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Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan

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ABSTRACT

Organochlorine pesticide (OCP) residues in 519 samples; comprising eggs, chicken and meat (lamb and beef), collected from Jordan were determined. All samples were analyzed for their residual contents of aldrin, dichlorodiphenyltrichloroethane and metabolites (DDTs), dieldrin, endosulfan isomers, endrin, hexachlorocyclohexane isomers (HCHs), heptachlor, heptachlor epoxide and hexachlorobenzene (HCB). The samples were Soxhlet extracted for 8 h in 250 mL petroleum ether. The cleanup of the samples was performed by Florisil column chromatography and analysis was done on a gas chromatography equipped with an electron capture detector (GC-ECD). The results indicated that 28% (38/134), 20% (23/115) and 49% (131/270) of the examined eggs, chicken and meat samples, respectively, were contaminated with OCP residues. HCHs and DDTs are the most prominently noticed compounds, as they were detected at a high incidence. On the other hand, heptachlor, heptachlor epoxide, HCB, aldrin and endrin compounds were only present in less than 7% of the analyzed samples. These residues are present despite complete ban on the use of OCPs for agricultural purposes in Jordan. No residues of *op′*-DDD, *op′*-DDT, dieldrin, α-endosulfan and β-endosulfan were detected.

1. Introduction

Organochlorine pesticides (OCPs) have been used in the public health sector for disease vector control and in agriculture to control crop pests for the past several decades in Jordan. They are characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. Therefore, they can accumulate in human body fats and the environment posing problems to human health (Ejobi et al., 1996). Based on reports of their persistent, toxicity and adverse harmful effects to wildlife and humans, many OCPs were banned or restricted from use or trade in Jordan. Since the early eighties, aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex and toxaphene, which are also important constituents of the toxin group known as persistent organic pollutants (POPs), have been banned or prohibited. While dichlorodiphenyltrichloroethane (DDT) was allowed to use for control of disease vector till 1995. Furthermore, the global Stockholm convention (SC) on POP was ratified and entered into force on August 2004. Although most of OCPs are no in longer use in Jordan, they are still being found as residues and they are occurring in food now as a result of environmental contamination. Studies have documented their presence in mother milk (Alawi et al., 1992; Nasir et al., 1998) and dairy products including milk, cheese, yoghurt, butter and labaneh (Salem et al., 2009). Products found more widely include DDTs, HCHs and endosulfan.

The OCP residues may concentrate in the adipose tissues and in the blood serum of animals leading to environmental persistence, bioconcentration and biomagnification through the food chain. Pesticide contamination of chicken and meat resulting from feeding a diet containing a low concentration of pesticides is a well-established fact (Noble, 1990; Aulakh et al., 2006). OCP residues in feed may be ingested by herbivores and eventually find their way into the animal body which ultimately results in the contamination of milk, meat, eggs, etc. consumed by human being. Thus human body also gets contaminated. So far it has been reported that humans are mainly exposed to OCPs through ingestion. A study conducted in Tianjin, China, revealed that inhalation and dermal contact contributed to only 5.1% and 13.5% of the total intakes of DDTs and HCHs by adults, while ingestion through diet was responsible for 94.9% and 86.5% of the total, respectively (Guo, 2004).

The importance of eggs, chicken and meat consumption as a source of OCP has been established worldwide (Al-Omar et al., 1985; Kannan et al., 1992a,b; Antonio et al., 1994; Osibanjo and Adeyeye, 1997; Barkatina et al., 1999; Aulakh et al., 2006; Darko and Acquaah, 2007; Corrigan and Seneviratna, 2008; Fontcuberta et al., 2008; Windal et al., 2009). Therefore, the awareness and need for regular screening of these foodstuffs is necessary and is in interest of both international trade and consumers. Eggs, chicken and meat are among the most popular food items on the Jordanian diet of many communities in Jordan and because of their high fat content, they have drawn our concern in terms of human exposure to OCPs. The problem of food contamination particularly by

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pesticides becomes more critical, especially given that data baselines on the status of the pesticide levels in foods consumed and their dietary intake by the different groups of the population are nonexistent. Reports are lacking on OCP residues in eggs, chicken and meat marketed in Jordan. Thus, this work was carried out to investigate the extent of contamination with aldrin, DDTs, dieldrin, endosulfan isomers, endrin, HCHs, heptachlor, heptachlor epoxide and HCB residues in eggs, chicken and meat, that will assist in a scientific assessment of the impact of OCPs on public health, agriculture and environment in Jordan.

2. Materials and methods

2.1. Sampling

A total of 519 samples; comprising 134 of eggs, 115 of chicken and 270 of meat (lamb and beef) of different brands were collected at random from various market places in Jordan between 2001 and 2007. All of the collected eggs and chicken were locally produced. On the other hand 78% (211/270) of the collected meat samples were imported from different countries including Australia, China and India. 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. They were analysed for residues of OCPs within 24 h from their arrival.

2.2. Reagents

All solvents of pesticide residue grade were purchased from Scharlau, Spain. OCPs standards (aldrin, DDE (*op*′, *pp*′), DDD (*op*′, *pp*′), DDT (*op*′, *pp*′), dieldrin, endosulfan (α, β), endrin, HCH (α, β, γ), heptachlor, heptachlor epoxide and HCB) were obtained from Dr. Ehrebstorfer 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. Glassware was washed with detergent, rinsed with purified water and acetone and were heated to 180 °C for 2 h.

2.3. Sample extraction and cleanup

Approximately 10–15 g of eggs and 25–40 g of chicken and meat were placed into a beaker containing 30 g anhydrous sodium sulfate and mixed thoroughly to remove water. The mixtures were Soxhlet extracted with 250 mL of petroleum ether at 50 °C for 8 h (Tao et al., 2009). Extracts were processed in a rotary evaporator at 40–60 °C to remove petroleum ether and passed over N_2 to ensure dryness. Fat contents were determined by weighting the samples before and after extraction. Pesticide residues were detected and determined according to the Association Of Official Analytical Chemists (AOAC, 1995) methods. Briefly three grams of the fat was dissolved into petroleum ether, partitioned with acetonitrile saturated with petroleum ether and back-extracted into petroleum ether. This mixture was dried over anhydrous sodium sulfate, and concentrated at 30 °C on a rotary vacuum evaporator to a volume less than 5 mL, transferred to Florisil column with anhydrous sodium sulfate (Kodba and Voncina, 2007). 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. Gas chromatographic analysis

The OCP residues were determined by analysis of samples using a Hewlett–Packard gas chromatograph (GC-HP 5890 Series II) equipped with a ^{63}Ni 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 280–300 °C, respectively. The sample volume injected was 1 μL. In the GC analysis, peaks were identified by comparing their retention times with those of the standards under the same operating conditions. The samples were also injected under the same conditions as done with the standards. The concentrations of various residues in each sample were reported as mg kg^{-1} on a fat basis.

2.5. Analytical quality control

All analyses were performed under United Kingdom Accreditation Service (UKAS) accreditation, following the 17025 ISO standard. Recovery analyses were carried out on eggs, chicken and meat samples fortified at 0.1 mg kg^{-1} of 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 analyses were also performed in order to check interference from the sample. All analyses were carried out in duplicate and the mean concentrations were calculated based on the total number of each sample. The detection limits, the average recoveries with their standard deviations (SDs) of OCPs are shown in Table 1. The average recoveries of OCPs in eggs, chicken and meat were from 76.2% to 99.8%, 85% to 104.6% and 85.8% to 107.8%, respectively, which indicates that the reproducibility of the method was satisfactory.

3. Results and discussion

3.1. Organochlorine pesticide residues in eggs, chicken and meat

A total of 519 samples obtained from different areas in Jordan during 2001–2007 were analysed of which 192 (37%) samples were found to be contaminated with different OCP residues. Out of the 192 samples contaminated with OCP residues, 15 (2.9%) samples exceeded the maximum residue limits (MRLs) when compared to the FAO/WHO Codex Alimentarius (FAO/WHO, 2006). All mean and range values of OCP residues in eggs, chicken and meat samples, on a fat basis (Table 2), are presented and discussed as below.

3.1.1. DDTs (dichlorodiphenyltrichloroethane and its metabolites)

The *op*′-DDD and *op*′-DDT were the only metabolites among the DDTs (*op*′-DDD, *pp*′- DDD, *op*′-DDE, *pp*′-DDE, *op*′-DDT, *pp*′-DDT) that were not detected in levels higher than the detection limit. Among all the metabolites of DDT found in the analyzed samples, *pp*′-DDE was the most dominant. The frequency of different metabolites of DDT residues in the analyzed samples on fat basis was of the order of *pp*′-DDE > *pp*′-DDT > *pp*′-DDD > *op*′-DDE.

Out of the 519 samples analyzed, 20 (15%), 8 (7%) and 74 (27%) eggs, chicken and meat samples analyzed, respectively, were positive for *pp*′-DDE, with an overall detection of 19.7% (102/519) among the analyzed samples. The mean values of the residual concentrations of *pp*′-DDE in the examined eggs, chicken and meat samples, were 0.031, 0.032 and 0.038 mg kg^{-1} fat, respectively.

Table 1
Percentage recovery in eggs, chicken and meat samples after fortification at 0.1 mg kg^{-1}.

Pesticide	Detection limit (mg kg^{-1})	Percentage recovery (mean ± SD)		
		Eggs	Chicken	Meat
Aldrin	0.004	88.7 ± 11.3	92.2 ± 3.3	90.5 ± 1.6
op′-DDD	0.004	83.7 ± 2.9	95.9 ± 1.8	107.8 ± 5.2
pp′-DDD	0.004	85.3 ± 3.7	104.1 ± 1.8	101.5 ± 4.7
op′-DDE	0.004	83.2 ± 2.1	92.3 ± 3.4	92.2 ± 1.1
pp′-DDE	0.004	83.3 ± 2.3	92.1 ± 2.9	92.6 ± 3.5
op′-DDT	0.005	83.6 ± 3.0	103.0 ± 3.5	97.5 ± 4.0
pp′-DDT	0.004	99.7 ± 3.5	104.6 ± 8.8	93.6 ± 5.4
Dieldrin	0.004	81.1 ± 1.9	93.9 ± 2.5	93.5 ± 2.1
α-Endosulfan	0.004	83.9 ± 1.4	93.3 ± 2.9	94.9 ± 2.3
β-Endosulfan	0.004	80.3 ± 2.5	94.9 ± 2.3	92.8 ± 3.4
Endrin	0.005	76.2 ± 1.1	85.0 ± 7.1	99.5 ± 1.8
α-HCH	0.004	81.9 ± 1.0	91.3 ± 2.4	92.1 ± 0.7
β-HCH	0.004	80.2 ± 0.5	93.0 ± 2.4	85.8 ± 0.6
γ-HCH	0.004	83.5 ± 0.6	92.6 ± 2.1	93.0 ± 1.1
Heptachlor	0.004	92.2 ± 1.3	96.5 ± 0.6	107.7 ± 1.9
Heptachlor epoxide	0.004	94.3 ± 1.6	92.5 ± 3.5	92.5 ± 3.5
Hexachlorobenzene	0.004	82.8 ± 0.7	91.3 ± 2.7	90.9 ± 0.9

Table 2
Organochlorine pesticides (mg kg^{-1} on fat basis) detected in eggs, chicken and meat.

Pesticides	Eggs (n = 134)			Chicken (n = 115)			Meat (n = 270)		
	Frequency	Mean	Range	Frequency	Mean	Range	Frequency	Mean	Range
Aldrin		<LOD			<LOD		1	0.470	
op′-DDD		<LOD			<LOD			<LOD	
pp′- DDD		<LOD			<LOD		6	0.045	(0.010–0.150)
op′-DDE	1	0.049			<LOD		2	0.030	(0.020–0.040)
pp′-DDE	20	0.031	(0.005–0.200)	8	0.032	(0.005–0.100)	74	0.038	(0.005–0.150)
op′-DDT		<LOD			<LOD			<LOD	
pp′-DDT	5	0.142	(0.010–0.600)	5	0.018	(0.008–0.040)	16	0.064	(0.010–0.500)
ΣDDTs	22	0.072	(0.005–0.600)	10	0.033	(0.005–0.100)	83	0.045	(0.005–0.500)
Dieldrin		<LOD			<LOD			<LOD	
α-Endosulfan		<LOD			<LOD			<LOD	
β-Endosulfan		<LOD			<LOD			<LOD	
Endrin	1	0.01			<LOD			<LOD	
α-HCH	5	0.197	(0.007–0.850)	11	0.029	(0.004–0.100)	21	0.053	(0.006–0.500)
β-HCH	8	0.238	(0.006–1.300)	5	0.038	(0.008–0.080)	14	0.028	(0.009–0.050)
γ-HCH	3	0.019	(0.006–0.030)	1	0.050		37	0.208	(0.005–2.890)
ΣHCHs	15	0.110	(0.006–1.300)	15	0.037	(0.004–0.110)	64	0.050	(0.006–0.630)
Heptachlor	5	0.058	(0.030–0.090)	3	0.052	(0.01–0.130)	10	0.070	(0.010–0.220)
Heptachlor epoxide	4	0.040	(0.020–0.050)	1	0.030		7	0.019	(0.010–0.050)
Hexachlorobenzene		<LOD		1	0.006		1	0.009	

<LOD: below limit of detection.

One sample of eggs out of 20 has *pp*′-DDE concentration higher than MRL for DDT (sum of *pp*′-DDT, *op*′-DDT, *pp*′-DDE and *pp*′-DDD) of 0.1 mg kg^{-1} fat (FAO/WHO, 2006).

The *pp*′-DDT was detected in 5 (3.7%), 5 (4.3%) and 16 (5.9%) of eggs, chicken and meat with mean concentration of 0.142, 0.018 and 0.064 mg kg^{-1} fat, respectively. Eggs have the highest concentration compared to chicken and meat. Out of the five contaminated eggs samples, concentration of *pp*′-DDT in three eggs samples was higher than MRL for DDT (sum of *pp*′-DDT, *op*′-DDT, *pp*′-DDE and *pp*′-DDD) of 0.1 mg kg^{-1} fat (FAO/WHO, 2006).

The *pp*′-DDD was found to be present in 6 (2.2%) of meat samples analyzed with concentration range varying between 0.01 and 0.15 mg kg^{-1} fat and average concentration being 0.045 mg kg^{-1} fat. However, *op*′-DDE was detected in one sample of eggs and two samples of meat. The mean values of the residual concentrations (mg kg^{-1} fat) of *op*′-DDE in the examined samples of eggs and meat in the present study, were 0.049 and 0.030, respectively.

3.1.2. HCHs (hexachlorocyclohexane isomers)

The predominantly accumulating and most active isomer of HCH, γ-isomer (lindane) was found to be present in 3 (2.2%), 1 (0.8%) and 37 (13.7%) eggs, chicken and meat samples analyzed, respectively. The mean values of the residual concentrations (mg kg^{-1} fat) of γ-HCH in the examined samples of eggs, chicken and meat in the present study, were 0.019, 0.050 and 0.208, respectively. Meat has the highest frequency and concentration of γ-HCH compared to chicken and eggs. However, the MRLs of 0.010–0.1 mg kg^{-1} fat, as designated for lindane (FAO/WHO, 2006) in eggs and meat, respectively, were violated in two samples of eggs and three samples of meat. More surprisingly, all meat samples that exceeded the MRL for lindane were imported from India. The presence of lindane above its MRL in five samples in the present study is viewed with serious concern, as it is carcinogenic in nature (IARC, 2006), and recently it has been implemented in the list of SC on POPs (COP-4, 2009).

The most persistent and metabolically stable β-isomer of HCH could be identified in 8 (6%), 5 (4.3%) and 14 (5.2%), with the mean concentration of 0.238, 0.038 and 0.028 mg kg^{-1} fat in eggs, chicken and meat, respectively. In addition, the α-isomer of HCH was detected in 5 (3.7%), 11 (9.6%) and 21 (7.8%) sample of eggs, chicken and meat with mean concentration of 0.197, 0.029 and 0.053 mg kg^{-1} fat, respectively.

The mean total HCH (∑-HCH) concentration was found to be 0.110, 0.037 and 0.050 mg kg^{-1} fat in eggs, chicken and meat con-

taminated samples, respectively. The present results revealed that the occurrence of different isomers of HCH residues in analyzed eggs, chicken and meat samples on fat basis was of the order of $\beta > \alpha > \gamma$, $\alpha > \beta > \gamma$ and $\gamma > \alpha > \beta$, respectively. However, the ratios and levels of HCH isomers are often used as indication of past or recent usage of technical HCH. As the γ-HCH is the most abundant isomer that detected in meat, this indicates a more recent use of products containing lindane.

3.1.3. Heptachlor and heptachlor epoxide

Heptachlor was detected in 5 (3.7%), 3 (2.6%) and 10 (3.7%) of eggs, chicken and meat samples with a mean concentration of 0.058, 0.052 and 0.070 mg kg^{-1} fat, respectively. On the other hand, heptachlor epoxide was found less frequently and at lower level compared to heptachlor. The MRLs of 0.05 and 0.2 mg kg^{-1} fat for heptachlor (sum of heptachlor and heptachlor epoxide) (FAO/WHO, 2006) in eggs and meat, respectively, were exceeded in five eggs and two meat samples imported from India.

3.1.4. Aldrin, dieldrin, endosulfan, endrin and HCB

Aldrin could not be detected in any of the analyzed samples, except in one meat sample with a mean concentration of 0.47 mg kg^{-1} fat and its MRL of 0.2 mg kg^{-1} fat (FAO/WHO, 2006) was exceeded in this sample. The presence of considerable level of aldrin in meat indicates the need for concern from the public health point of view because of its much higher toxicity than other OCPs (Tanabe et al., 1991). Residues of dieldrin and α- and β-endosulfan have not been detected in levels higher than the detection limit in any of the samples. Endrin was detected in one sample of eggs with a mean concentration of 0.01 mg kg^{-1} fat. HCB has been detected in one sample of chicken and meat with mean concentration of 0.006–0.009 mg kg^{-1} fat, respectively. However, Nasir et al. (1998) found high mean levels of HCB in human milk from Jordan. On the other hand, HCB was not found in any of the dairy samples in Jordan (Salem et al., 2009).

3.2. Incidence of contamination with the different organochlorines

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 of >50%. A concern raised in recent years is whether or not HCHs and DDTs are still being used illegally in agriculture or livestock breeding in Jordan. Likewise, HCHs and DDTs have been found to be the principal contaminants in camel, cattle and sheep carcasses slaughtered in Sharki Province, Egypt (Sallam and Morshedy, 2008). On the other hand, heptachlor, HCB, aldrin and endrin compounds were detected at a low incidence and they were only present in less than 7% of the analyzed samples. The high detection limits and the low quantities of these compounds could mask the occurrence of these OCPs in the analyzed samples. Generally, the incidence of contamination of the examined samples by the OCPs followed the order of ∑DDT > ∑HCH > heptachlor > HCB > aldrin & endrin (Fig. 1).

3.3. Variations in organochlorine among eggs, chicken and meat

The incidence of contamination of all OCPs detected in meat was higher than those detected in chicken and eggs (Fig. 2). The results indicated that 28% (38/134), 20% (23/115) and 49% (131/270) of the examined eggs, chicken and meat samples, respectively, were contaminated with OCP residues. The order of contamination in the analyzed samples was meat > eggs > chicken. However, 39% (9/23) eggs and 4.6% (6/131) meat contaminated samples showed concentration above the MRLs of the FAO/WHO (2006). The high levels of OCP residues measured in eggs in this study are surprising and raise questions about pesticides banned for more than

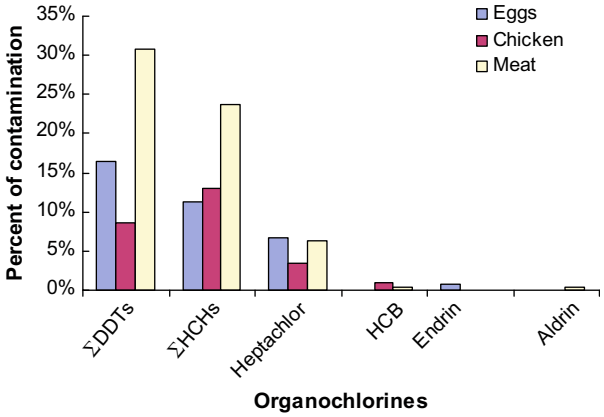

Fig. 1. Distributions of organochlorine pesticides analyzed in samples of eggs, chicken and meat collected from Jordan.

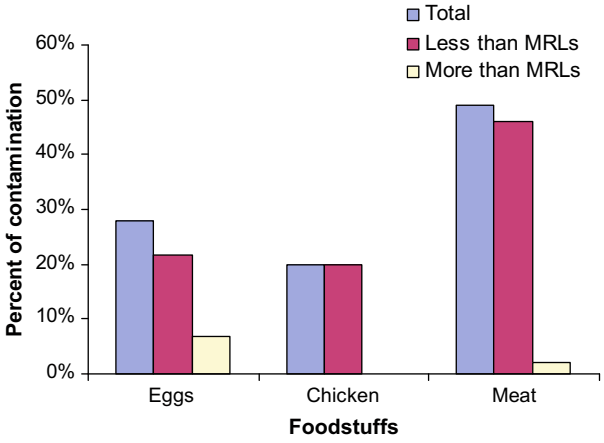

Fig. 2. Percent of contamination of examined eggs, chicken and meat samples with organochlorine pesticides analyzed.

20 years in Jordan. DDT is usually not detected anymore or only at trace level in commercial eggs, as observed in Spain (Fontcuberta et al., 2008), in Belgium (17.30 ng g^{-1} fat) (Van Overmeire et al., 2006) or in Sweden (6.6 ng g^{-1} fat) (Darnerud et al., 2006). However, high level of DDT (457 ng g^{-1} fat) was found in home-produced eggs in Belgium (Van Overmeire et al., 2009).

From the present investigation it clearly emerged that imported meat had high pesticide content. In Jordan, more than 85% of the meat consumed, particularly fresh and frozen lamb and beef, are imported from other countries of the world, and primarily from developing countries where there is limited or no control over the use and/or control of pesticide residues in foods. However, such types of meat are always cooked before consumption, a procedure thought to cause a reduction in pesticide content. Indeed, Sallam and Morshedy (2008) showed that heat treatment of meat (boiling for 1.5 h) produced overall reductions of 40.4%, 55.0%, 32.4%, 33.5%, 29.2% and 38.2% in DDTs, lindane, dieldrin, aldrin, endrin and HCB contents, respectively. This could be attributed to the volatility of these compounds and to the elimination with the fat rendering induced by high temperatures.

Finally, the most important conclusions and recommendations stemming from the study are summarized. In general, people must be aware that eggs, chicken and meat might contribute (in addition to other known sources as dairy products) substantially to the intake of OCPs through food consumption. People should adapt their pattern of food consumption (including a limitation of the con-

sumption of these foodstuffs) to minimize their intake of OCP residues. Although the Jordanian government has imposed a ban or restricted the use of various pesticides, there is need to continue the monitoring study of the OCP and other pesticide residues in foodstuffs from the view point of human food safety. Based on our results, the Jordanian Food and Drug Administration (JFDA) acted expeditiously to identify and control contaminated Indian meat. As a result of this action, currently, all Indian lamb and beef meat must be tested for the presence of OCP residues in authorized food testing laboratories before distribution into commercial markets in Jordan.

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