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Original Research Article

Organochlorine pesticides residues in butter, olive oils and corn oils

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ABSTRACT

Dietary fat and oil is important for many body processes. The present investigation was carried out to determine the concentrations of organochlorine pesticides in butter, olive and corn oil. A total of 125 samples (75 butter, 25 each of olive oil and corn oil) were collected from El Minia Governorate, Egypt. Levels of these compounds were determined by gas chromatography with electron capture detector (GC-ECD). The results indicated that 30.4%(38/125), 24.8%(31/125), 21.6%(27/125), 21.6%(27/125), 16.8%(21/125), 14.4%(18/125), 14.4%(18/125), 12.8%(16/125), 9.6%(12/125), 8.8%(11/125), 8%(10/125), 1.6%(2/125) and 0.8%(1/125) of the examined samples were contaminated with Heptachlor, Endrin, Aldrin, Dichlorodiphenyldichloroethylene(p,p'-DDE), Dichlorodiphenyldichloroethane(p,p'-DDD), Gamma hexachlorocyclohexane(Gamma HCH), Heptachlor epoxide, Dieldrin, Endosulfan, methoxychlor, Alpha hexachlorocyclohexane(Alpha HCH), Delta hexachlorocyclohexane(Delta HCH) and Gamma Chlordane, respectively. None of the examined samples revealed the presence of Dichlorodiphenyltrichloroethane (p,p'-DDT) and 11 samples contained organochlorine residues above European Union maximum residue limits (EU MRL)..

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1. Introduction

Edible fats and oils comprise one of the three major classes of foods, the other being carbohydrates and proteins. Fat and oils are the most concentrated form of energy as they yield 9 calories/g. It contributes texture, flavor, taste and increase the diet palatability. However fat and oils are good sources of essential fatty acids linoleic (omega-6), linolenic (omega-3) and

vitamin E. In addition to act as the transport vehicle for fat soluble vitamins and hormones (Swanson, Block, & Mousa, 2012). Moreover, fat and oil can also contain chemical hazards as organochlorine pesticides (OCPs), which constitute a risk factor for the health of the consumer (Mansour, 2004).

Human exposure to OCPs is mainly attributed to the food chain. The contamination of food, including fat and oil, by OCPs is a worldwide phenomenon. OCPs get accumulated in fat-rich dairy products, such as butter (Waliszewski et al., 1997) and edible oil (Bajpai, Shukla, Dixit, & Banerji, 2007). Although the studied of OCPs has been restricted or banned in many countries, several studies have been documented their presence in butter (Prachar et al.,1995; Pandit and Sahu ,2002; Battu et al.,2004 and Salem et al.,2009) and edible oil (Darnerud et al., 2006;Fontcuberta et al., 2008; Herceg Romanić et al., 2011and Qin et al., 2011). This fact indicates that the butter and edible oils are an important exposure route for persistent pollutants in general.

Organochlorine pesticides are compounds of pressures and partitioning behavior under ambient conditions, that 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 the 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, Vom Saal, & Soto, 1993) and carcinogenic substances (Mansour, 2004).

Extensive use of organochlorine pesticides in Egypt throughout the 1960s and 1970s; mainly against termites and soil insects prompted the Government to issue legislation to ban them since the 1980s (El-Sebae and Soliman, 1982 and Soliman et al., 2003) Furthermore, Egypt signed the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) on May, 2002 and entered into force on May, 2004. At present, there is no manufacturing formulation, import and legal use for any OCPs in Egypt(Ibrahim & El-Ruby, 2007).

As far as we know that a very little studies are currently known about the levels of OCPS in butter and oils in Upper Egypt. Therefore this study was designed to provide information on the concentration of OCPs in butter, olive and corn oil in El Minia Governorate, Egypt. The results will help in a scientific assessment of the implications of pesticide residues with regards to human risks in Egypt.

2. Materials and methods

Collection of samples

A total 125 samples: (50 farmer’s butter, 25 each of imported butter, olive oil and corn oil) were collected from El Minia Governorate, Egypt.

Chemicals and reagents

Acetone, acetonitrile, anhydrous sodium sulfate, dichloromethane, diethyl ether, n-hexane, petroleum ether and Florisil (60/100 mesh) from Sigma, USA. Analytical standards of OC pesticides: Alpha HCH, Gamma HCH, Delta HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDE, p,p'-DDD and p,p'-DDT, methoxychlor were obtained from Dr. Ehrenstorfer, Augsburg in Germany, with purities larger than 98.5%.

Sample preparation, extraction and cleanup

Three grams of the butter or oil were dissolved into 40 ml petroleum ether. This was partitioned three times into acetonitrile saturated with petroleum ether (3 × 30 ml). The acetonitrile fraction, after dilution with saline (600 ml), was again partitioned into petroleum ether (3 × 100 ml). After that it was dried over anhydrous sodium sulfate, and concentrated at 30℃ on a rotary vacuum evaporator to a volume less than 5 ml to be used for Florisil cleanup(Kodba & Vončina, 2007).

Cleanup of the extracted samples, to remove the residual fat was performed by transferring the extract into a glass chromatographic column (25mm 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 then the extracted sample was transferred onto the column. The column was eluted with 300 ml eluent (20% dichloromethane + 80% petroleum ether) and the collected eluate was concentrated to dryness on a rotary vacuum evaporator and dissolved in hexane to a volume of 5 ml (Salem et al., 2009).

Sample freeze

An aliquot of each extract was stored in a freezer at -20 ℃ for ensure full cleanup then each extract was transferred to injection vials to be ready for the analysis with the electron capture gas chromatography. GC/ECD [Gas Chromatography (Hewlett-Packard Model 6890) equipped with Electron Capture Detector Ni , Agilent Technologies, Inc. 2850 Centerville Road Wilmington, DE 19808-1610]; GC-MS/MS [Gas Chromatography coupled with tandem Mass Spectrometry, Trek Equipment Corp. Sausalito, CA 94965].

Recovery studies

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.

Target Hazard Quotient (THQ)

For quantitative risk assessment Target Hazard Quotient was calculated (THQ) (the ratio of the potential exposure to a substance and the level at which no adverse effects are expected). The hazard ratios were assessed by this Equation THQ=EDI/RFD Where RFD (reference oral dose) (mg/kg-day) for each organochlorine (ORD, 2017).EDI (Estimated daily intake) (mg/kg-day) calculated by this equation (Mahmoud et al., 2016).

EDI=(C×W)/Bw Where C (mg/kg) is the concentration of OCP residue in contaminated samples, W represents the daily average consumption of food and Bw represents the body weight. The average daily consumption per adult person (70 kg BW) was considered to be 8, 1.1 and 0.3 g of butter, olive oil and corn oil respectively (Food Balance Sheets, 2014). A hazard ratio greater than one indicates that there could be potential human health risks(Jiang et al., 2005).

Statistical Analysis:

SPSS pocket program for windows (version 14, 2006) was used for statistical analysis. Values of different parameters were expressed as the mean ± standard error (SE); mean calculated for positive result only. Data obtained were statistically analysed for descriptive statistics, Q squares and ANOVA test at significant level of $P < 0.05$.

3. Results

Generally, the incidence of organochlorine pesticides in butter and oil samples collected from El Minia Governorate, Egypt were found to be present in 16(64%), 49(98%), 25(100%) and 24(96%) of the imported butter, farmer butter, olive oil and corn oil samples respectively (Fig. 1).

The chemical concentrations in the examined samples followed the order of Endrin > Aldrin> p,p'-DDE> Heptachlor epoxide > Heptachlor > Endosulfan> Gamma HCH > Dieldrin > methoxychlor > p,p'-DDD > Alpha HCH > γ-Chlordane > Delta HCH > p,p'-DDT.

According to the samples which contained organochlorine more than MRL, imported butter has a high ratio (32%) then the olive oil (8%) followed by corn oil (4%).

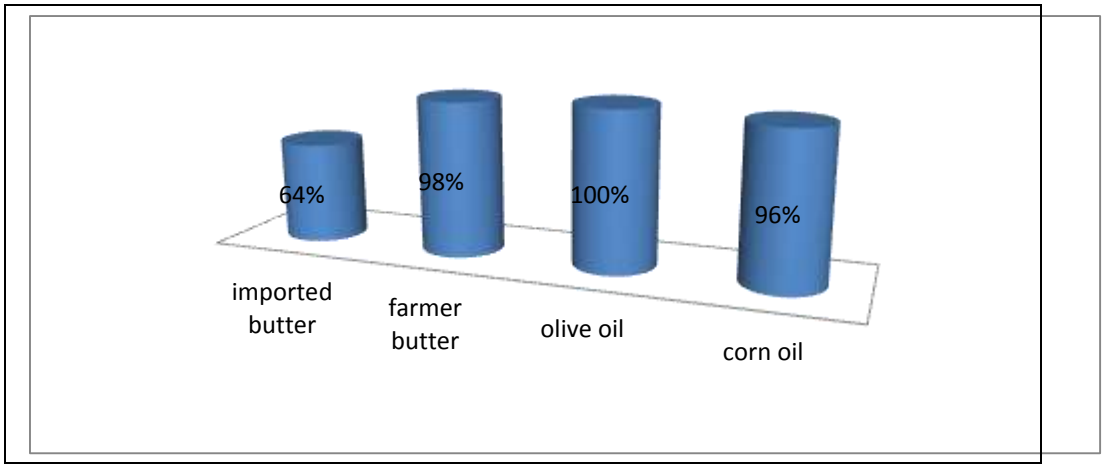

Fig. 1 Incidence percentage of OCPs in butter and oil samples.

Table 1: Residues of organochlorine insecticides in the imported and farmer butter samples (mg/kg)

	Imported butter				Famer butter			
pesticides detected	No.	%	MEAN.	the samples exceed MRL*	No.	%	MEAN.	the sample exceed MRL
Alpha HCH	0	0	ND	0	10	20	0.000221± 0.00005	0
Gamma HCH	2	8	0.0055± 0.003182	0	6	12	0.0002± 0.00005	0
Delta HCH	0	0	ND	0	2	4	0.00015± 0.00003	0
Heptachlor	9	36	0.00111± 0.000111	1	13	26	0.000292± 0.00007	0
Heptachlor epoxide	3	12	0.008± 0.004243	0	5	10	0.000124± 0.000036	0
Aldrin	3	12	0.0176667± 0.00557	2	18	36	0.002089± 0.0004711	0
γ-Chlordane	0	0	ND	0	1	2	0.001±0.001	0
Endosulfan	0	0	ND	0	12	24	0.001492± 0.000354	0
Dieldrin	0	0	ND	0	16	32	0.0009± 0.00047	0
Endrin	5	20	0.0146± 0.006439	5	11	22	0.000809± 0.0003152	0
p,p'-DDE	4	16	0.01565±0.00935	1	8	16	0.00155± 0.0006383	0
p,p'-DDD	1	4	0.001±0.001	0	10	20	0.00068± 0.000197	0
p,p'-DDT	0	0	ND	0	0	0	ND	0
Methoxychlor	0	0	ND	0	11	22	0.001155 ± 0.000235	0

ND = not detected, *: there were one sample contained two OCP residues

(p,p'-DDE and aldrin)

Table 2: Estimated daily intake (EDI) and target hazard quotient (THQ) for the mean values of organochlorine insecticide residues in the imported and farmer butter samples (mg/kg b.w)

	Imported butter		Farmer butter	
Pesticides detected	**EDI**	**THQ**	**EDI**	**THQ**
Alpha HCH	0	a	0.000000025	a
Gamma HCH	0.000000629	0.002095238	0.000000023	0.000076190
Delta HCH	0	a	0.000000017	a
Heptachlor	0.000000127	0.000253966	0.000000033	0.000066743
Heptachlor epoxide	0.000000914	0.070329670	0.000000014	0.001090110
Aldrin	0.000002019	0.067301714	0.000000239	0.007958095
γ-Chlordane	0	0	0.000000114	0.000228571
Endosulfan	0	0	0.000000171	0.000028419
Dieldrin	0	0	0.000000103	0.002057143
Endrin	0.000001669	0.005561905	0.000000092	0.000308190
p,p'-DDE	0.000001789	a	0.000000177	a
p,p'-DDD	0.000000114	a	0.000000078	a
p,p'-DDT	0	0	0	0
Methoxychlor	0	0	0.000000132	0.000026400

a: Not assessed under the IRIS Program

Table 3: Residues of organochlorine insecticides in the olive and corn oil samples (mg/kg)

	Olive oil				Corn oil			
pesticides detected	No.	%	MEAN.	the samples exceed MRL*	No.	%	MEAN.	the sample exceed MRL
Alpha HCH	0	0	ND	0	0	0	ND	0
Gamma HCH	0	0	ND	0	10	40	0.00026± 0.000056	0
Delta HCH	0	0	ND	0	0	0	n.d.	0
Heptachlor	8	32	0.000338± 0.00005	0	8	32	0.0002625± 0.00006	0
Heptachlor epoxide	9	36	0.000322± 0.0001011	0	1	4	0.0003±0.0003	0
Aldrin	6	24	0.0003± 0.00007	0	0	0	ND	0
γ-Chlordane	0	0	ND	0	0	0	ND	0
Endosulfan	0	0	ND	0	0	0	ND	0
Dieldrin	0	0	ND	0	0	0	ND	0
Endrin	10	40	0.00058± 0.0002843	2	5	20	0.00094± 0.000766	1
p,p'-DDE	0	0	ND	0	15	60	0.00078± 0.000354	0
p,p'-DDD	10	40	0.00038± 0.000186	0	0	0	ND	0
p,p'-DDT	0	0	ND	0	0	0	ND	0
Methoxychlor	0	0	ND	0	0	0	ND	0

ND : not detected

Table 4: Estimated daily intake (EDI) and target hazard quotient (THQ) for the mean values of organochlorine insecticide residues in the olive and corn oil samples (mg/kg b.w)

	Olive oil		Corn oil	
pesticides detected	**EDI**	**THQ**	**EDI**	**THQ**
Alpha HCH	0	a	0	a
Gamma HCH	0	0	0.000000001	0.000003392
Delta HCH	0	a	0	a
Heptachlor	0.000000005	0.000010583	0.000000001	0.000002055
Heptachlor epoxide	0.000000005	0.000387777	0.000000001	0.000090321
Aldrin	0.000000005	0.000156556	0	0
γ-Chlordane	0	0	0	0
Endosulfan	0	0	0	0
Dieldrin	0	0	0	0
Endrin	0.000000009	0.000030267	0.000000004	0.000012264
p,p'-DDE	0	a	0.000000003	a
p,p'-DDD	0.000000006	a	0	a
p,p'-DDT	0	0	0	0
Methoxychlor	0	0	0	0

a: Not assessed under the IRIS Program

4. Discussion

From the results recorded in Table (1), it cleared that seven organochlorine insecticides were detected in the examined imported butter samples including Gamma HCH, Heptachlor, Heptachlor epoxide, Aldrin, Endrin, p,p'-DDE and p,p'-DDD at percentages of 8, 36, 12, 12, 20, 16 and 4%, respectively, and with a mean values of 0.0055± 0.003182, 0.00111± 0.000111, 0.008± 0.004243, 0.0176667± 0.00557, 0.0146± 0.006439, 0.01565±0.00935 and 0.001 mg⁄kg, respectively.

Comparing our results with the European Union maximum residue limits (EU MRL) that reported MRLs of Alpha HCH, Gama HCH, Delta HCH, total Heptachlor, Aldrin, Endosulfan, Dieldrin , Endrin, total DDT and methoxychlor as 0.01, 0.01, 0.01, 0.004, 0.006, 0.05, 0.006, 0.0008, 0.04 and 0.01mg/kg, respectively(European Commission, 2005). Eight imported butter samples contain organochlorine residues above EU MRL, five samples from them contained Endrin residues, and one sample contained Aldrin, and other two samples one of them contained p,p'-DDE and Aldrin while the last contained heptachlor epoxide.

The THQ and the EDI of imported butter in Table (2) THQ for Gamma HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane,

Endosulfan, Dieldrin, Endrin, p,p'-DDT and Methoxychlor were0.002095238, 0.000253966, 0.070329670, 0.067301714, 0, 0, 0, 0.005561905, 0 and 0 respectively, It was cleared that none of the obtained results exceeded one for THQ that indicated safety results(Jiang et al., 2005).

From the results recorded in Table (1), it cleared that all organochlorine insecticides were detected in the examined farmer butter samples except p,p'-DDT, including Alpha HCH Gamma HCH, Delta HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane , Endosulfan, Dieldrin, Endrin, p,p'-DDE, p,p'-DDD and Methoxychlor at percentages of 20, 12, 4, 26, 10, 36, 2, 24, 32, 22, 16, 20 and 22%, respectively, with a mean values of 0.000221± 0.00005, 0.0002± 0.00005, 0.00015± 0.00003, 0.000292± 0.00007, 0.000124± 0.000036, 0.002089± 0.0004711, 0.001± 0.001, 0.001492± 0.000354, 0.0009± 0.00047, 0.000809± 0.0003152, 0.00155± 0.0006383, 0.00068± 0.000197 and 0.001155 mg⁄kg, respectively. All examined farmer's butter samples were below the MRL.

Higher levels of organochlorine insecticide residues were obtained by (Waliszewski et al., 1997; Amr, 1999); Pandit & Sahu, 2002; Pardio, Waliszewski, Landin, & Bautista, 2003; El-Aziz, 2005 and Witczak & Abdel-Gawad, 2014). While in (Aksoy et al., 2011) detected β-HCH in 3 of 88 samples at a mean level of 0.001 mg/kg. On the other hand (Prachar et al., 1995) found that the examined butter samples from Slovakia were contaminated with HCB and DDT at a mean values of 0.004 and 0.01 mg/kg, respectively but (Salem et al., 2009) in Jordan found that DDT and β-HCH were detected in 8 and 16%, with the mean values of 0.009 and 0.019 mg/kg, respectively.

On contrast, lower levels of some organochlorine insecticide residues were obtained by (Aksoy et al., 2011);(Waliszewski et al., 1997);(El-Aziz, 2005) in Turkey who could not detect DDT, α- HCH, Lindane, Aldrin or HCB and (Salem et al., 2009) in Jordan also could not detect α-HCH, γ- HCH, Aldrin, Heptachlor or HCB and this may be attributed to presence of these residues at very low values below the detection limits.

The THQ and the EDI of farmer butter in Table (2) THQ for Gamma HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDT and Methoxychlor were 0.00007619, 0.000066743, 0.00109011, 0.007958095, 0.000228571 , 0.000028419, 0.002057143, 0.00030819, 0 and 0.0000264 respectively, It is cleared that none of the obtained results exceeded one for HQ that indicated safety results(Jiang et al., 2005).

From the results recorded in Table (3), it cleared that 5 organochlorine insecticides were detected in the examined olive oil samples including Heptachlor, Heptachlor epoxide, Aldrin, Endrin and p,p'-DDD at percentages of 32, 36, 24, 40 and 40 %, respectively, and with a mean values of 0.000338± 0.00005, 0.000322± 0.0001011, 0.0003± 0.00007, 0.00058± 0.0002843 and 0.00038± 0.000186 mg ⁄kg, respectively. Comparing our results with the European Union maximum residue limits (EU MRL) it was found that two olive oil samples contain Endrin residues above EU MRL.

The THQ and the EDI of olive oil in Table (4) THQ for Gamma HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDT and Methoxychlor were 0, 0.000010583, 0.000387777, 0.000156556, 0, 0, 0, 0.000030267, 0 and 0 respectively, It is cleared

that none of the obtained results exceeded one for THQ that indicated safety results(Jiang et al., 2005).

From the results recorded in Table (3), it cleared that 5 organochlorine insecticides were detected in the examined corn oil samples including Gamma HCH, Heptachlor, Heptachlor epoxide, Endrin and p,p'-DDE at percentages of 40, 32, 4, 20 and 60 %, respectively, with a mean values of 0.00026± 0.000056, 0.0002625± 0.00006, 0.0003± 0.0003, 0.00094± 0.000766 and 0.00078± 0.000354 mg ⁄kg, respectively. Comparing our results with the European Union maximum residue limits (EU MRL) There were one corn oil sample contain Endrin residues above EU MRL.

Higher levels of organochlorine insecticide residues in corn oil samples were obtained by (Bajpai et al., 2007;Qin et al., 2011 and Kannan, Tanabe, Quynh, Hue, & Tatsukawa, 1992). On contrast, lower levels of some organochlorine insecticide residues were obtained by (Škrbić & Predojević, 2008 and Fontcuberta et al., 2008).

The THQ and the EDI of corn oil in Table (4) THQ for Gamma HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDT and Methoxychlor were0.002095238, 0.000003392, 0.000002055, 0.000090321, 0, 0, 0, 0.000012264, 0 and 0 respectively, It is cleared that none of the obtained results exceeded one for THQ that indicated safety results(Jiang et al., 2005).

Organochlorine has harmful effects as they can cause injury to human health as well as to the environment. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects and cancer(Longnecker and Lucier, 1997). Furthermore epidemiological studies indicate that OCPs compounds may influence the concentrations of thyroid hormones. Organochlorine pesticides may be associated with increased prevalence of diabetes and associated with delays in neurodevelopment during early childhood(Pardío et al., 2012).

OCPs can be taken up by crops from the contaminated soil or the polluted air, and transferred into different tissues of the plants. For oil crops, they may accumulate into the oil seeds easily and consequently exist in the oils because of their lipophilicity (Li et al., 2014).

From the public health point of view, we observed high percentage 8.8% (11/125) of samples exceeding the MRLs in this study, these compounds represent a potential risk to human health because of their accumulation properties in human fat tissue and presence of different residues which may be synergize together and cause health hazards to human beings. The overall results from this study showed that butter and edible oil are an important route for OCPs since they contained high fat content and consumption of these products contribute in high degree to human exposure to organochlorine insecticides.

Periodical surveys and studies for detection of organochlorine insecticides to estimate the state of food and environmental contamination with such compounds are recommended with Application of prompt regulatory actions to prevent possible illegal use of organochlorine insecticides in Egypt.

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