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PREVALENCE OF SOME ORGANOCHLORINE PESTICIDE RESIDUES IN MARKET MILK

(With 2 Tables and One Figure)

By

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مدى تواجد بقايا بعض المبيدات الكلورينية العضوية فى اللبن المباع

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تعتبر المبيدات من اهم الملوثات الكميائية للالبان والتى لها اثار ضارة على صحة الانسان وانتاج الالبان وخاصة بقايا بعض المبيدات الكلورينية العضوية. لذا أجريت هذة الدراسة على عدد ٥٠ عينة عشوائية من اللبن الخام المباع وذلك لتقدير مدى تواجد وكمية متبقيات المركبات الكلورينية العضوية بها وذلك باستخدام جهاز كروماتوجراف الغاز. وتشمل الفاهكساكلوروسيكلوهكسان والندان و د.د.ت و د.د.د. و د.د.اى والالدرين والدايلدرين والهبتاكلور والهبتاكلور ايبوكسيد. وقد اثبتت النتائج ان متبقيات مبيدات كلا من الفاهكساكلوروسيكلوهكسان والندان و د.د.ت و د.د.د. و د.د.اى والهبتاكلور والهبتاكلور ايبوكسيد هى اكثر المتبقيات تواجدا فى عينات اللبن الخام المباع بالاسواق التى تم فحصهاحيث تواجدت بمستويات متفاوتة. بينما كان مبيد الدايلدرين متواجد فى عدد قليل من العينات المختبرة. على الجانب الاخر اظهرت هذة الدراسة ان متبقيات مبيد الالدرين لم يمكن الكشف عنة فى جميع العينات المفحوصة. وقد تمت فى هذة الدراسة مقارنة مستويات متبقيات المبيدات الحشرية التى تم الكشف عنها بالحدود القصوى المسموح بتواجدها والمحددة من قبل FAO/WHO حيث وجد ان مبيدات الندان و د.د.ت و د.د.اى والهبتاكلور ايبوكسيد قد زاد عن الحد المسموح به فى ٩٧,٧% ، ٢,١% ، ٢,٢% ، ٢,٥% من العينات المفحوصة على التوالى. هذا وقد تم مناقشة الاهمية الصحية لتواجد هذة المتبقيات باللبن الخام المباع بالاسواق وكذلك الاشتراطات الصحية الواجب مراعتها فى انتاج الالبان.

SUMMARY

Monitoring of organochlorine pesticide residues level in raw market milk in Sharkia province, Egypt was carried out. A total of 50 random market raw milk samples were collected for detection of organochlorine pesticide residues using electron capture detector gas chromatography. The achieved results from this study revealed that pesticide residues which most often found in examined milk samples were ∝-HCH, γ-HCH "lindane", P-P-DDT, P-P-DDD, P-P-DDE, Heptachlor and

Heptachlorepoxide, while residues of dieldrin could be detected in a few number of samples, on the other hand residues of aldrin could not be detected in all examined samples. The detected levels of organochlorine pesticide residues were compared with maximum residues limits of FAO/WHO.Moreover, the public health importance of such toxic residues and suggestive control measures to safe guard human health were discussed.

Key words: *Milk, organochlorine pesticide, lindane, DDT, heptachlor.*

INTRODUCTION

The organochlorine pesticides were used extensively in Egypt during past 30 years, these compounds characterized by persistence, high absorbency and high accumulation in fatty animal and human tissues and are excreted in milk, represent a potential hazard to public health due to the harmful biological effects. Therefore, they are banned completely since 1970s (Deiana and Fatichenti, 1992). The major sources for the contamination of milk with pesticide residues are fodder, soil and veterinary treatments (IDF, 1979). Obviously, the extensive use of pesticides has created many problems, one of this problem, is the contamination of food products with such toxic residues (FDA, 1993).

In spite of their effect on environment, the risk of pesticides is still prominent because of the huge amount used for agricultural and industrial purposes and the lack of proper protective measures during handling and application. As a result, pesticides are nowadays present in all compartments of the environment, which explains the great interest in early warning and monitoring system of these compounds (IDF, 1991 and Osweiler, 1996).

Up to date, no entirely safe pesticides have been developed, so the presence of significant amounts of organochlorine pesticide residues in milk is undesirable. As consequence these compounds have been reviewed by regulatory governmental agencies, including Food and Agriculture Organization for establishing an acceptable daily intake (ADI) and tolerance limits for pesticides (FAO/WHO, 1993). Therefore, it is plausible to throw light on the presence of organochlorine pesticide residues in raw market milk.

MATERIALS and METHODS

1- Collection and preparation of milk samples:

Fifty random raw milk samples (250 ml each), were collected from street peddlers and dairy shops in Sharkia Province, Egypt. Each sample was placed into clean and dried glass container and immediately transferred to the laboratory as soon as possible in ice box to be examined for presence of pesticide residues.

If analysis delayed for a few days, 10 ml milk was perfectly mixed in small glass container with screw capped stoppers and kept deeply frozen at-40°C for organochlorine pesticide residues analysis.

2-Glass wares:

All glasses were prepared for analysis according to steps described by Suzuki *et al.* (1979).

3-Chemicals and solvents:

- Analytical grade of anhydrous sodium sulphate was dried at 135 °C for several days prior to use.
- Florisil, PR grade (60-100 mesh) was prepared by extensive washing with water, drying at 100 °C and then overnight firing at 550 °C, the washed, fired Florisil was kept at 135 °C till use.
- Acetonitrile, ethanol, N-hexane, benzene, ethylacetate were distilled from glass wares subjected to general purity test.

4- Gas Liquid chromatography (GLC):

ATIUNICAM-610 series gas chromatography with splitless injector, equipped with an electron capture detector and ATI UNICAM chromate jet integrator was used. The injector was connected to 30 m x 0.22 mm I.D. Silica capillary column containing DP-17 with 0.25 um film thickness. Nitrogen was used as a carrier gas, at flow rate of 2ml/min. The injector temperature was 260°C and the detector temperature was 300°C. Splitless injector was performed at oven temperature of 250°C. The organochlorine pesticide residues were quantitatively determined by comparison with standard solution injected under identical GLC conditions.

5- Pesticides reference standards:

Generally 0.01 gm was accurately weighed and quantitatively transferred to a 100 ml. volumetric flask as a stock standard solution using n-hexane. An aliquot from each of these stock standard solutions was diluted to a proper concentration from which a 5 ul. Injection gives a half full scale deflection peak. These working standard solution were used in determining the retention times of each of the investigated organochlorine pesticides. All reference standards were kindly provided by the National Food Administration, Food Research dept. Uppsala, Sweden.

6- Organochlorine pesticides under investigation:

Investigated pesticides includes different isomers of Hexachlorocyclohexane (α-HCH & γ-HCH "lindane"), DDT complex

(DDT P-P, DDD P-P & DDE P-P), Heptachlor, Heptachlorepoxide, Aldrin and Dieldrin.

7- Extraction and clean-up procedures:

The method used for extraction and clean-up procedures was described by Suzuki *et al.* (1979).

8- Recovery experiment:

Duplicate sub-samples from raw milk were subjected to all the previously mentioned steps of extraction, clean up and gas liquid chromatographic determination. To one of the duplicate sub-samples an a aliquot of the mixture contained the organochlorine pesticides standards was added. The amounts recovered from each pesticide was calculated and compared to the other duplicate to which no pesticides were spiked. The difference between the duplicates indicates the actual recovery of the pesticides.

Retention times and recovery percent of organochlorine pesticides from fortified milk samples.

Pesticides	Retention time (minutes)	Fortified level (ppm)	Recovery percent
∝-HCH	5.22	1.6	90.12
γ-HCH (Lindane)	5.21	0.2	84.30
P-P'-DDT	11.69	5.0	91.80
P-P'-DDD	10.05	5.0	100.14
P-P'-DDE	10.29	4.0	88.90
Aldrin	6.15	0.8	84.14
Dieldrin	8.79	1.6	98.90
Heptachlor	5.63	0.4	89.90
Heptachlorepoxide	7.29	0.6	96.15

RESULTS

Table 1: Prevalence and levels of organochlorine pesticide residues in examined raw milk samples (ppb).

Pesticides	Incidence (Positive samples)		Levels(ppb)		
	No./50	%	Min.	Max.	Average
∝-HCH	45	90	0.36	3.6	0.64
γ-HCH (Lindane)	44	88	1	53	42
P-P' –DDT	47	94	3.2	59	9
P-P' -DDD	28	56	0.56	29	3.67
P-P' –DDE	45	90	2.6	66	20.2
Aldrin	0.0	0.0	0.0	0.0	0.0
Dieldrin	8	16	0.52	2.93	1.63
Heptachlor	33	66	0.43	4.7	1.7
Heptachlorepoxide	40	80	0.31	39.2	4.4

Fig. 1: Levels of organochlorine pesticide residues in positive samples of raw milk.

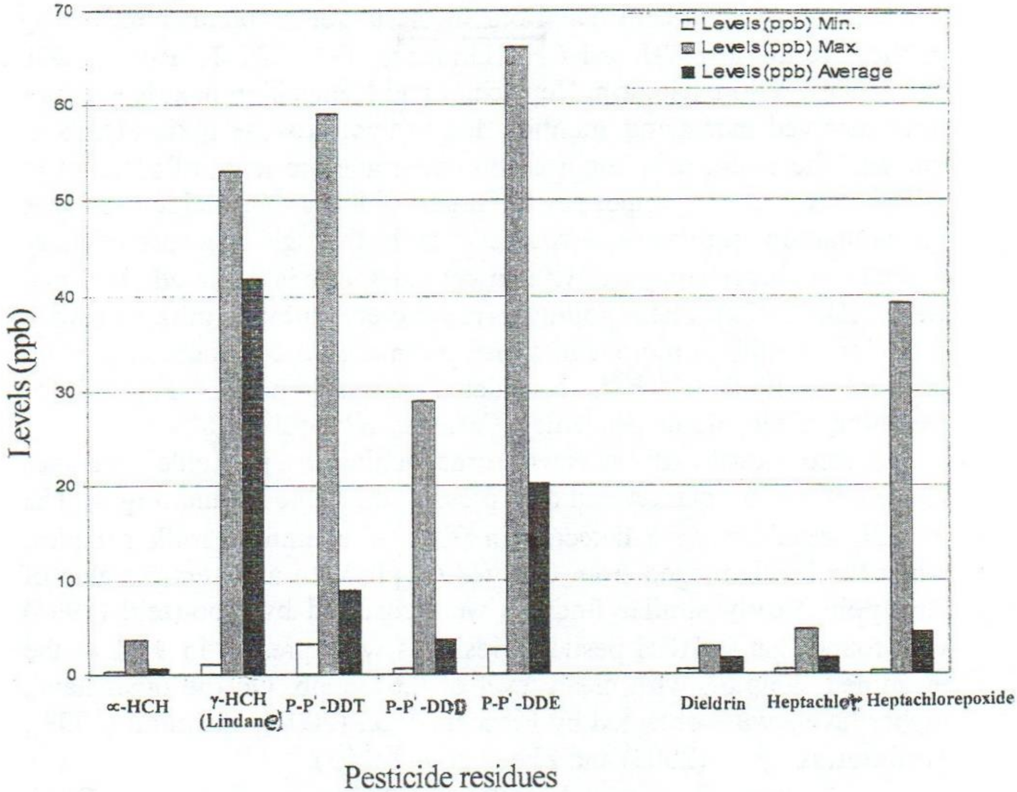

Table 2: Organochlorine pesticide residues of positive market milk samples on a basis of maximum residues limits (MRL*)

Pesticides	Maximum residues limits (MRL) (ppb)	Within MRL		Over MRL	
		No.	%	No.	%
∝-HCH	50	45	100	0.0	0.0
γ-HCH (Lindane)	10	1	2.3	43	97.7
P-P'- DDT	50	46	97.9	1	2.1
P-P'–DDD	50	28	100	0.0	0.0
P-P'–DDE	50	44	97.8	1	2.2
Aldrin	6	0.0	0.0	0.0	0.0
Dieldrin	6	8	100	0.0	0.0
Heptachlor	6	33	100	0.0	0.0
Heptachlor epoxide	6	39	97.5	1	2.5

* The maximum residue limit of organochlorine pesticide residues in milk proposed by FAO/WHO (1993).

DISCUSSION

Contamination of milk and milk products with organochlorine pesticide residues is considered one of the major problems confronting public health. ∝-HCH, γ-HCH (Lindane), P-P' –DDT, P-P' –DDD, P-P' –DDE, Aldrin, Dieldrin, Heptachlor and Heptachlorepoxide residues have received increasing attention due to their adverse toxic effects in human. Therefore, it is important to determine the level of residues in milk, since it is exposed to organochlorine pesticide residues contamination from various sources mainly through exposure of dairy animals to direct spraying with insecticides or via polluted food and water (IDF, 1979). These pollutants are excreted into the milk by simple diffusion as milk is more acidic than plasma, also considerable portion of milk is lipid which is considered as an important channel for excretion of fat soluble pesticides (Casarett and Doull, 1980).

The level of selected organochlorine pesticide residues concentration in market milk is present in Table 1 and Fig.1. The ∝-HCH residues were detected in 90% of examined milk samples, while the levels ranged from 0.36 to3.6 ppb, with an average value of 0.64 ppb. Nearly similar findings were reported by Abouzeid (1994) who found that ∝-HCH pesticide residues were present in 94% of the examined samples, with mean level of 0.613 ppb. On the other hand, higher levels were obtained by Francois *et al.* (2002); Bayoumi (2003); Waliszewski *et al.* (2003) and Zhong *et al.* (2003).

Likewise, the obtained results revealed lower values of ∝-HCH, this may be due to highest accumulation factors (5.4%), as well as, low excretion percentage. The range of accumulation factor depends more on the level of the contamination of the feed and time period of the feeding (IDF, 1991).

It is obvious clear from the results illustrated in Table 1 that γ-HCH (lindane) was present in 88% of the examined samples. The residues levels ranged from 1.0-53.0 ppb, with an average value of 42.0 ppb. Similar results were obtained by El-Hoshy (1997) and Francois *et al.* (2002). More or less similar levels were reported by Abouzeid (1994); Cerkvenik *et al.* (2000) and Pardio *et al.* (2003).

According to the maximum residues limits recorded by FAO /WHO (1993), the results presented in Table 2, revealed that 97.7% of the examined milk samples containing lindane beyond the maximum residues limits. The sources of food contamination by lindane were identified by Viccellio *et al.* (1998) used as pesticide, scabicide and pedialocides. It was the last organochlorine pesticides to be banned from agriculture practice in France in 1988, and it has been banned in Canada, United states, China, Soviet Union and Austalia in 1971, 1976, 1983, 1990 and 1994, respectively, (Li, 1999 and Francois *et al.,* 2002). Lindane still used extensively in a number of African countries, India and Brazil (Li, 1999), derived from many industries activities (Frank and Ripley, 1990) and high persistence of lindane in the environment (Uhnak *et al.,* 1986).

P-P'-DDT is considered one of the most important pollutant in the environment, with a potential toxicity for all biological systems, as it accumulates in human tissues. The levels of p-p'-DDT concentration were ranged from 3.2 to 59 ppb, with an average value of 9 ppb. 94% of examined market milk samples containing p-p' DDT (Table 1 & Fig.1). Nearly similar findings were reported by Abouzeid (1994), who recorded that the mean level of p-p'- DDT was 8.902 ppb in examined milk samples. On the other hand, high figures were recorded by several workers (Cerkvenik *et al.,* 2000; Francois *et al.,* 2002; Waliszewski *et al.,* 2003 and Zhong *et al.,* 2003). Lower levels were recorded by Aman and Bluthgen (1997), they mentioned that p-p' DDT was detected with a mean level of 7.67 ppb.

DDT is considered one of the most important pollutants in the environment and distributed widely in different classes of animal feeds. The major sources of DDT in the environment arises from application of DDT for control of pests in the animal feed, flies and ectoparasites. Although DDT was banned in the early 1970, it was still manufactured in the United states and exported at rate of one ton/day for developing and tropical countries in 1994 (Smith, 1995).

It is evident from the results illustrated in Table 1 and Fig.1 that p-p'-DDD residues were detected in 56% of the examined market milk samples. The levels ranged from 0.56 to 29 ppb, with an average value of 3.67 ppb. Nearly similar findings were obtained by Abouzeid (1994). On the contrary, Ali *et al.* (1993) and El-Hoshy (1997) recorded much higher levels of p-p'-DDD in the examined raw milk samples.

Inspection of Table 1 and Fig. 1 revealed that p-p'-DDE residues were detected in 90% of samples. The values ranged from 2.6-66 ppb, with an average level of 20.2 ppb. More or less similar levels were obtained by Awasthi and Ahuja (1995); Wong and Lee (1997); Storelli *et al.* (2001); Francois *et al.* (2002) and Bayoumi (2003). The level of DDT in milk is lower than its metabolites p-p'-DDE. These results proven that p-p'-DDT undergo several metabolic degradation to its metabolites as p-p'-DDE (IDF, 1991). This data might indicate the old use of DDT, aging DDT stored in food results in higher p-p'-DDE concentration as derivatives (Fries *et al.*, 1972 and El-Marsafy *et al.*, 1999).

The results displayed in Table 2 revealed that 2.1%, 0% and 2.2% of market milk samples contained p-p' DDT, p-p'-DDD and p-p'-DDE, respectively, beyond the maximum residues limits recorded by FAO/WHO (1993).

It is obvious clear from the results presented in Table 1 and Fig. 1 that dieldrin was detected in 16% of samples. The concentration varied from 0.52 to 2.93 ppb, with an average value of 1.63 ppb. Similar results were obtained by Bluthgen *et al.* (1984) and Abouzeid (1994). Considerable higher levels were recorded by El-Hoshy, (1997); Wong and Lee (1997) and Francois *et al.* (2002). On the other hand, Elafi *et al.* (1997) reported that dieldrin was not detected in any of analyzed milk samples.

Results presented in Table 2 indicated that all examined milk samples contained dieldrin within the permissible residues limits recorded by FAO/WHO (1993). Although dieldrin is used in egyptian agriculture since 1970, it was detected at lower levels in any of analyzed raw milk samples according to FAO/WHO (1993). This may be due to that the dieldrin was destroyed by acid produced by soil bacteria (UNEP/IOC/IAEA, 1986).

The results shown in Table 1 and Fig.1 revealed that aldrin could not be detected in all the examined raw milk samples. Similar results were recorded by Abouzeid (1994) and El-Hoshy (1997).

The absence of aldrin in the examined raw milk samples could be attributed to its continuous degradation into dieldrin within the living tissues through the different metabolic processes (Dogheim *et al.*, 1988).

Heptachlor concentrations in the examined market milk samples was clear in Table 1 and Fig.1. The results revealed that heptachlor residues were detected in 66% of samples. The levels ranged between 0.43 and 4.7 ppb, with an average of 1.7 ppb. Also, the results shown in Table 2 revealed that non of market milk samples were beyond the maximum residues limits for heptachlor recorded by FAO/WHO (1993). Nearly similar levels were recorded by Abouzeid (1994). Considerable higher levels were reported by Francois *et al.* (2002) and Bayoumi (2003).

Heptachlorepoxide concentration in the examined raw milk samples were clear in Table 1 and Fig.1 the results proved that 80% of examined market milk samples contained heptachlorepoxide residues at level ranged from 0.31 to 39.2 ppb, with an average value of 4.4 ppb. Likewise, the results shown in Table 2 cleared that 2.5% of the examined market milk samples were beyond the maximum residues limits for heptachlorepoxide recorded by FAO/WHO (1993). The obtained results substantiate what had been reported by Frank *et al.* (1985). On the other hand, Mahieu *et al.* (1982); Wong and Lee (1997) and Francois *et al.* (2002) recorded much higher levels of heptachlorepoxide in the examined raw milk samples.

The significant high value of heptachlorepoxide than heptachlor isomer, it could be attributed to its higher stability. Nearly similar findings were reported by Dogheim *et al.* (1988); Venant *et al.* (1990) and Abouzeid (1994).

On analyzing the results presented, in this study one can recognize that the only concentration of γ-HCH (Lindane), p-p'-DDT, p-p'-DDE and heptachlorepoxide residues in raw milk samples were considerably higher than the maximum residues limits recorded by FAO/WHO (1993). The presence of low levels of pesticide residues in raw milk may be attributed to efforts by Governmental agencies to monitor the level of these residues, enacting of food safety laws and enforcing all regulation that help for limiting usage of such pesticides; moreover, strict hygienic measures should be imposed during milk production and handling. In addition, education, training and information on pesticides safety involving improved formulation and application techniques help to minimize the residues level in different varieties of foods.

From the public health point of view, organochlorines have been related to an increase in the incidence of some kinds of tumors, such as leukemia and solid tumors. Reproductive effects, due to anti-androgenic and estrogenic action, the incidence of abortion and the frequency of prematurely, have also been observed. The accumulation of the organochlorines in the adipose tissues is positively correlated to the increase in aging and could be implicated in development of aging diseases, such as Parkinson's disease (Nunes Monica and Tajara Eloiza, 1998). DDT is suspected to being a human carcinogen, this suspicion is based on the fact that DDT has been shown to cause liver tumor in mice (Edward, 1993). Likewise, Helleday *et al.* (1996) concluded that DDT has adverse effect on human health in terms of inducing genetic recombination which is known to provoke a number of diseases including cancer. Furthermore, Langnecker *et al.* (1997) reported that high level exposure to selected organochlorines (including DDT)) in man appeared to cause abnormal functioning of the liver, skin and nervous system. Ruehlnam *et al.* (1998) found that DDT,o-p inhibits the L-type Ca channel in vascular smooth muscle cells and evoke rapid relaxation of coronary vasculature. Thus inhibition of calcium influx in vascular smooth muscle cells explaining the acute vasodilator action of DDT,o-p. Regarding the effect of pesticides on the immune system, Banerjee *et al.* (1996) concluded that pesticide exposure may play a greater role in a suspected fragile immune system and may result in altered disease susceptibility. They proposed that pesticide chemicals influence humeral immunity while having no detectable effect on cell mediated immunity. The immune dysfunction is related to dose and duration of exposure. They also concluded that lymphocyte dysfunction may be an integral part of pesticide induce immunosuppressant. They quietly cleared that pesticides induce immunomodulation endangers human and animals.

From the economic point of view organochlorine residues in milk even in small quantities have adversely affect and depress the profitability of milk industry. Organochlorine pesticides residues and their metabolites in milk have been implicated in the processibility of milk (Konrad and Gabrio, 1976), who detected that the clotting property of yoghurt and ripening process of cheese was not impaired by pesticides at concentration 100 ppm. Moreover, Abouzeid (1994) reported that the organochlorine pesticides had little or no effect on acid production in low concentration (less than 4 ppm), while it inhibits acid production at high concentrations.

In conclusion, the results of this study revealed that the levels of organochlorine pesticide residues in the examined market raw milk samples were high and beyond the maximum residues limits in most of the examined samples which may constitute a possible public health hazards. The predominant compounds in milk were lindane, DDT, DDE and heptachlorepoxide. These data can be used as guideline and a base line for future monitoring for organochlorine residues in milk in the Governorated should be done to deal with public health aspects and effects should be directed to minimize the organochlorine pesticides contamination in milk.

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