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Monitoring of some organochlorine residues in raw bovine milk in the west Delta area, Egypt

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Abstract

Background: For decades, the use of organochlorine (OC) pesticides has had a detrimental effect on the environment and human health. Contamination of soil, water, and air has also resulted in contaminated milk.

Aim: The purpose of this study was to investigate if any OC residues dichlorodiphenyltrichloroethane (DDT, Dieldrin, Endrin, and Lindane) were present in raw bovine milk from West Delta, Egypt.

Methods: 200 fresh raw cow milk samples (500 ml of each sample) collected from three different governorates, west Delta, Egypt, for determination of OC pesticides residues using gas chromatography with an Agilent 6890A model gas chromatograph equipped with a 63Ni microelectron capture detector.

Results: The obtained results revealed that åDDT, dieldrin, endrin, and lindane were detected in Alexandria, Behera, and Matrouh at incidence levels (22.7%, 30.7%, and 10%), (20%, 20%, and 16%), (9.33%, 13.3%, and 16%), and (12%, 10.7%, and 14%) with mean values of 232.2 ± 163.6, 156.4 ± 134.6 and 100.4 ± 85.9; 91.3 ± 61.2, 95.3 ± 59.8 and 57.6 ± 3.33; 15.7± 3.86, 15.1 ± 3.96 and 20.1 ± 7.33; 33.7 ± 10.6, 36.9 ± 5.51 and 52.2 ± 21.8 ng/g fat, respectively. El-Behera was the most contaminated province with an incidence level of 53.3% with a mean value of 136.8 ± 128.0 ng/g fat, followed by Alexandria at 44% with a mean value of 173.7 ± 155.5 ng/g fat, and finally, Matrouh 40% with a mean value of 74.5 ± 56.5 ng/g fat.

Conclusion: This research demonstrated that milk samples contain varying levels of OC pesticide residues, which can be hazardous to consumer health. Therefore, to safeguard consumers, especially children, and the elderly, OC pesticide residues in milk must be closely monitored.

Keywords: Bovine milk, Gas chromatography, Organochlorine.

Introduction

Organochlorine (OC) pesticides are highly toxic, long-lasting pollutants that can harm the environment and human health. These compounds can cause chronic toxicity more quickly than other pesticides, such as organophosphorus pesticides (Abou Donia *et al.*, 2010). OC pesticides have been found to contaminate milk, posing a serious risk to infants, children, and adults who consume it. In recent years, this has become a major concern due to the vast milk consumption (Fontcuberta *et al.*, 2008). Previous studies showed that over 90% of the typical human intake of polychlorinated biphenyls and OC compounds during the past few years came from food of animal origin (Dirtu and Covaci, 2010).

OC pesticides, especially dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCHs), together with the cyclodienes such as aldrin, dieldrin, chlordane, and heptachlor were found in milk and dairy products during the last decades, and they were mentioned in different studies (Tsiplakou *et al.*, 2010; Aslam *et al.*, 2013; Nasef *et al.*, 2019). DDT was one of the most widely used organochloride pesticides because it could kill various insects, was inexpensive to produce, and had no odor (Lallas, 2001).

In this regard, foods high in fat, such as meat, fish, and dairy products, are the main way people are exposed to OCs (Jayaraj *et al.*, 2017). Milk stands out among them because, in addition to its derivatives being widely used in the human diet, it is an essential nutrient for children's growth. Therefore, to reduce the risk to human health, it is necessary to continuously evaluate OCP levels, especially in milk (Luzardo *et al.*, 2012; Avancini *et al.*, 2013).

Compounds containing OC are lipophilic and undergo minimal metabolization in living things. Therefore, these chemicals accumulate and stay in adipose tissue due to environmental exposure (Falandysz *et al.*, 2004). Additionally, pesticides are biomagnified in the food chain, which leads to chronic toxicity after prolonged

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exposure (Borga *et al.*, 2001). Although the long-term effects of pesticides from contaminated food on human health are poorly understood, there is growing proof that they are genotoxic, carcinogenic, and disturb hormonal processes (Ledoux, 2011).

Due to water contamination, the application of pesticides to treat ectoparasites directly on the animal, intake of polluted pastures and meals, and pesticide residues, it is possible to find pesticide residues or their metabolites in milk (Bajwa and Sandhu, 2014). Milk is used as an indicator to assess the persistence of chemicals in agriculture and environmental pollution since the presence of these substances in milk may pose a concern to public health.

Maximum residue levels (MRLs) for pesticide residues in products of plant and animal origin have been defined to safeguard consumers and advance commerce. The MRLs for the target°C pesticides dichloro-diphenyl-trichloroethane and endrin in milk have been set by legislation in the European Union at 40 and 0.8 ng/g, respectively (EC, 2005).

In Egypt, OC is still widely used in Egypt's agriculture, despite the country's ban on its use. Therefore, this study aimed to monitor the levels of OC residues (DDT, Endrin, Dieldrin, and Lindane) in raw cow's milk from three provinces in West Delta, Egypt.

Materials and Methods

Collection of samples

200 fresh raw cow's milk samples were gathered randomly from local markets in Egypt's West Delta region, including Alexandria (75 samples), El-Behera (75 samples), and Matrouh (50 samples) provinces in Egypt. Samples were kept frozen at −20°C before analysis.

Chemical and reagent

Hexane, petroleum ether for chromatography, and 60/100 mesh pesticide reagent grade Florisil were obtained from Mallinckrodt Backer (Kentucky, USA). Florisil was previously activated at 150°C/12 hours and deactivated by adding 2% Milli-Q water before use. Standard OC pesticides (DDT, dieldrin, endrin, and lindane) were obtained from Ultra Scientific (North Kingstown, RI). All other reagents used were of analytical reagent grade. All glassware used was previously washed with distilled water, rinsed with hexane and acetone alternately, and dried at 150°C to assure chemical cleanliness.

Sample extraction and clean-up

Sample extraction and clean-up were done according to the method mentioned by Heck *et al.* (2007). Fat samples were extracted and purified, following the method described by Sandmeyer (1992). To begin, 250 ml of milk was centrifuged at 17.300 rpm for 15 minutes at 4°C. The milk fat was then combined with 25 g of anhydrous sodium sulfate and 100 ml of petroleum ether before being filtered through anhydrous sodium sulfate and evaporated under a vacuum. The resulting purified fat residue was transferred to a glass vial and stored at 20°C until the compounds were purified.

Compounds are purified, according to Martinez *et al.* (1997). In brief, 1 g of fat sample was mixed with 3 ml of n-hexane and applied to a chromatographic column containing 15 g of florisil and anhydrous sodium sulfate. The eluate was filtered through anhydrous sodium sulfate, evaporated to dryness in a rotary evaporator, dissolved in 1 ml of n-hexane, and analyzed for OC pesticide determinations by gas chromatography with an Agilent 6890A model gas chromatograph equipped with a 63 Ni microelectron capture detector. The carrier gas was nitrogen (1.5 ml/minute), and the oven temperature was set at various levels. All samples were analyzed in duplicate, and the results represent the arithmetic means.

Analysis of data

To analyze the data, version 20.0 of the Statistical Package for Social Sciences software from IBM Corp. USA was utilized. The Kruskal–Wallis test was employed to perform a nonparametric comparison of all milk samples across cities, while Fisher's Exact test was used to evaluate the comparison based on frequencies (%) of detection.

Results

Data presented in Table (1) reveal that the most contaminated raw cow's milk with DDT was found in El Behera province, with an incidence rate of 30.7% and a higher maximum value of 547.1 ng/g fat. While the incidence rate in Alexandria province was 22.7% with a higher mean value of 232.2 ± 163.6 ng/g fat, and Matrouh province had the lowest incidence rate of 10% with a lowest mean value of 100.4 ± 85.9 ng/g fat. In addition, most contaminated raw cow's milk samples in different provinces were above the permissible limit established by the European Commission.

Dieldrin residues in raw cow's milk collected from both Alexandria and El-Behera provinces had the same incidence level 20, 20% with mean values of 91.3 ± 61.2 and 95.3 ± 59.8 ng/g fat, respectively, and Matrouh has the lowest incidence 16% with mean values of 57.6 ± 3.3.3 ng/ g fat. Regarding Endrin residues, Matrouh was the most contaminated province with an incidence level of 16% with a mean value of 20.1 ± 7.33 ng/g fat, followed by El-Behera with a level of 13.3% with a mean value of 15.1 ± 3.96 ng/g fat and Alexandria has lowest incidence 9.33% with a mean value of 15.7 ± 3.86 ng/g fat. All positive samples contaminated with dieldrin and endrin exceeded the permissible limit established by European Commission lindane residues, Matrouh was the most contaminated province with an incidence level of 14% with a mean value of 52.2 ± 21.8 ng/g fat, followed by Alexandria with a level 12.0% with a mean value of 33.7 ± 10.6 ng/ g fat and El-Behera has lowest incidence 10.7% with a mean value of 36.9 ± 5.51 ng/ g fat and all positive samples exceeding the permissible limit.

Table 1. Concentration (ng/g fat) and frequency of detection of certain OC in raw milk samples obtained from small-scale farmers in West Delta, Egypt.

			All samples			Contaminated samples			
Insecticide	**Governorate**	***N***	**Min–Max**	**Mean (SD)**	**Median (Q1–Q3)**	***N* (%)**	***N* (%) >MRL**	**Mean (SD)**	**Median (Q1–Q3)**
åDDT	Alexandria	75	nd–541.8	52.6 (123.9)	0 (0–0)	17 (22.7)	16 (21.3)	232.2 (163.6)	153.5 (120.6–301.1)
	Behera	75	nd–547.1	48.0 (103.2)	0 (0–32.3)	23 (30.7)	18 (24.0)	156.4 (134.6)	147.7 (41.6–198.8)
	Matrouh	50	nd–239.1	10.0 (39.1)	0 (0–0)	5 (10.0)	4 (8.00)	100.4 (85.9)	63.5 (51.7–124.5)
	P1 = 0.021, P2 = 0.022								
Dieldrin	Alexandria	75	nd–210.3	18.3 (45.4)	0 (0–0)	15 (20.0)	15 (20.0)	91.3 (61.2)	68.3 (45.6–137.4)
	Behera	75	nd–211.9	19.1 (46.4)	0 (0–0)	15 (20.0)	15 (20.0)	95.3 (59.8)	68.3 (52.0–141.3)
	Matrouh	50	nd–129.1	9.20 (24.8)	0 (0–0)	8 (16.0)	8 (16.0)	57.6 (33.3)	56.3 (30.9–66.1)
	P1 = 0.716, P2 = 0.873								
Endrin	Alexandria	75	nd–21.1	1.46 (4.72)	0 (0–0)	7 (9.33)	7 (9.33)	15.7 (3.86)	15.4 (13.2–19.3)
	Behera	75	nd–21.9	2.01 (5.33)	0 (0–0)	10 (13.3)	10 (13.3)	15.1 (3.69)	14.9 (13.1–17.8)
	Matrouh	50	nd–31.3	3.22 (7.95)	0 (0–0)	8 (16.0)	8 (16.0)	20.1 (7.33)	19.5 (13.7–26.4)
	P1 = 0.479, P2 = 0.506								
Lindane	Alexandria	75	nd–44.8	4.04 (11.6)	0 (0–0)	9 (12.0)	8 (10.7)	33.7 (10.6)	37.5 (31.3–38.3)
	Behera	75	nd–44.3	3.94 (11.6)	0 (0–0)	8 (10.7)	8 (10.7)	36.9 (5.51)	36.7 (32.9–41.8)
	Matrouh	50	nd–90.7	7.31 (19.8)	0 (0–0)	7 (14.0)	7 (14.0)	52.2 (21.8)	42.5 (37.0–72.6)
	P1 = 0.787, P2 = 0.823								
åOCP	Alexandria	75	nd–586.8	76.4 (134.1)	0 (0–108.0)	33 (44.0)	–	173.7 (155.5)	130.7 (61.6–210.3)
	Behera	75	nd–592.8	73.0 (115.6)	19.8 (0–107.8)	40 (53.3)	–	136.8 (128.0)	95.3 (39.8–213.1)
	Matrouh	50	nd– 39.1	29.8 (50.9)	0 (0–39.9)	20 (40.0)	–	74.5 (56.5)	63.3 (30.3–114.8)
	P1 = 0.158, P2 = 0.300								

(åOCP): per sample sum of DDT; (dieldrin): endrin and lindane residues; (Min–Max): minimum–maximum; (Q1): 25th percentile; (Q3): 75th percentile. (MRL): Maximum residue level as per the European Commission (EC) Regulation No 299/2008 (EU): åDDT: 40 ng g^{-1} fat, dieldrin: 6 ng g^{-1} fat, endrin: 0.8 ng g^{-1} fat, lindane: 10 ng g^{-1} fat; (SD): Standard deviation; (nd): non–detectable. (P1): Probability values result from the nonparametric comparison among governorates (Kruskal–Wallis test) including all samples; (P2): Probability values result from the comparison among frequencies (%) of detection (Fisher's Exact).

In general, El-Behera province was the most contaminated province with OC residues in raw cow's milk, with an incidence level of 53.3%, followed by Alexandria, with an incidence level of 44%, and Matrouh province has the lowest incidence rate of 40%.

Gas chromatography with a microelectron capture detector exhibited excellent repeatability, as indicated by the coefficient of variation below 10%. Limits of detection and quantification were determined using the average blank values method. Mean recoveries ranged from 88.7% to 103.6% for OC pesticides (Table 2).

Discussion

OCPs were still widely used for agricultural and human pest control management in some tropical nations in the early 1980s despite being illegal. Due to OCs slow breakdown rate and lengthy half-life, which give them the property of high environmental stability, this contaminated the environment and the food chain. Additionally, due to their lipophilic nature, milk animals store pesticides in tissues rich in fat, then transferred them to milk fat (Aslam *et al.*, 2013).

A higher incidence of contamination with total DDT in Behera than in the other two provinces may result from the expansion of agricultural land and its extensive use. The lower level of DDT in raw milk was recorded by Sharma *et al.* (2007) in bovine milk collected from India was 36.7 ng/g with an incidence of 97%; Darko and Acquaah (2008) in cow's milk in Ghana was 12.53 ng/g; Ismail and Elkassas (2016) in buffalo milk in Egypt was 22.6 ng/g. At the same time, a higher level was reported by Nasef *et al.* (2019) in raw milk collected from three districts (A–C) in Alexandria provinces 217, 251, and 306 ng/g with lower incidence rates of 10%, 6.66%, and 10%, respectively.

According to permissible limits established by EC (299/2008) (åDDT, 40 ng g^{-1} fat), 21.3%, 18%, and 8.0% of examined raw cow's milk samples exceed this limit. Sharma *et al.* (2007) found that 35 bovine milk samples in India out of 147 samples (23.81%) exceeded the permissible limits recommended by FAO.

According to the permissible limits established by EC 299/2008 (6 ng/g), all contaminated samples with dieldrin exceeded the limit. A low level of dieldrin was reported by Darko and Acquaah (2008) in cow's milk in Ghana was 1.32 ng/g. A higher level was reported by Nasef *et al.* (2019), who found that dieldrin was detected in 10% and 6.66% of examined raw milk with a mean value of 147 and 163 ng/g, respectively, in distract "A" and "B" in Alexandria city. In Giza, Egypt, a study by Ahmed and Zaki (2009) indicated that the prevalence of dieldrin in raw milk was 55.5%, with a mean value of 2,966 ng/g. On the other hand, Salem *et al.* (2009) could not find dieldrin residues in milk samples taken in Jordan.

Table 2. Mean recoveries of OC pesticides.

Pesticide	Recovery rate (%)
DDT	95.2
Dieldrin	88.7
Endrin	103.6
Lindane	91.4

Animals quickly convert endrin pesticide to dieldrin. As a result, total dieldrin was defined as the sum of the residues from both endrin and dieldrin pesticides (Smith, 1991).

According to the permissible limit established for endrin by EC 299/2008 (0.8 ng/g), all contaminated cow's milk samples exceeded the limit. A lower endrin figure was reported by Ismail and Elkassas (2016) in Kafr El-Sheikh governorate, who found that the mean concentration of endrin was 10.3 ng/g with an incidence rate of 65%. On the contrary, due to the restricted use of these insecticides, (El-Asuoty *et al.*, 2017) could not find endrin in any of the analyzed raw milk samples in the El-Behera governorates.

Lindane is seen as a major issue due to its carcinogenic properties, which can have a detrimental effect on the functioning of other vital organs in the body (Vettorazzi, 1975). According to European Commission (299/2008) it is stated that the permissible limit of lindane was (10 ng/g fat); there are 10.7%, 10.7%, and 14.0% of contaminated samples exceeding this limit.

A higher level of lindane in raw milk was reported by Shaker and Elsharkawy (2015), who found that lindane was 165 ng/g (north) and 97 ng/g (south) in samples from dairy farms and 155 ng/g (north) and 187 ng/g (south) in samples from dairy shops, Assiut. Regarding higher incidence, Gamma-HCHs, also known as lindane, were discovered by Battu *et al.* (2004) in 53.3% of liquid milk samples. Also, Abou Donia *et al.* (2010) found -HCH in 50% of buffalo milk samples.

Overall, a higher incidence of contaminated raw milk samples with investigated OC pesticide residues was found in El-Behera with an incidence rate of 53.3% while Alexandria at 44% and Matrouh at 40% with a mean value of 136.8 ± 128.0, 173.7 ± 155.5, and 74.5 ± 56.5 ng/g fat in El-Behera, Alexandria, and Matrouh, respectively. This is due to intensive agricultural activities in El-Behera province, which often involve OC pesticides. These pesticides can easily contaminate the soil and water, leading to higher contamination levels in milk produced in the region.

According to Fernandez *et al.* (2008), the quantification of various pesticides in milk can be efficiently achieved through gas chromatography with microelectronic-capture detection, which is a rapid and uncomplicated method.

The presence of OC pesticide residues in milk at levels higher than the established maximum residue limits is a cause for concern, as these substances can be hazardous to human health and the environment, potentially causing carcinogenicity, reproductive impairment, developmental and immune system changes, and endocrine disruption (IOMC, 2002).

Conclusion

Residue levels of investigated OC pesticides were higher than the European Commission MRLs in raw bovine milk samples taken from three different provinces in Egypt. To conclude, Behera governorate was the most contaminated province with investigated OC pesticides, followed by Alexandria and then Matrouh, due to intensive agricultural activities in Behera than in other governorates, and this means that there is a greater likelihood of pesticide use in the area than in Alexandria and Matrouh Governorates. To safeguard people and reduce pesticide exposure for consumers, institute monitoring systems for OC pesticide residues in milk intended for human consumption.

Authors contributions

Both authors contributed to the study. Both authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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