



Banned Organochlorine Pesticides Residues in Camel Milk, Meat, and Liver: A Case Study from Jordan

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Abstract

Although most of the organochlorine pesticides have been banned from use and trade in Jordan, their residues remain still present in different environmental and food matrices. Because of the need to clarify the current status of pesticide contamination in Jordan, the present study has investigated the extent of contamination in camel milk, meat, and liver. One hundred eighty samples of camel milk, meat, and liver have been analyzed for their residual contents of dichlorodiphenyltrichloroethane and related metabolites, hexachlorohexane isomers, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, and hexachlorobenzene. These samples were Soxhlet-extracted, cleaned up using florisil-column chromatography, and analyzed using a gas chromatographic system equipped with the electron-capture detector. *Results:* 31.7, 35, and 38.8% of the examined milk, meat, and liver samples, respectively, were contaminated with organochlorine pesticides. In general, obtained results have confirmed that pesticide contamination is still a significant concern when speaking of environmental samples and food in Jordan. More research is needed in this ambit. The pesticide contamination appears relevant enough in camel milk and liver samples, suggesting the need for reliable maximum residue levels where absent.

Paper type: Research paper

Keywords: camel, chromatography, Jordan, liver, meat, milk, organochlorine, pesticide.

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Introduction

The global population is increasing by 70 million human beings per annum, and the most relevant part is located in developing countries. Many challenges, including microbial and chemical contamination, traceability and authenticity issues, new foods, etc., have forced the modern food and beverage industries to modify their procedures and operations to increase yields of production, possibly in a sustainable way (Al-Dalain *et al.*, 2020; Barone and Parisi, 2020; Facciola *et al.*, 2019; Haddad and Parisi, 2020; Mania *et al.*, 2017-2018; Omar *et al.*, 2020; Parisi, 2019-2020; Parisi *et al.*, 2020a,b). The consequent demand for food and beverage products has determined the increase of food production worldwide, with several consequences, including the increase of pesticide uses against pests of various types (Popp *et al.*, 2013). Pesticides are toxic compounds with recognized toxicity: however, hazardous effects on human health can vary from acute to chronic toxicity. Some of these compounds showed to be of deep concern to human beings and the environment; consequently, countries and different organizations have progressively stopped their registration and related use in the agricultural sector (Codex Alimentarius Commission 2019; IARC 2006). Organochlorine pesticides (OCPs) represent a significant part of the cheaper (off-patent) and older pesticides. OCPs are used intensively to manage agricultural and public-health insect pests for several decades in Jordan and worldwide. In detail, OCPs are recognized as a significant source of food contaminants and a significant concern for human health due to their residual effects resulted from their persistence, low water solubility, and lipophilic compounds, leading to their bioaccumulation in fatty tissues. Because of their properties, OCPs can transfer themselves and increase their higher trophic levels through the food chain.

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OCP residues become an exciting factor in environmental pollution and related monitoring actions: their toxic effects are observed in humans and animals (Ahmad *et al.*, 2010). Most concerning OCPs such as aldrin, chlordane, dieldrin, endrin, heptachlor, and hexachlorobenzene (HCB) are used in Jordan for agricultural purposes when speaking of pest control. However, these compounds are banned from use and trade since the early 1980s. As a single example, dichlorodiphenyltrichloroethane (DDT) has been used to control insects of public health concern such as mosquitoes until 1995. Besides, several OCPs have carcinogenic effects: for this reason, they have been classified as endocrine-disrupting chemicals (Salem *et al.*, 2009). DDT, HCB, and several hexachlorohexane (HCHs) isomers have been classified as "group 2B" (possibly carcinogenic to human) as agents, mixtures, and exposure circumstances, by the International Agency for Research on Cancer (IARC 2006). Although most parts of OCPs have been banned from use and trade in Jordan, their residues remain still detected at present in different environmental matrices like animal products (Al-Antary *et al.*, 2016), human milk (Allawi *et al.*, 2013), eggs, chicken, meat (Ahmad *et al.*, 2010), dairy products (Salem *et al.*, 2009), sheep milk (Allawi and Al-Hawadi, 2005) sheep liver and kidney (Allawi and Al-Hawadi, 2008), soil (Al-Nasir, 2000), fish (Alawi *et al.*, 1995), honey (Al-Rifai and Akeel, 1997), grapes and homemade wine (Al-Nasir *et al.*, 2001), and also adipose tissues and fodder (Allawi and Al-Hawadi, 2005). Because of the critical importance of pesticide contamination in foods and beverages so far, the present study has aimed to investigate the extent of contamination concerning several analytes such as DDT, dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), HCH, HCB (and related isomers or compounds), aldrin, dieldrin, endrin, and heptachlor residues in camel milk, meat, and liver. This work follows other studies started in 1993 to monitor organochlorine residues in Jordan. The Ministry of Environment in Jordan funded four research studies during the 1993-2003 period to clarify the current status and establish database-line overtime to assist policy makers and suitable direct efforts concerning contaminated commodities and geographic areas of Jordan (Allawi *et al.*, 2013).

1 Materials and Methods

1.1 Sampling

180 samples of camel related products, 60 samples of milk, 60 samples of meat, and 60 samples of the liver, have been collected from slaughterhouses in different geographical regions of Jordan in the 2013-2015 period and subjected to residue analyses:

- Maan (about 220km south of Amman, the capital of Jordan, latitude, and longitude coordinates: 30.194958° and 35.734241°, respectively)
- Sahab (in the neighborhood of Amman, latitude and longitude coordinates: 31.844851° and 36.045490°, respectively)
- Ar-Ramtha (about 90km northwest of Amman, latitude and longitude coordinates: 32.5587300° and 36.0081600°, respectively).

About 50ml of camel milk have been collected in cleaned and dry glass bottles, kept in dry ice during transportation to the laboratory, then stored at -20°C until analysis. In the case of meat and liver, 250g of each have been kept separately in polyethylene bags and transported into dry-ice-filled boxes during transportation to the laboratory; subsequently, these samples have been stored at 4°C until analysis within 24 hours from their arrival.

1.2 Apparatus

Gas chromatography GC (HP 5890) equipped with an ⁶³Ni electron capture detector, using an HP-5 capillary column (30m x 0.32 mm i.d. with 0.25µm film thickness). All used analytical-grade solvents for pesticide residue analysis. Petroleum ether, (40-60°C) Analar (BDH, England), dichloromethane, (Jansen Chimica, Belgium), n-hexane (Riedel de-Haan, Germany), acetone, Analar (BDH, England), ethanol, (GCC, U.K.), diethyl ether (GCC, U.K.), ammonia solution (25%) (Riedel de-Haan, Germany), cyclohexane (Scharlau, Spain), ethyl acetate (Lab-scan, Ireland), toluene (Riedel de-Haan, Germany). Organochlorine pesticides (OCP's) used internal standards *o,p*-DDT, *p,p*-DDT, *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, *p*, DDD, α -HCH, β -HCH, γ -HCH, aldrin, dieldrin, endrin, heptachlor, heptachlorepoxyde, HCB, and isodrin have been obtained from Dr. Ehrestorfer GmbH laboratory (Germany). Analytical-grade anhydrous sodium sulfate (Fluka, Switzerland) has been heated at 550°C for 3hrs and kept in a closed container.

1.3 Analysis of camel milk samples

1.3.1 Determination of fat content.

Ten grams of milk samples were mixed with 2ml of 25% ammonia, 25 ml of diethyl ether, and 25ml of petroleum ether then added into a separatory funnel equipped with polytetrafluoroethylene (PTFE) stopcock. The mixture was mixed thoroughly. Subsequently, the organic layer was separated, and the extraction procedure was repeated twice. Organic extraction layers containing fat were pooled and filtered through anhydrous sodium sulfate. After solvent evaporation to dryness at 30°C and 200mbar in a pre-weighed round

bottom flask, the same flask and inner residues were kept overnight in a desiccator, then re-weighted. The percentage of milk fat content was finally calculated from the weight difference between the empty round bottom and the flask after desiccation.

1.3.2 Pesticides extraction.

25g of florisil (3% water) were poured into a chromatography column (50x2cm with PTFE stopcock) containing 100ml of petroleum ether. The remainder of petroleum ether was reduced to about 50ml after settling florisil down. 10g of the milk sample were mixed with 25g of florisil poured onto the chromatographic column. Subsequently, the column was eluted with 300ml of petroleum ether-dichloromethane (80:20, v/v). The eluate was collected in a 500ml- round-bottomed flask and evaporated to dryness at 35°C and 12mbar. The remaining solvent was evaporated under a nitrogen stream. The residues were dissolved in 2 ml- *n*-hexane containing 0.5ppm isodrin as internal standard and 1µL of the final extract injected onto the gas-chromatographic (GC) column.

1.3.3 Analysis of camel-meat and liver samples.

25g of homogenous samples of meat or liver were mixed with 50g of anhydrous sodium sulfate in a mortar. The mixture was then transferred to a thimble and extracted with 250ml of petroleum ether in a Soxhlet apparatus for six hours. Extracts were evaporated using a rotary evaporator nearly to dryness. The remaining solvent was evaporated under a nitrogen stream. The residue was left in a desiccator for half an hour; then, the fat residue was weighed to obtain the percentage amount of fat matter in the sample.

Subsequently, the extract from the previous step was dissolved in petroleum ether (10ml) and transferred with a Pasteur pipette to the florisil column in order to remove the residual fat. The column was then eluted with 300ml of petroleum ether- dichloromethane (80:20, v/v). Eluates were evaporated using a rotary evaporator to dryness under a nitrogen stream. The residues were dissolved in 2ml of *n*-hexane contain 0.5ppm isodrin as internal standard and 1µL of the final extract injected onto the GC-column.

1.4 Gas-chromatographic analysis.

The analysis of OCPs in samples was carried out by using an HP 5890GC system equipped with ⁶³Ni-electron capture detector and an HP-5 capillary column (30mx0.32mm i.d. with 0.25µm film thickness). The carrier gas was helium (flow rate, 2ml/min), while the Argon-methane mixture was employed as makeup gas (30ml/min). GC-running conditions were 300 °C (injection temperature) and 300°C (detector). The oven temperature started at 80°C (2.2 minutes), 80-175°C (rate:30°C/min), then raised to 225°C (rate: 10°C/min), and held at 225 °C (2 min). The injected sample volume was 1µl with a split ratio of 1:25. The identification of organochlorine pesticides in analyzed samples was carried out by comparing their retention time with those in the standard mixture, concerning the internal standard retention time under the same injection conditions. Organochlorine pesticide residues were quantitatively determined using the relative peak area of the sample chromatogram and the relative concentration. The concentration of OCPs residues in each sample was finally reported as mg/kg on a fat basis.

1.5 Recovery tests and detection limits

Extraction and cleanup methods were evaluated by spiking blank samples with known concentrations of organochlorine pesticides mixture. The analysis of spiked samples was carried out according to the above-mentioned procedures. The average OCP recovery was 89.6-102.3% for milk samples and 88.1-97.8% for meat and liver samples. The detection limit was calculated for each compound as a signal to noise ratio 3:1 from the standard mixture's chromatogram after dilution several times. Results of detection limits varied from 0.004 to 0.005mg/kg.

2 Results and Discussion

In general, results revealed that 53 of the analyzed samples (29.4%) were contaminated with OCP residues. Also, 19 and 2 (31.7 and 3.3%) of milk and meat samples, respectively, exceeded maximum residue limits (MRLs) as defined by the Codex Alimentarius Commission (Codex Alimentarius Commission 2019) (**Table 1**). The Codex Alimentarius Commission has not defined MRLs for camel liver. The mean values of OCP residues in milk, meat, and liver samples on a fat basis are presented in **Table 2**.

Table 1 Number of camel milk and meat contaminated samples with organochlorine pesticides exceeding MRL (mg/kg on fat basis) (Codex Alimentarius Commission 2019). Total milk samples: 60; total meat samples: 60

Pesticide	Milk		Meat	
	Codex MRL	No. of samples exceeding MRL	Codex MRL	No. of samples exceeding MRL
DDT	0.02	9	5	0
Aldrin and Dieldrin	0.006	3	0.2	0
Lindane (γ -HCH)	0.01	4	0.1	2
Heptachlor	0.006	3	0.2	0

Table 2 Organochlorine pesticides (mg/kg) on fat basis) detected in camel milk, meat, and liver.

	Milk (N=60)			Meat (N=60)			Liver (N=60)		
	Frequency	Mean	Range	Frequency	Mean	Range	Frequency	Mean	Range
HCB	1	0.016±0.002	-	3	0.016±0.005	0.007-0.25	3	0.199±0.07	0.017-0.36
α -HCH	3	0.035±0.011	0.017-0.048	3	0.016±0.005	0.009-0.026	4	0.02±0.094	0.015-0.48
β -HCH	6	0.12 ±0.068	0.015-0.25	9	0.14±0.091	0.018-0.34	10	0.255±0.178	0.007-0.68
γ -HCH	4	0.26 ±0.135	0.080-0.728	5	0.21±0.134	0.019-0.728	5	0.36±0.174	0.026-0.79
Σ HCH	8	0.236±0.158	0.015-0.765	13	0.179±0.163	0.009-0.767	15	0.34±0.26	0.007-0.93
Heptachlor	3	0.07 ±0.025	0.025-0.13	2	0.027±0.007	0.016-0.037	5	0.049±0.02	0.015-0.075
Heptachlorepoxyde	5	0.030±0.013	0.010-0.05	4	0.04±0.015	0.025-0.07	6	0.12±0.065	0.018-0.31
Σ Heptachlor+epoxyde	7	0.05 ±0.029	0.018-0.130	5	0.04±0.019	0.025-0.086	10	0.097±0.068	0.015-0.31
Aldrin	n.d	-	-	n.d	-	-	1	0.016±0.002	-
Dieldrin	3	0.02 ±0.008	0.010-0.040	n.d	-	-	n.d	-	-
Σ Aldrin+Dieldrin	3	0.20 ±0.008	0.010-0.040	n.d	-	-	1	0.016±0.003	-
Endrin	n.d	-	-	n.d	-	-	1	0.01	-
<i>o,p'</i> -DDT	n.d	-	-	n.d	-	-	n.d	-	-
<i>p,p'</i> -DDT	n.d	-	-	n.d	-	-	n.d	-	-
<i>o,p'</i> -DDE	3	0.04 ±0.013	0.027-0.06	2	0.025±0.006	0.020-0.027	3	0.045±0.017	0.01-0.085
<i>p,p'</i> -DDE	8	0.057±0.041	0.010-0.19	8	0.077±0.050	0.016-0.190	9	0.136±0.096	0.017-0.36
<i>o,p'</i> -DDD	1	0.120±0.021	-	n.d	-	-	n.d	-	-
<i>p,p'</i> -DDD	2	0.056±0.014	0.047-0.065	2	0.135±0.042	0.039-0.23	3	0.14±0.063	0.016-0.34
Σ -DDTs	12	0.067±0.052	0.010-0.190	9	0.10±0.065	0.016-0.23	12	0.15±0.115	0.017-0.36

n.d= not detected

2.1 Dichlordiphenyltrichloroethane and its metabolites (DDTs)

DDT is a chlorinated aromatic hydrocarbon that is suspected to be carcinogenic. It was released in late 1945 for public sale when insecticides were commonly known as "economic poisons" as they proved to improve agricultural production through combating agricultural insect pests (Conis, 2017). After publishing Silent Spring in 1962, in which Rachel Carson documented the detrimental effects of long-term use of DDT and other insecticides for human health, the Environmental Protection Agency (EPA) stopped its registration in 1972, banning its use (Paull, 2013). Jordan, like other countries, banned DDT for agricultural uses, but DDT remained allowed for the active control of insect vectors of public health concerns such as mosquitoes until 1995 (Salem *et al.*, 2009).

Due to DDT chemical properties, as it persists in the environment for extended periods, this molecule can still be found in our foods. In the present study on camels of Jordan, 12, 9, and 12 (20, 15, and 20%) of milk, meat, and liver samples, respectively, have been found positive for DDTs, which represented an overall detection of 33 (18.3%) of the total analyzed samples (Table 2). The mean value of DDTs concentration has been calculated to be 0.067, 0.10, and 0.15mg/kg fat in milk, meat, and liver contaminated samples, respectively (Table 2). The *o,p*- and *p,p*-DDT analytes were the only metabolites DDT metabolites (with concern to the group concerning *o,p*-DDT, *p,p*-DDT, *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, and *p,p*-DDD) that were not detected at levels higher than detection limits. *p,p*-DDE was the most dominant analyte among all DDT metabolites found in analyzed samples. Eight, eight, and nine (13.3, 13.3, and 15%) of considered samples, respectively, have been found positive for *p,p*-DDE, with an overall detection of 13.9% (25

on a total number of 180 samples), as shown in Table 2. The frequency of different metabolites of DDT residues in analysed samples on a fat basis was found in the following order: *p,p*-DDE>*o,p*-DDE>*p,p*-DDD>*o,p*-DD (Table 2). Moreover, nine camel milk samples had concentrations higher than MRLs for DDT (as the sum of *o,p*-DDT, *p,p*-DDT, *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, and *p,p*-DDD), as shown in Table 1. On the other side, the recorded concentration of DDT-related analytes in both meat and liver samples has been found lower than the concentration of camel meat and liver recorded in *Sharkia* (Egypt), where it was detected in 56.7% of samples with a mean of 13.9 and 34.6 ng/kg of meat and liver, respectively (Sallam and Morshedy, 2008). Also, the obtained result has been lower than concentrations recorded in sheep liver and milk found in Jordan, as reported in other studies (Allawi and Al-Hawadi, 2005-2008).

2.2 Hexachlorocyclohexane isomers (HCHs)

α -, β -, γ -, and δ -HCHs have been found in at least 146, 159, 189, and 126, respectively of the 1,662 current or former National Priorities List (NPL) sites placed by EPA to identify serious hazardous waste sites in the United States of America (USA) for long-term federal cleanup activities, according to the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR 2005). Even though use of HCHs was officially banned in Jordan in 1981, they still may be detected in dairy milk (Salem *et al.*, 2009). In the present study on camels in Jordan, HCHs isomers (α , β , and γ) have been detected in eight, 13, and 15 (13.3, 21.6, and 25.0%) of milk meat and liver, respectively (Table 2). The mean values of HCHs residual concentrations (mg/kg fat) in examined samples were 0.236, 0.179, and 0.34, respectively (Table 2). The most active and essential HCH isomer, γ -isomer (lindane), has been found in four milk samples with a mean concentration of 0.260mg/kg fat, exceeding its MRL value (0.01mg/kg fat) on four occasions (Table 1). In addition, it has been detected in five meat samples with a mean concentration of 0.21mg/kg fat; its MRL of 0.100mg/kg fat has been surpassed in two samples (Tables 1 and 2).

2.3 Heptachlor and Heptachlorepoide

Heptachlor is a chlorinated dicyclopentadiene insecticide that is persistent in the environment for extended periods, with consequent accumulation in the food chain. Its use has been banned or severely restricted in many countries since the 1980s, but it is still detected as a contaminant in some food commodities (Kielhorn *et al.*, 2006). In this study, heptachlor has been detected in three milk samples with a mean concentration of 0.07mg/kg fat, exceeding its MRL (0.006mg/kg fat) in three samples (Tables 1 and 2). Two meat samples have been found contaminated with a mean 0.027mg/kg fat without exceeding the related MRL of 0.1mg/kg fat (Tables 1 and 2). In addition, heptachlor has been detected in five liver samples with a mean of 0.049mg/kg fat (Table 2). Prolonged exposure to this insecticide is reported to causes toxic effects when speaking of the human central nervous system; heptachlor is also associated with liver damages. Its classification as a Group 2B molecule is evidence of carcinogenicity in animals, while there is not sufficient evidence in humans (WHO, 2003). Heptachlor epoxide has been detected in five, four, and six (8.3, 6.7, and 10%) samples with a residual concentration of 0.05, 0.04, and 0.12mg/kg fat in milk, meat, and liver samples, respectively (Table 2). Recently, Alawi and Al-Hawadi had detected heptachlor and heptachlor epoxide in sheep milk samples of Jordan with a mean concentration of 0.56 and 1.21mg/kg fat. These values were higher than the obtained results concerning camel milk (Alawi and Al-Hawadi, 2005).

2.4 Aldrin, Dieldrin, Endrin, and HCB

The Diels-Alder cycloaddition between hexachlorocyclopentadiene and norbornadiene produces the aldrin molecules (Jubb, 1975; Nestorovska-Krsteska and Zdravkovski, 2006). According to the Stockholm Convention on Persistent Organic Pollutants, the European Union (EU) banned its use (PSD, 2020); besides, the USA canceled aldrin registration in 1974. With concern to our study, aldrin has been detected in one liver sample with a concentration of 0.016 mg/kg fat (Table 2). Another analyte, endrin, has been found in one liver sample with a concentration of 0.010mg/kg fat (Table 2). Moreover, in three milk samples, dieldrin has been detected with a mean concentration of 0.020mg/kg fat (Table 2). Finally, HCB has been detected in one milk sample with a concentration of 0.016mg/kg fat and three meat and liver samples with a mean concentration of 0.016 and 0.199mg/kg fat, respectively (Table 2). It has been reported that 40% of camel meat and liver samples contained dieldrin in 40% in Egypt with low concentrations of 0.15 and 3.07ng/kg, respectively (Sallam *et al.*, 2008). With relation to Jordan, aldrin, dieldrin, endrin, and HCB were detected in sheep-milk samples with frequency 48, 14, 10, and 40%, respectively, with a mean concentration of 0.021, 0.158, 0.684, and 0.161mg/kg fat, respectively (Alawi and Al-Hawadi, 2005).

Conclusions

In general, obtained results have confirmed that pesticide contamination is still a significant concern when speaking of camel milk, meat, and liver in Jordan. OCPs are frequently revealed in analyzed samples, with peculiar contamination in liver samples when speaking of β -, γ -, Σ -HCHs, heptachlorepoxy alone, and in combination with heptachlor, endrin, and the sum of DDTs. On the other side, milk samples are notably contaminated if compared with meat and liver samples when speaking of α -HCH, dieldrin alone and in combination with aldrin, and heptachlor. Meat samples show intermediate contamination, in general.

Obtained results have also demonstrated that OCP contamination sometimes exceeds MRLs for pesticides concerning milk and meat samples (with evident predominance for milk contamination). There are no proposed MRLs for pesticides in liver samples (the observed contamination would suggest the need for MRLs). On the other side, several OCPs appear to be found with low values than reported results in the literature, including concentrations recorded in sheep liver and milk found in Jordan. However, these results suggest that the environmental persistence of researched OCPs is still remarkable; consequently, more research is needed in this ambit, including camel-derived products and tissues and other animal-derived food products.

Nomenclature

ATSDR	=Agency for Toxic Substances and Disease Registry	[-]
DDD	=Dichlorodiphenyldichloroethane	[-]
DDE	=Dichlorodiphenyldichloroethylene	[-]
DDT	=Dichlorodiphenyltrichloroethane	[-]
HCB	=Hexachlorobenzene	[-]
HCH	=Hexachlorocyclohexane	[-]
IARC	=International Agency for Research on Cancer	[-]
MRL	=Maximum residue limit	[-]
OCP	=Organochlorine pesticides residues	[-]

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