Evaluation of Organochlorine pesticide and Fates in the Women Milk in Al- Muthanna Province-Iraq

Eyhab R.M.AL-Samawy**

Wafaa Abdulmutalib Naji*

Department of biology, College of Science, AL-Muthanna University, Iraq *
Department of histology, College of Medicine, AL-Muthanna University, Iraq **

Keywords: Organochlorine pesticide, fates, rural, urban, Correlation.
Received (February) **, Accepted** (June).

ABSTRACT

The objective of this study was to investigate Organochlorine pesticide (OCPs) and Fates (F) levels in the breast milk of lactating healthy women who were living in Al Muthanna province of Iraq according to the region and age of mother and to determine the relationship between the selected parameters under the study. Seventy breast milk samples collected between January 2015 to March 2015 from healthy lactating women at different stages of lactation, from 5 days to 72 weeks postpartum in morning. The breast milk samples were collected by self-milking. (OCPs) in milk samples were measured by High Performance Liquid Chromatography (HPLC), while the fats were done by soxhlet extractor. Results of the current study (according to the minitab statistical program) revealed a very high significant variation between the levels of (OCPs) and (F) in the breast milk of mothers in the rural (R) and urban (U) regions ($P< 0.001$), and there are positive relationship between the both parameter in the samples of milk and the concentration of (OCPs) and (F) in women aged < 25 years lower than the concentration of (OCPs) and (F) in women aged ≥ 25 years. This high difference was statistically significant for (F) ($P < 0.01$), but non-significant for (OCPs).

تقييم المبيدات الكلورينية والدهون في حليب النساء في محافظة المثنى –العراق

وفاء عبد المطلب ناجي*
إيهاب رزاق محسن السماوي**

قسم علوم الحياة, كلية العلوم , جامعة المثنى, العراق*
قسم التشريح , كلية الطب , جامعة المثنى , العراق**
الكلمات المفتاحية: المبيدات العضوية الكلورينية, الدهون, الريف , المدينة, الأرتباط.

الخلاصة

الهدف من هذه الدراسة هوالكشف عن مستويات المبيدات الكلورينية و الدهون في عينات الحليب البشري من النساء المرضعات الاصحاء اللاتي يعشن في محافظة المثنى- العراق تبعاً للموقع وعمر الأم و تحديد العلاقة بين هذه المتغيرات.جُمعت سبعين عينة من الحليب من الامهات المرضعات في الفترة بين شهر كانون الثاني 2015 □تى شهر آذار 2015 وعلى مرا□ل مختلفة من الرضاعة، من 5 يوم الى 75 أسبوع بعد الولادة صبا□ا. وجمعت عينات الحليب يدوياً و قيست تراكيز المبيدات بواسطة الجهاز الضوئي العالي الأداء و تراكيز الدهون

بجهاز مستخلص السوكسليت. أظهرت نتائج الدراسة الحالية(تبعا الى برنامج منيتاب الأ□صائي) فرقاً معنوياً عالي جداً في تراكيز المبيدات الكلورينية والدهون في عينات الحليب للأمهات في المناطق الحضرية مقارنةً مع المناطق الريفية ($P\leq 0.001$) وأن العلاقة طردية ما بين المبيدات و الدهون في عينات الحليب وتركيز المبيدات والدهون في□ليب الأمهات ذوات الأعمار < 25 سنة وأقل منها في اللاتي تكون أعمارهن ≤ 25 سنة والفرق المعنوي عالي بالنسبة للدهون ($P\leq 0.01$) و غير معنوي للمبيدات الكلورينية.

Introduction

A pesticides are any chemicals used to control pests (any unwanted flora or fauna, e.g., weeds, insects, fungi, rodents) [4]. They are belong to the group of persistent organic pollutants and these pollutants can persist in the environment for long periods of time due to their resistance to bio-degradation [13]. They are transported through biotic and abiotic means to foods, are absorbed for the most part orally, are metabolized and distributed, accumulating in adipose tissue. In lactation processes, they are excreted in milk through the mobilization of fats [10] and [7].

The most vulnerable time of exposure to organochlorines appears to be during the embryonic and fetal period [18; 20]. Perinatal the exposure to these compounds my induce several adverse effects such as lower birth weight [17], neurodevelopmental delay [16].

Materials and Methods:-

Samples collection

Seventy milk samples which were obtained from healthy lactating mothers whose infants were inpatient in Al- Samawah feminine and children teaching hospital of Al-Muthanna province- Iraq in their reproductive age ranged between 14-40 years, with different period of lactation. The breast milk samples were obtained by manual suckling between 10 Am and 11 Am during the day. The mothers provided 5 ml of milk directly into sterilized tubes. All samples were frozen immediately after collection and kept at (−20 °C) until they were analyzed.

Extraction of fats [12]

1- The samples were dried by placed in water bath on 60 ^{o}C and then were crushed for small volumes by mill.
2- The samples were put in oven at (150 mm Hg) and temperature (70 C^{o}) for 30 min.
3- (5 gm) of sample was put into a cellulose extraction thimble of soxhlet apparatus.
4- (30 ml) of a mixture of Chloroform and methane 1:2 is used as a solvent.
5- Condenser was connected to Soxhlet and evaporated solvent was compensated from time to time.
6- The mixture (solvent and oil) was collected for 30 min.
7- The solvent was evaporated under vacuum (150 mm Hg) and in temperature (70 C^{o}) by using rotary evaporator.
8- The amount of oil was calculate from the extraction of neutral oil by dissolving (2 g) of Crude extracted oil in (20 ml) of Chloroform

9- The mixture was put in flask and the solvent was evaporated on a rotary evaporator (50 C°) and the remaining is neutral fats.
10- The fat was calculated by the following equation:-

$$\text{Fatty (mg/l)} = \frac{(tw1 - tw2) \times 1000}{V \text{ ml}}$$

Whereas:-
tw1 = the weight of the beaker with the mixture.
tw2 = the weight of the empty flask.

Extraction of (OCPs) [19]

1- (2 ml) methanol was added to (4 ml) of milk sample and then stirred for 5 min.
2- (50 g) sodium oxalate was added to mixture and then Continue stirring.
3- The mixture was centrifuged for 15 min and taking the organic layer. The process was repeated for three times to get the largest amount of the organic layer.
4- The volume of mixture was reduced to (1 ml) by putting in rotary evaporator.
5- (0.5 ml) of sulfuric acid was added and centrifuged for 10 min.
6- The process was repeated for twice to get the largest amount of the organic layer and then (1 ml) of hexane was added.
7- The mixture was dried by stream of nitrogen gas.
8- The dry matter was melt by (1ml) of hexane and then purified by silica.
9- (10 ml) of (hexane 15ml / methanol 40 ml / isopropanol 45 ml) were added.
10- The mixture was dried by stream of nitrogen gas
11- The dry matter was melt by (1ml) of hexane
12- The samples are now ready for HPLC analysis.

Conditions of the Liquid Chromatograph

The conditions of separation of Organochlorine pesticides by HPLC represented in the following table :-

The solvent type	Hexane 15 ml / 40 ml methanol / isopropanol 45 ml: Fluka
Injector	Injector Rheodye (7211)
Device control	Automatic system controller (S11-6A)
The amount of the model used by injection	Injection Loop (5 µL)
The type and number of pumps	Two shimadzu model (LC-6A Pumps)
Degree elementary column temperature(T1)	(250) C for two minutes
The final column degree heat (T2)	(300) C for two minutes

The rate of temperature rise	(42) c / min
Injector temperature	(186) c
Detector Temperature	(203) C
Nitrogen gas flow rate	1.0 mL/min
The dimensions of the column	-E C18 (5 µm, 0.25 × 30 mm)
Direct injection of the column	On column injection
Liquid phase	Liquid phase gradient elution: Solvent A buffer phosphate 0.01m PH6.0, Solvent B acetonitrile
Solid phase	Solid phase C .18 shimpack (L OD)
Sensitivity of the device	(213 × 103) Attenuation
Speed paper Date	(min/1 cm)
Type Detector	Uv- visible detector SPd-6AV Equipped with fliw cell 8m 206 nm

The Results

1. Determination of (OCPs) and (F) in human milk according to region.

Analysis of breast milk samples which were obtained from 70 lactating mothers, showed that the total mean ± SD of (OCPs) concentration was 5.8793 ± 2.116 µg/l in which (OCPs) concentration in the milk samples of rural region was 4.056± 0.908167 µg/l, minimum value 1.06 µg/l and maximum value 5.38 µg/l. (OCPs) concentration in the milk samples of urban region was 7.963143± 0.649542 µg/l, minimum value 7.17 µg/l and maximum value 9.51 µg/l.

The total mean ± SD of (F) concentration was 2.9172± 0.581609 µg/l in which (F) concentration in the milk samples of rural region was 2.499± 0.449329 µg/l, minimum value 1.02 µg/l and maximum value 3.16 but (F) concentration in the milk samples of urban region was 3.395143± 0.252474 µg/l, minimum value 3.09 µg/l and maximum value 3.98 µg/l. These result showed in the current table.

Table 1: Showed the levels of (OCPs) and (F) in the samples:

Group / parameter	T (U+R)	U	R
(OCPs)	5.879333 ± 2.116447	7.963143 ± 0.649542	4.056 ± 0.908167
Min. P.	1.06	7.17	1.06
Max. P.	9.51	9.51	5.38

F	2.9172 ± 0.581609	3.395143 ± 0.252474	2.499 ± 0.449329
Min. F.	1.02	3.09	1.02
Max. F.	3.98	3.98	3.16

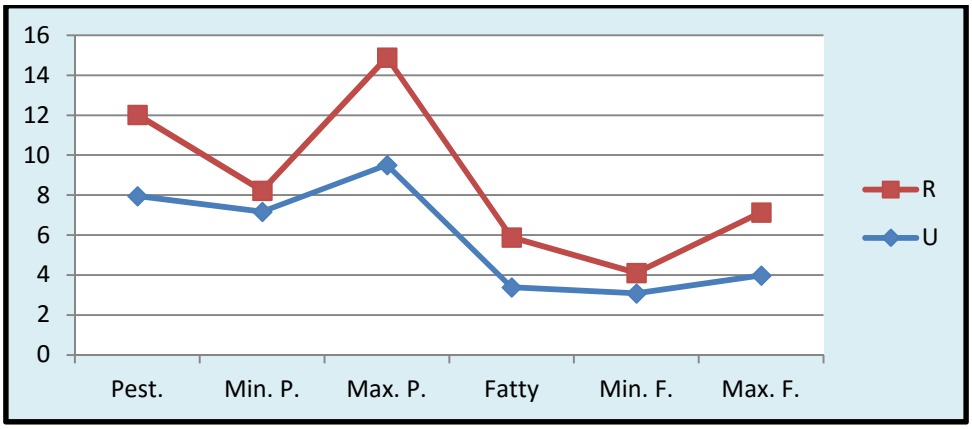

Fig.1: The variations between rural and urban in the levels of (OCPS) and (F) in the mother's milk.

2. Relationships between (OCPs) and (F) concentrations.

The current study revealed a significant positive correlation between (OCPs) and (F) at 0.953015 as the total of the values , 0.999834853 in rural samples and 0.993486383 in urban.

Table 2: the values of the correlations between (OCPs) and (F):

F / P	U	R	T(U+R)
U	0.999835		
R		0.993486	
T(U+R)			0.953015

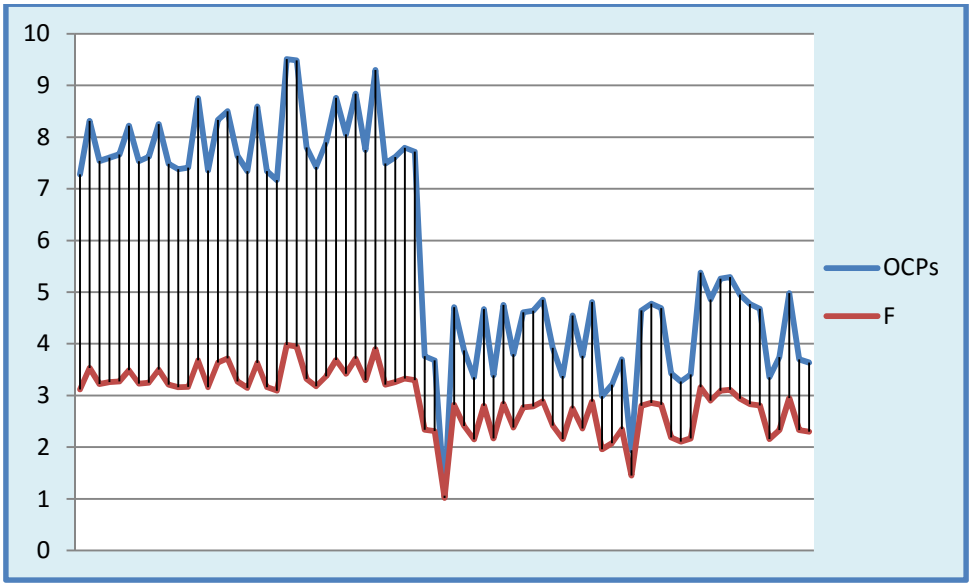

Fig 2: The correlations between (OCPs) and (F) concentrations.

3. Determination of (OCPs) and (F) concentrations in human milk according to mother's age.

The mean of (OCPs) and (F) concentrations in women aged < 25 y were 5.715263 µg/l, 2.743947µg/l respectively, while the mean of (OCPS) and (F) concentrations in women aged ≥ 25 y were 6.047838 µg/l, 3.095135 µg/l respectively.

In the present study, the mean concentration of (OCPs) and (F) in women aged < 25 y was lower than that in women aged ≥ 25 y as presented in fig. 3. This difference was statistically significant for (F) ($P < 0.01$), but non-significant for (OCPs).

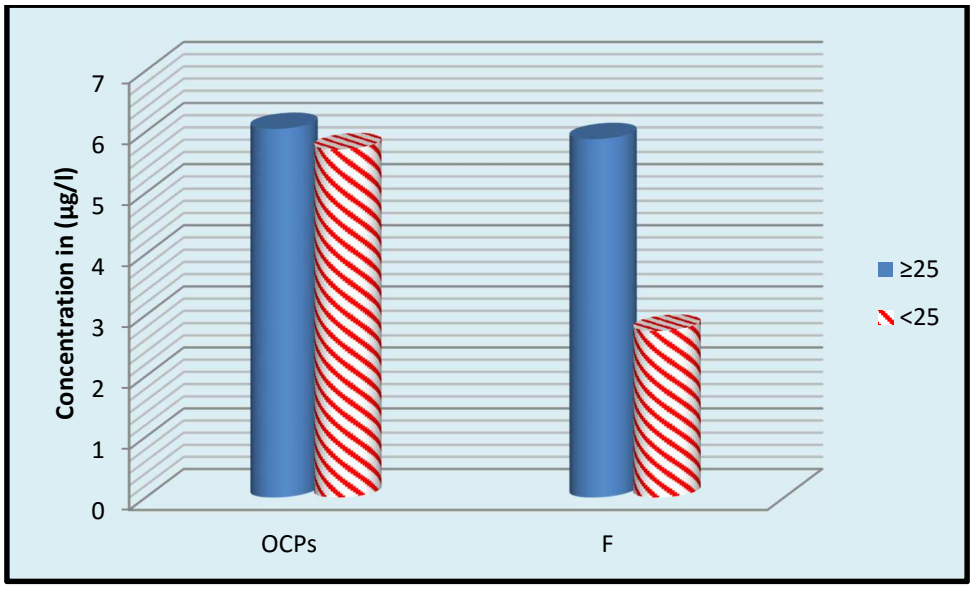

Fig. 3: The variations in the levels of (OCPS) and (F) in the mother's milk according to the age.

Discussion

The use of pesticides became very relevant in an attempt to control and eradicate crop pest and also to produce quality and bumper harvest to feed the ever growing population. Over the years, human population has been on the increase thus the territory of these pests became larger and larger hence the need to look for stronger and more effective alternatives to meet food security and to survive from disease vector organisms [8]. It is estimated that as much as 45% of the world's crop is destroyed by plant pest and disease [3].

They have no idea of the half-lives of these chemicals nor the dangers they pose when misused. The environment is contaminated with pesticides because of their massive use in both the agriculture and public health sectors. [11].

Breast milk monitoring programs have been performed in several countries for investigating geographical and temporal trends in human exposure to persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) .[1]

Researchers are interested in whether body levels organochlorine pesticides affect the characteristics of breast tumors, or breast cancer survival rates. Other researchers are looking at differences in genes that control the ability to detoxify chemicals in the body. Some of these differences in genes, called polymorphisms, may affect the ability of a person to make or break down estrogen, or handle chemicals from the environment.[2].

The breast, a lipid-rich tissue acts as a depot or reservoir of lipophilic pesticide by virtue of physiochemical interactions of the cellular component with the pesticide [11].

Organochlorine pesticides and its breakdown products accumulate and are stored over time in a woman's body fat, and can appear in human breast milk[2], these accumulation over time in the body of humans and may cause serious illness [14] ;therefore, the concentration of (OCPs) was high in the ages of women ≥ 25.

In the present study the mean concentration of Organochlorine pesticides and fats in human milk of lactating women in urban regions was higher than that in rural regions, this rise in urban region may be due to the higher pollution source included higher in traffic emission (vehicle exhaust particles, tire wear particles, weathered street surface particles, brake lining wear particles), industrial emission (power plants, coal combustion, metallurgical industry, auto repair shop, chemical plant, etc.), domestic emission, waste disposal, a weathering of building and pavement surface [21 ; 5].

The previous studies supported the view that women living in urban areas with heavy road traffic and industrial activity had pollutions higher than women living in rural areas [6; 9; and 15].

Considering the high levels of Organochlorine pesticides in breast milk in this study, It is important to apply direct strategies and solutions of protection against contaminants in order to reduce their levels in biological fluids , and to decrease a opportunity for estimation of total chemical intake by infants during breast-feeding.

References

[1] Anuschka, P.; Tatjana N. S.; Georg B. & Janneche, U. S. (2004). Temporal Changes of PCBs, PCDD/PCDFs and Chlorinated Pesticides in Human Milk from Murmansk, Russia, and Tromsø, Norway. **Organohalogen Compounds**. 66:2761-2765.

[2] BCERF. (2001).Cornell University Program on Breast Cancer and Environmental Risk Factors in New York State. **Pesticides and Breast Cancer Risk, An Evaluation of DDT and DDE**. Revised, March, 2001.

[3] Bhanti, M. & Taneja, A. (2007). Contamination of vegetables of difference seasons with organophosphorous pesticides and related health risk assessment in Northern India. **Chemosphere**. 69(1):63-68.

[4] Cohn, B.A.; Wolff, M.S.; Cirillo, P.& Sholtz, R.I.(2007). DDT and breast cancer in young women: new data on the significance of age at exposure. **Environ Health Perspect**. 115:1406–1414.

[5] Faiz, Y.; Tufail, M.; Javed,M.T.;Chaudhry, M.M.& Naila-Siddique. (2009). Road dust pollution of Cd, Cu, Ni, Pb and Zn along Islamabad expressway, Pakistan, **Micrchemical J**. 92(2): 186-192.

[6] Frkovic, A.; Kras, M.& Alebic-Juretic,A. (1997).Lead and cadmium content in human milk from the Northern Adriatic area of Croatia. Bull. Environ. Contam.**Toxical**. 58(1): 16-21.

[7] Hernandez, A. B.; Ron, A.; Melba, A.; Amstadter, H. S.; Resnick, K. S.; Wendy, M. & Dean, G. K. (2010). Prevalence and Correlates of Emotional, Physical, Sexual, and Financial Abuse and Potential Neglect in the United States: The National Elder Mistreatment Study. **Am J Public Health**. 100(2): 292–297.

[8] Hodgson, A. (2003). The high cost of pesticide poisoning in northern Ghana. **Pestic. News**. 62(3): 4-8.

[9] Leotsinidis, M.; Alexopoulos,A. &Kostopoulou-Farri, E.(2005). Toxic and essential trace elements in human milk from Greek lactating women: association with dietary habits and other factors. **Chemosphere**. 61:238-247.

[10] Mishra, J. P. ; Vaithiyanathan, S. ; Mishra, S. ; Prasad, R. ; Misra, A. K., (2002). Effect of transinoculation of subabul (Leucaena leucocephala) leaves fed goat rumen liquor into sheep rumen on haematobiochemical parameters in sheep. **Indian J. Small Rumin**., 8 (1): 19-22

[11] Mussalo-Rauhamaa, H. (1991). Partitioning and levels of neutral organochlorine compounds in human serum, blood cells and adipose and liver tissue. **Sci. Tut. Environ**. 103: 159-175.

[12] Nata a Fidler, Thorsten Sauerwald, Anja Pohl, Hans Demmelmair, and Berthold Koletzko.(2000).Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. **Journal of Lipid Research** 41.1376-1383.

[13] Oehme, FW. & Smith, EA. (1991). Acrylamide and polyacrylamide: a review of production, use, environmental fate and neurotoxicity. **Rev Environ Health** 9:215–228.

[14] Ogundiran, M.B.; Ogundele,D.T.; Afolayan, P.G.&Osibanjo,O.(2012). Heavy Metals Levels in Forage Grasses Leachate and Lactating Cows Reared around Lead Stage Dumpsites in Nigeria. **Int. J. Environ**.Re 6(3): 695-702.

[15] Ozcetin, M.; Yilmaz, R.; Mendil, D.;Kocyigit, R.& Gedik, S.(2013). Presence of Toxic Heavy Metals in Human breast Milk. **J. Clin Anal Med**.4(2):89-92.

[16] Ribas-Fito, N.; Ramon, R.; Ballester, F.; Grimalt, J., Marco, A. & Olea, N. (2006). Child health and the environment: the INMA Spanish Study. **Paediatr Perinat Epidemiol** 20:403–410.

[17] Siddiqui, MK.; Srivastava, S.; Srivastava, SP.; Mehrotra, PK.; Mathur, N. & Tandon, I. (2003). Persistent chlorinated pesticides and intra-uterine foetal growth retardation: a possible association. **Int Arch Occup Environ Health** 76:75–80.

[18] Toft, G.; Axmon, A.; Giwercman, A.; Thulstrup, AM.; Rignell-Hydbom, A. & Pedersen, HS. (2005). Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross-sectional study. **Environ Health** 4:26.

[19] Weisenberg, E., I. Arad, F. Graver and Z. Sahm, (1985). Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch. **Environ. Contam. Toxicol**., 14: 517-521.

[20] Wojtyniak, BJ.; Rabczenko, D.; Jonsson, BA.; Zvezday, V.; Pedersen, HS. & Rylander, L. (2010). Association of maternal serum concentrations of 2,2', 4,4'5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p´-DDE) levels with birth weight, gestational age and preterm births in Inuit and European populations. **Environ Health** 9:56.

[21] Zhou,J.; Ma,D.; Pan,J.; Nie,w. & Wu,K. (2008) . Application of multivariate statistical approach to identify heavy metal sources in sediment and waters: acase study in Yangzhong China, **Environ Geo** 54:373-380.