099</td>
<td>0.010</td>
<td>Nag and Raikwar (2008)</td>
</tr>
<tr>
<td>India/Maharashtra</td>
<td>0.015</td>
<td>0.011</td>
<td>0.015</td>
<td>0.004</td>
<td>Pandit et al. (2002)</td>
</tr>
<tr>
<td>Mexico/Medellin</td>
<td>0.039</td>
<td>0.013</td>
<td>0.023</td>
<td>0.049</td>
<td>Pardio et al. (2003)</td>
</tr>
<tr>
<td>Mexico/Paso San Juan</td>
<td>0.018</td>
<td>0.013</td>
<td>0.017</td>
<td>0.022</td>
<td>Pardio et al. (2003)</td>
</tr>
<tr>
<td>Mexico/Talixcoyn</td>
<td>0.024</td>
<td>0.031</td>
<td>0.069</td>
<td>0.128</td>
<td>Pardio et al. (2003)</td>
</tr>
<tr>
<td>Slovakia/Bratislava</td>
<td>0.051</td>
<td>0.005</td>
<td>0.006</td>
<td>0.004</td>
<td>Prachar et al. (1995)</td>
</tr>
<tr>
<td>Spain/Léon</td>
<td>0.005</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.003</td>
<td>Lozada et al. (1996)</td>
</tr>
<tr>
<td>Jordan</td>
<td>0.027</td>
<td>0.060</td>
<td>0.073</td>
<td>n.d.</td>
<td>Present study</td>
</tr>
</tbody>
</table>

n.a.: Not analyzed.

n.d.: Not detected.

![Fig. 1](image1.png)  
**Fig. 1.** Percent of contaminated samples with different organochlorine pesticides analyzed.

![Fig. 2](image2.png)  
**Fig. 2.** Percent of dairy products contaminated with organochlorine pesticides analyzed.


