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EFFECT OF SOME FACTORS ON CONCENTRATIONS OF ORGANOCHLORINE RESIDUES IN MOTHERS' MILK AT SHARKIA GOVERNORATE, EGYPT

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ABSTRACT

Effect of some factors such as mothers' age, body mass index, mothers' participation in agricultural work, duration of mothers' participation in agricultural work, number of participation hours per day, parity, number of breast feeding per day and dietary habits on organochlorine pesticide (O.C.Ps) and polychlorinated biphenyls (PCBs) residues concentrations in mothers' milk was studied. This study was conducted on 60 lactating mothers selected randomly from the attendants to El-Hokamaa Maternal & Child Health Center in Zagazig City(urban area) and El-Saadeen Hospital in Menia Al-Kamh Center(rural area) at Sharkia governorate, Egypt. All mothers filled a pre-constructed questionnaire and milk samples were taken from them for determination of OCPs and PCBs concentrations. The obtained results showed a significant positive correlation between sum DDTs levels and mothers' age, duration of mothers' participation in agricultural work, number of participation hours per day in agriculture and average number of consumption of meat, fish, eggs and dairy products per week (foods of animal origin). A significant positive correlation was also found between sum PCBs levels and average number of weekly consumption of meat, fish and eggs. The mean concentrations of p,p'-DDT and sum DDTs were significantly higher in mothers' milk who participated in agricultural work, while the mean concentrations of heptachlor, aldrin and chlordane were significantly higher in mothers' milk who didn't participate in agricultural work. The mean concentrations of p,p'-DDE, p,p'-DDT, sum DDTs and sum PCBs were significantly higher in mothers' milk who fed their infants ≤ 10 times/day.

INTRODUCTION

The stability and lipophilic nature of organochlorine pesticides and polychlorinated biphenyls (PCBs) have led to their high persistency in the environment despite restrictions on their use (Landrigan *et al.,* 2002). They accumulate in the food chain and transfer to human body by diets, contact and inhalation. The ingestion of contaminated food is considered to be the main source for human exposure to these compounds (Schinas *et al.,* 2000). Alawi *et al.,* (1992) stated that human milk is one of the excretion pathways for organochlorine residues from mother's body. At the same time, it is one of the first natural ways of intake of these pollutants by the breast-fed newborn.

There are many factors affecting the level of chemical residues in human milk including fat content of human milk, fluctuations of contamination levels during lactation, maternal age, weight, racial group, whether or not the infant was born prematurely, maternal diet and dietary habits, cigarette-smoking, seasonal variations and direct use of pesticides by the women (Jensen and Slorach, 1991). Also, Harris *et al.,* (2001) indicated that age, parity / length of previous lactation, fat mobilization from maternal stores and the time of sampling were identified as the most likely factors to be

considered when assessing transfer of organochlorine pesticide residues into breast milk.

The aim of this work was to study the effect of some factors on concentrations of organochlorine residues in mothers' milk at Sharkia governorate, Egypt.

MATERIALS AND METHODS

Study design: This comparative cross-sectional study was conducted from July 2006 till March 2007 aiming at estimation of the concentration of organochlorine compounds in the breast milk and study some factors associated with these compounds among sixty lactating mothers from El-Hokamaa Maternal & Child Health Center in Zagazig City (30 samples from urban area) and El-Sadeen Hospital (Pediatric section) in Menia Al-Kamh Center (30 samples from Rural area).

Sample selection: Sharkia governorate is divided into 11 cities and 13 centers. From these cities and centers, Zagazig City (representive urban area) and Menia Al-Kamh Center (representive rural area) were chosen by a simple random sample. By multistage random sample, El-Hokamaa Maternal and Child Health Center was chosen from Zagazig city and by simple random sample, El-Sadeen Village was chosen from 42 villages in Menia Al-Kamh Center. Sixty lactating mothers, 30 from El-Hokamaa Maternal & Child Center and 30 from El-Sadeen Hospital, were chosen randomly from visitors to these center and hospital. All mothers who participated in the study gave their informed consent. Milk samples (20 – 30 ml) were collected in sterilized glass bottles and transported to the laboratory in an ice box which contained freezing brine solution. All samples were stored at –20°C till analysis. Mothers were asked to complete a questionnaire to obtain details about the mother such as residence area, mothers' age, weight (kg), height (m), mothers' participation in agricultural work, duration of mothers' participation in agricultural work, number of participation hours per day, parity, number of breast feeding per day and number of weekly consumption of foods.

The organochlorine pesticides under investigation were p,p'-DDT, p,p'-DDE, p,p'-DDD, α-BHC, β-BHC, γ-BHC, δ-BHC, heptachlor, heptachlor epoxide, chlordane, aldrin, endrin and endosulfan. The following PCBs were targeted no. 8, 18, 44, 70, 101, 105, 138, 152, 153, 180, 192 and 194.

Extraction:

Milk samples were extracted according to the method of Suzuki *et al.*, (1979). Before extraction, milk samples were homogenized and kept in water bath at 30°C for 10 min. A ten ml milk sample was put into a 50 ml centrifuge tube with n-hexane, acetonitril and ethanol (20, 5 and 1 ml, respectively). Samples were mixed by vigorous shaking for 1 min. followed by centrifugation for two min. at 2000 r.p.m. The n-hexane phase was filtered through anhydrous sodium sulphate and the sample was extracted twice with 20 ml n-hexane. The combined extracts were concentrated to 5 ml using the rotary evaporator. The quantity of the extract was determined by transferring 1 ml extract to a small beaker, which had been previously weighted, and n-hexane was evaporated using a gentle stream of air. The beaker was heated

at 120 °C for 30 min., cooled to room temperature and weighed again. The quantity of extract was calculated from the weight differences. The remaining 4 ml n-hexane extract was submitted to florisil column clean-up.

Cleanup:

A florisil column was prepared according to the method of De Lappe *et al.*, (1983). A ball of glass wool pre-extracted with n-hexane was placed loosely in the buttom of 22 x 300 mm. chromatographic column to give the base of florisil. Florisil was activated at 250°C for 12–15hr. Placed in a desiccator until cool, deactivated with 0.5% H_2O, stored in a sealed container in a desiccator overnight, and then used within 72 hr. Columns were rinsed with n-hexane. Samples extracts were transferred to the columns which contained 7g. florisil and topped by two cm. anhydrous sodium sulphate. Sufficient n-hexane to elute the polychlorinated biphenyls (PCBs) and p,p'-DDE, but not p,p' DDT, was added and collected as fraction I. Sufficient 30% methylene chloride in n-hexane (v/v) to elute p,p'-DDT and chlordane, but not dieldrin, was added and collected as fraction II. Sufficient 50% methylene chloride in n-hexane (v/v) to elute all dieldrin and endrin was added and collected as fraction III.

Quantitative determination:

Organochlorine pesticide and PCBs residues were determined at the Central Laboratory of Pesticides, Agriculture Research Center, Ministry of Agriculture, Cairo. Organochlorine pesticide and PCBs residues were determined by gas liquid chromatograph equipped with Ni^{63} electron capture detector (ECD).

Organochlorine pesticide and PCBs residues components was identified by comparing their retention times with those of the standards quantified by extrapolation of corresponding sample peak areas with those from standard curves prepared for each pesticide standard. Small variations are corrected by obtaining fresh chromatograms of the standard mixture after every nine injection. Standard solutions were prepared for each pesticide of concentrations ranging from 0.01 to 0.04 ppm and then 1 µl was injected into the GC. Peak areas of standard solutions were plotted against their concentrations.

Data management and analysis:

The data collected were computerized and statistically analyzed using SPSS version 11.0 computer program (Norusis, 1997). Quantitative data were expressed by means & standard deviations (X ± SD) and were compared using student t test. Correlations between data were evaluated using pearson linear correlation coefficient (r). The significance level was considered at P-value < 0.05.

RESULTS AND DISCUSSION

1. Mothers' age:

The relationship between organochlorine residues values and the mothers' age is plotted in Fig. (1), where a significant positive correlation was found between δ-BHC, p,p'-DDE p,p'-DDT and sum DDTs levels and mothers' age.

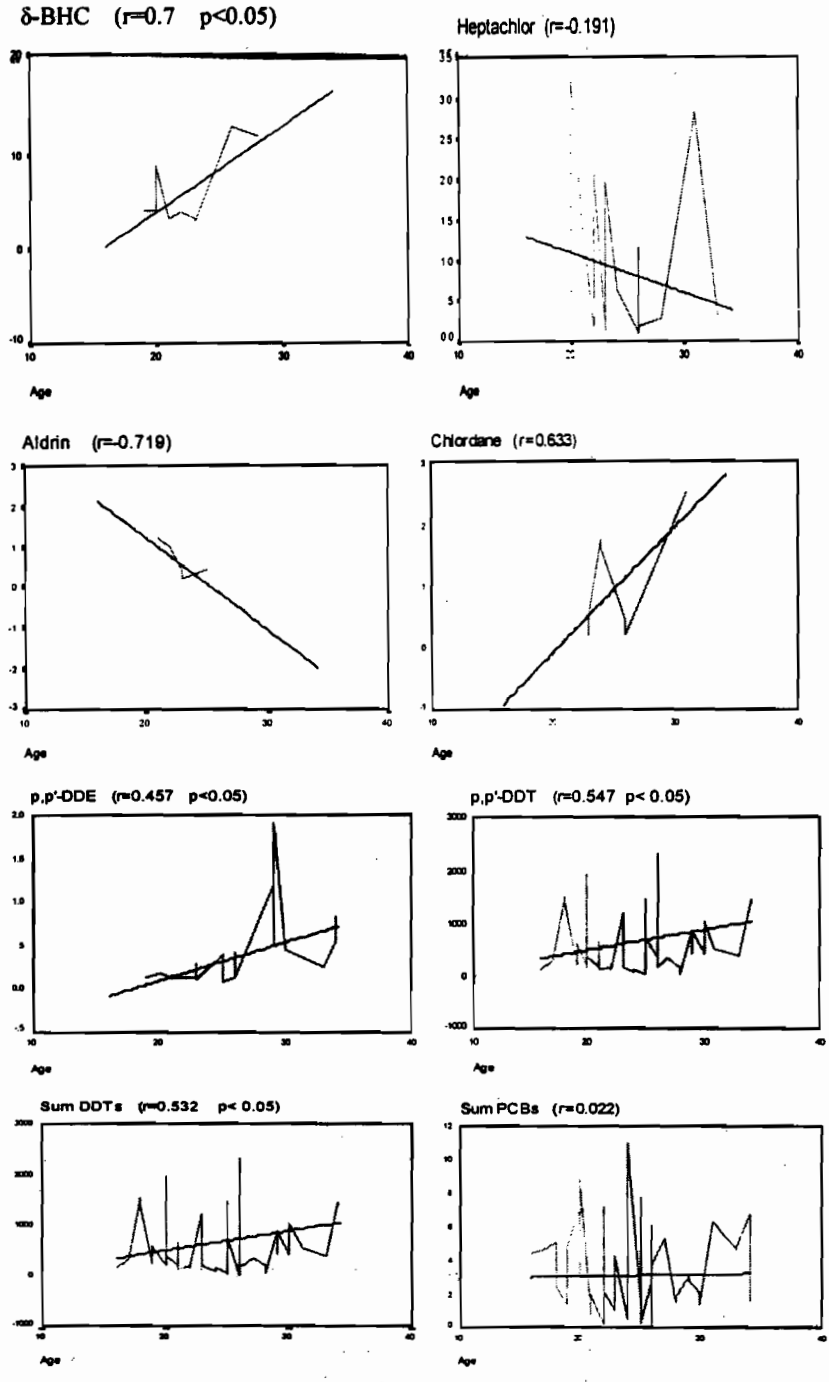

Fig. (1): Correlation between age of mothers (years) and organochlorine residues concentrations (ng/g milk fat) in their milk.

This is presumably because dietary food intake is a major source to increase the body burden of these contaminants (Albers *et al.*, 1996) and OC. contaminants are resistant to metabolism in the body and bioaccumulate with age (Sudaryanto *et al.*, 2006). This is in agreement with the study of Brunetto *et al.*, (1996), Romero *et al.*, (2000), Dagher *et al.*, (1999) and Sudaryanto *et al.*, (2006) and in contrast with the study of Okonkwo *et al.*, (1999), Mohammed *et al.*, (1999), Cok *et al.*, (2004), Kunisue *et al.*, (2004) and Tsydenova *et al.*, (2007) .

Also, it can be seen from Fig. (1) that, although no significant correlation was found between levels of chlordane & sum PCBs and mothers' age, chlordane and sum PCBs levels tended to increase with increased mothers' age. These results are in agreement with those obtained by Kostyniak *et al.*, (1999) and Sudaryanto *et al.*, (2006) and in contrast with the study of Tsydenova *et al.*, (2007) who found that, concentrations of PCBs and chlordane were significantly correlated with age. The possible reason for absence significant correlation observed between levels of some OC. and mothers' age may be ban production and use of organochlorine compounds.

2. Body mass index (BMI):

Results in Table (1) demonstrate that, no significant correlation was found between BMI [weight (kg)/(height (m))^2] of mothers and heptachlor, aldrin, chlordane, p,p'-DDE, p,p'-DDT, sum DDTs and sum PCBs levels, except δ-BHC and heptachlor epoxide which had significant negative correlation with BMI ($p < 0.01$) this may be due to the δ-BHC and heptachlor epoxide were detected only in nine and three analyzed samples. This agrees with the work of Dorea *et al.*, (1997) who found no significant correlation between DDT and body mass index. Also this is agree with the work of Takekuma *et al.*, (2004) who found no significant correlation between ∑PCBs and body mass index.

Table (1): Correlation between Body mass index (BMI) of mothers and organochlorine residues in their milk.

Organochlorine residues	BMI	
	r	P-value
δ-BHC	-0.801	0.009*
Heptachlor	0.039	0.873
Heptachlor epoxide	-1.000	0.007*
Aldrin	0.481	0.274
Chlordane	-0.099	0.799
p,p'-DDE	-0.195	0.374
p,p'-DDT	-0.204	0.131
Sum DDTs	-0.191	0.155
Sum PCBs	-0.099	0.451

*P < 0.01

3. The participation in agricultural work:

Table (2) shows the means and standard deviations of organochlorine residues according to participation in agricultural work. It could be notices from Table (2) that, the mean concentrations of p,p'-DDT and sum DDTs were significantly higher in mothers' milk who participated in agricultural work (732.677 and 709.988 ng/g milk fat, respectively) than in mothers' milk who

didn't participate in agricultural work (386.573 and 386.582 ng/g milk fat, respectively). This is probably because of their intensive usage in both agricultural activities and malaria control programs (Sudaryanto *et al.*, 2006), while the mean concentrations of heptachlor, aldrin and chlordane were significantly higher in mothers' milk who didn't participate in agriculture (1.456, 0.863 and 1.500 ng/g milk fat, respectively) than in mothers' milk who participated in agriculture (0.157, 0.336 and 0.368 ng/g milk fat, respectively).

Table (2): Means and standard deviations of organochlorine (O.C.) residues in mothers' milk according to mothers' participation in agricultural work.

O.C. (ng/g milk fat)	Mothers' participation in agricultural work		t-test	P-value
	Participate (no=28) $\bar{X}$ ± SD	Not participate (no=32) $\bar{X}$ ± SD		
δ-BHC	7.013 ± 4.19	3.100 ± 0.28	1.3	>0.05
Heptachlor	0.157 ± 0.07	1.456 ± 1.08	-3.5	< 0.01
Aldrin	0.336 ± 0.09	0.863 ± 0.43	-2.4	< 0.05
Chlordane	0.368 ± 0.15	1.500 ± 0.97	-2.6	< 0.05
p,p'-DDE	0.398 ± 0.44	0.110 ± 0.01	0.9	> 0.05
p,p'-DDT	732.677 ± 605.91	386.573 ± 367.43	2.5	< 0.05
Sum DDTs	709.988 ± 609.99	386.582 ± 367.44	2.3	< 0.05
Sum PCBs	2.734 ± 2.11	3.505 ± 2.85	-1.2	> 0.05

4. Duration and hours of mothers' participation in agricultural work:

Correlation between duration of participation in agricultural work in years & number of participation hours/day and organochlorine residues is presented in Table (3). With regard to duration of participation in agricultural work in years, it is clear from Table (3) that, there was a significant positive correlation between duration of participation in agricultural work in years and levels of p,p'-DDT and sum DDTs, but no significant correlation was observed between duration of participation and δ-BHC, heptachlor, heptachlor epoxide, aldrin, p,p'-DDE and sum PCBs. A significant negative correlation was found between duration of participation in agricultural work and chlordane.

Table (3): Correlation between duration & hours of mothers' participation in agricultural work and organochlorine residues in mothers' milk.

Organochlorine residues	Duration of participation in years		Number of participation hours/day	
	r	P-value	r	P-value
δ-BHC	0.365	0.334	0.419	0.261
Heptachlor	-0.424	0.070	-0.499	0.030*
Heptachlor epoxide	0.464	0.693	0.510	0.659
Aldrin	-0.700	0.080	-0.856	0.014*
Chlordane	-0.755	0.019*	-0.912	0.001**
p,p'-DDE	0.178	0.416	0.239	0.171
p,p'-DDT	0.381	0.004**	0.309	0.021*
Sum DDTs	0.324	0.014*	0.262	0.049*
Sum PCBs	-0.116	0.376	-0.166	0.206

*P < 0.05 **P < 0.01

With regard to number of participation hours/day, it is evident from Table (3) that a significant positive correlation was found between number of participation hours/day and levels of p,p'-DDT, and sum DDTs, while a significant negative correlation was found between number of participation hours and levels of heptachlor, aldrin and chlordane. Also, no significant correlation was found between the rest of organochlorine residues and number of participation hours.

5. Parity:

It is observed from Figures (2 and 3) that, no significant differences were found between primipara and multipara in the mean concentrations of organochlorine residues in both rural and urban samples.

Sudaryanto *et al.*, (2006) studied the relationship between parity and DDTs concentrations in primipara and multipara from Indonesia, and found that concentrations of DDTs tended to decrease in human milk with increased parity ($p < 0.05$) in Jakarta (urban) and Purwakarta (rural).

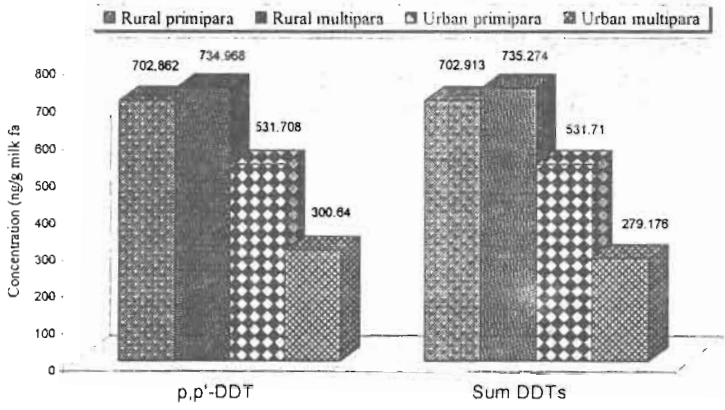

Fig. (2): The mean concentrations of p,p'-DDT and sum DDTs in rural & urban mothers milk according to parity.

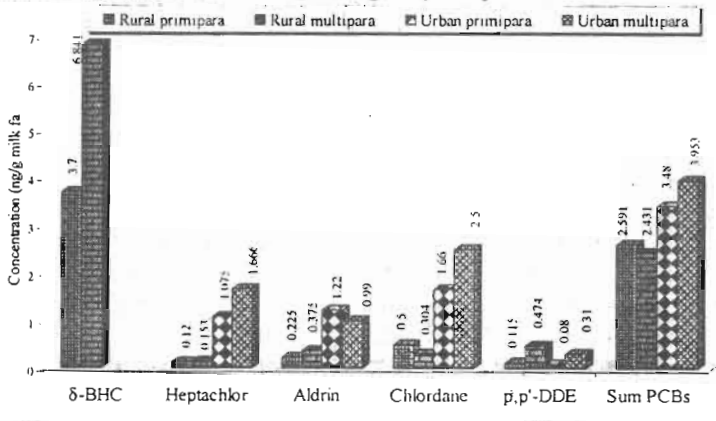

Fig. (3): The mean concentrations of δ-BHC, heptachlor, aldrin, chlordane and sum PCBs in rural & urban mothers' milk according to parity.

6. Number of breast feeding per day:

It is evident from Table (4) that, the mean concentrations p,p'-DDE, p,p'-DDT, sum DDTs and sum PCBs were significantly higher in mothers who fed their infants ≤ 10 times/day (0.509, 698.937, 699.087 and 3.707 ng/g fat, respectively) than those who fed their infants > 10 times/day (0.118, 301.105, 284.429 and 1.829 ng/g fat, respectively).The same Table also indicates that, there were no significant differences between the rest of organochlorine residues in lactating mothers who fed their infants ≤ 10 times day and those who fed their infants > 10 times.

These results are almost similar with those reported by Al-Saleh *et al.*, (1998), who found that the p,p'-DDE and p,p'-DDT concentration in lactating mothers who fed their infants less than 6 times a day was significantly higher than p,p'-DDE and p,p'-DDT in lactating mothers who fed their infants more than 6 times a day. This confirms the phenomenon that secretion of DDT; DDE and DDD in breast milk is a fast mechanism of eliminating these lipophilic compounds.

Table (4): Relationship between organochlorine residues (O.C.) and number of the breast feeding per day.

O.C. (ng/g milk fat)	Number of the breast feeding per day		t-test	P -value
	≤ 10 times no=31 $\bar{X}$ ± SD	> 10 times no=29 $\bar{X}$ ± SD		
δ-BHC	8.178 ± 4.52	3.600 ± 0.60	1.9	> 0.05
Heptachlor	1.026 ± 1.07	0.145 ± 0.05	1.6	> 0.05
Aldrin	0.585 ± 0.428	0.532 ± 0.40	0.2	> 0.05
Chlordane	1.023 ± 0.91	0.340 ± 0.23	1.0	> 0.05
p,p'-DDE	0.509 ± 0.48	0.118 ± 0.02	2.3	< 0.05
p,p'-DDT	698.937 ± 582.44	301.105 ± 267.74	2.6	< 0.05
Sum DDTs	699.087 ± 582.48	284.429 ± 269.28	2.8	< 0.01
Sum PCBs	3.707 ± 2.59	1.829 ± 1.67	2.9	< 0.01

7. Dietary habits:

The ingestion of contaminated food is considered to be the main source for human exposure to organochlorine compounds (Schinas *et al.*, 2000), so the relationship between sum DDTs and sum PCBs levels in mothers' milk and dietary habits of mothers participating in the study was investigated. It could be observed from Table (5) that, a significant positive correlation was found between sum DDTs levels and average number of consumption of meat, fish, eggs and dairy products per week (foods of animal origin). These results are in quite agreement with those of Dagher *et al.*, (1999), Torres-Arreola *et al.*, (1999) and Schinas *et al.*, (2000). On the other hand some studies have not identified any trends or significant correlation between consumption of animal products and organochlorine residue levels in milk (Kostyniak *et al.*, 1999 and Burke *et al.*, 2003).

The same Table shows that, a significant positive correlation was found between sum PCBs levels and average number of consumption of meat, fish and eggs per week. These results are in quite agreement with those of Kostyniak *et al.*, (1999) and Takekuma *et al.*, (2004).

It could be noticed from Table (5) that no significant correlation was found between sum PCBs levels and consumption of dairy products. Also, no significant correlation between sum DDTs and sum PCBs levels and consumption of vegetable and fruits or legumes and cereals (foods of plant origin). These results are in quite agreement with those of Stuetz *et al.*, (2001) and Burke *et al.*, (2003) who found that no relation between consumption of rice, fruit and vegetables and pesticide residue levels, while our results disagree with those obtained by Schinas *et al.*, (2000). In contrast to this study, Dagher *et al.*, (1999) found that no correlation between DDE content in breast milk and consumption of legumes and cereals, but a significant positive correlation was found between consumption of vegetables and fruits and DDE content.

Table (5): Correlation between sum DDTs and sum PCBs levels in mothers' milk and average number of food consumption per week.

Foods	Average number of food consumption/week	Sum DDTs		Sum PCBs	
		r	P-value	r	P-value
Meat	.1.53	0.341	0.009**	0.401	0.001**
Fish	0.93	0.376	0.004**	0.269	0.037*
Eggs	2.15	0.375	0.004**	0.359	0.005**
Dairy products	3.15	0.307	0.020*	0.043	0.745
Vegetables & fruits	15.5	-0.031	0.808	-0.027	0.839
Legumes & cereals	25.8	0.157	0.244	-0.099	0.450

*P < 0.05 **P < 0.01

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تأثير بعض العوامل على تركيزات المتبقيات الكلورينية العضوية فى لبن الأم بمحافظة الشرقية – جمهورية مصر العربية.

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تم دراسة تأثير بعض العوامل على تركيزات المتبقيات الكلورينية العضوية فى لبن الأم مثل عمر الأم و مؤشر كتلة الجسم و المشاركة فى الأعمال الزراعية و مدة المشاركة فى الأعمال الزراعية و عدد ساعات المشاركة فى اليوم و عدد مرات الولادة و عدد مرات الرضاعة فى اليوم والعادات الغذائية.

وقد اشتملت الدراسة على ٦٠ من الأمهات المرضعات تم اختيارهن عشوائيا من مركز رعاية الأمومة و الطفولة بقسم الحكماء فى مدينة الزقازيق (عينة حضر) و مستشفى السعديين بمركز منيا القمح (عينة ريف) بمحافظة الشرقية بجمهورية مصر العربية. وقد أبدت كل أم موافقتها على استكمال إستمارة إستبيان و أخذ عينة لبن لتقدير تركيزات متبقيات المبيدات الكلورينية العضوية(O.P.Cs) و الفينولات الثنائية المتعددة الكلوريدات (PCBs).

وأشارت النتائج إلى وجود ارتباط إيجابى معنوى بين معدلات sum DDTs و كلا من عمر الأم و مدة المشاركة فى الأعمال الزراعية و عدد ساعات المشاركة وعدد مرات استهلاك الأغذية من أصل حيواني مثل اللحوم و الأسماك والبيض و الألبان و منتجاتها فى الإسبوع، ووجد أيضا ارتباط إيجابى معنوى بين معدلات sum PCBs و عدد مرات استهلاك اللحوم و الأسماك والبيض فى الإسبوع. و كان متوسط تركيزات sum DDTs أعلى معنويا فى لبن الأمهات اللاتى شاركن فى الزراعة من الأمهات اللاتى لم يشاركن فى الزراعة بينما متوسط تركيزات heptachlor وaldrin و chlordane كانت أعلى معنويا فى لبن الأمهات اللاتى لم يشاركن فى الزراعة.وأيضا كان متوسط تركيزات p,p'-DDE و p,p'-DDT و sum DDTs وsumPCBs أعلى معنويا فى لبن الأمهات اللاتى أرضعن أطفالهن ≥ ١٠ مرات يوميا من الامهات اللاتى أرضعن أطفالهن أكثر من ١٠ مرات يوميا.