Organochlorine Pesticide Levels in Lactating Women of Karachi, Pakistan

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Abstract

Forty breast milk samples were drawn from lactating mothers for the analysis of organochlorine compounds. Among the 40 lactating women 15 mothers (6 secundripara and 9 multipara) were selected with the aim to compare the level of organochlorine compounds in colostrum and mature milk. For this purpose mother's milk samples were collected in two different phases 1-7 days postpartum milk (colostrum) and 13-35 days postpartum milk (mature). We observed compounds of hexachlorocyclohexane group, they tend to decrease from colostrum (0.186 mg L^{-1} to mature (0.154 mg L^{-1}) milk but diphenylaliphatic group shows variation among these compounds. In the present study women were further classified according to the number of child they had born. The study comprised of 15 primipara, 15 multipara and 10 secundripara. During the study main focus was on primipara and multipara. Women of multipara status were also found to have higher (0.508 mg L^{-1}) level of organochlorine residues than primipara (0.429 mg L^{-1}). The rate of deposition of pesticides in forty milk samples were found in the following order with respect to different age groups as 17-24 > 25-32 < 33- 40.The chi square analysis at $p < 0.05$ shown the organochlorine levels were higher in the 33-40 year old group.

Keywords: Organochlorine compounds, breast milk, colostrum, mature, primipara, multipara, age factor

Introduction

Organochlorine pesticide (OCP) residues are universal environmental contaminants. They have the ability to degrade over a long time and to deposit in human and animal fatty tissues (1).Human breast milk is considered the best nutrition for newborn or infants. Because of its unique composition it provides all essential nutrients which are responsible for immunological and physiological development. Human milk is gradually contaminated by environmental toxic chemicals like organochlorine compounds that have been banned more than two decades ago, but these hazardous chemicals are still found in lactating women. Mother's milk may be contaminated by food chain and environmental pollution. These toxic compounds penetrate in the body all the way by skin, inhalation and oral route; also they are engrossed and disseminated to different human body organs (1 Organochlorines may transmit from mother to their newborn babies by breast milk. After birth, the infants are moreover revealed to comparatively greater persistent organic pollutants. The pre and post-natal deposition of these toxic chemicals causes adverse effect on human health. Previous studies show the effect of motor and mental development on infants (2-4). However, mother's feed is an important source of all the essential nutrients, immunological components and growth of infants (5, 6).

The colostrum is the initial milk secreted after labor. This release emission steadily converts in to mature milk and the transition complete after 14 days. Human colostrum has comparatively high protein, less fat and a lactose solution than mature milk. It also consists of immunoglobulin and other important immune factors (7, 8).

Since OCPs accumulate in the human body by bonding with the lipid content of milk. Composition of mother's milk and quality of lipid also differs due to mother's body mass index, daily food intake, habit of smoking and weight loss during lactation (9-11). Body burden of OC in expecting mothers' babies are transferred transplacentally and after birth by breast feeding, numerous risk aspects have been studied which affects on OCPs concentration eg; parity (greater level observed in primipara), mother's age, sample collection timing and nutritional habits (12, 13).

The aim of the study was to measure organochlorine residues in those women who are indirectly exposed to these organic pesticides. We intended to give valuable facts and figures on contamination level found in women of Karachi, Pakistan which was not well observed. Further more, interest could be established to compare the difference between colostrum and mature milk.

Such report of the pesticide concentration in human milk samples is in fact stands among the earlier research works could ever be done in country like Pakistan.

MATERIALS AND METHODS

The study was undertaken after endorsement from participants institutional review boards and in agreement with an assurance filed.

Subjects

The study subjects were lactating women (n=40) from the Karachi city. The enrolment/selection of mothers was managed on an intentional basis. This study focused on women who gave birth during September 24th to December 4th 2011. All women accomplish recruitment criteria for this project like age higher than 17 years, no serious illness during this pregnancy and reside in city for seven or more years. Forty milk samples were further divided according to parity and nature of milk.

Sample collection

The pregnant women who came to register their names for delivery in Karachi Gynae hospitals were considered. After approval by hospitals administration and physicians, we approached participants and took their interviews by a trained interviewer. After a brief history taken from these women they were followed for their expected days of delivery. They had been asked about their consent for the milk sample collection. They had been provided a questionnaire for this purpose. The breast pump had been provided to the women and lady nurse collected their samples in sterilized vials of 10ml.

The women were selected according to the number of children they delivered. The primipara (n=15) were those who had only one baby. Those women who had more than two children were considered as multipara (n=15). The breast milk samples were collected from pimiparas and multiparas without any distinguish of type of milk due to small sample size.

We intended to observe the accumulation rate of pesticide residues in different intervals. For this purpose the mother's milk was collected during two different intervals 1-7 days postpartum milk (colostrum), and 13-35 days postpartum milk (mature milk). The colostrums and mature milk were collected from 6 secundripara and 9 multipara.

The samples were collected from both types of mothers delivered by caesarean and normal delivery. The collected milk samples were instantaneously labeled with identification code and stored at -20 °C.

Chemicals

A standard mixture of organochlorine compounds i.e Mix -71 was purchased from Accu Stanadard, USA and Dr. Ehresnstorfer's laboratory, Germany which was contains 21 **(**Aldrin, Endrin, Heptachlor, Heptachlor exo-epoxide, Heptachlor endo-epoxide, Alpha HCH, Beta HCH, Gamma HCH, Methoxychlor, 4,4 DDD, 4,4 DDE, 4,4 DDT, 2,4-DDD, 2,4-DDE, 2,4-DDT, Endosulfan, Alpha Endosulfan, Beta Endosulfan, Dieldrin, HCB**)** organochlorine compounds. Pesticide-scan grade solvents and reagents were used. Methanol, sodium oxalate, diethyl ether, and hexane (Merck) were used for sample extraction. Silica Sep-Pak (Merck) was used for sample clean-up. Silica gel (70-230 mesh Merck) was heated at 130°C for 24 hours and activated with 10% (w/v) deionized water for prior to use as a cleaning agent.

Glassware

All glassware were cleaned with detergent, washed with water, dipped in acid overnight and washed with distilled water again and kept in oven at 200°C for 4 h. All the glassware was passed through hexane before use.

Extraction

4mL **of** milk samples was shake well in a glass jar than added half of same volume of methanol. After thoroughly mixed together in a shaker for 5 min. 0.1 g sodium oxalate was then added to the mixture and again allowed to shake well by vortex mixer. The process of extraction was performed by using approximately 10 mLof diethyl ether and n-hexane in a proportion of 1:1. This extract was centrifuged at 3000 rpm for 15 min. The supernatant containing organic phase was collected separately and treated with diethyl ether and hexane again as above. Likewise in the same manner three organic phases were collected and applied to a vacuum concentrator to concentrate up to the volume of 1 mL. To 1mL then 0.5 mL of concentrated sulphuric acid was added and the mixture was centrifuged for 10 min at 3000 rpm. After centrifugation the acidic residues were extracted two times with 1 mL of n-hexane. Later on the collected organic phases were dried through a flow of nitrogen gas. These dried residues were dissolved in 1mL hexane for cleaned up.

Clean up

Through silica the organic phases were purified along with 2 mL of n-hexane. This extract was eluted with 10 mL hexane along with an equal volume of hexane: methanol: isopropanol (45:40:15:v v v).These elutes were then dried through nitrogen stream after this the dried residue was finally dissolved in 1 mL hexane and analyzed on GC-ECD (Manufacturer?).

Gas Liquid Chromatography (GLC)

Organochlorines were analyzed by Gas Chromatograph Perkin Elmer Clarus-500 with the ^{63}Ni selective electron-capture detector. Nitrogen was used as carrier gas at 30 mL min^{-1} was used as the carrier and makeup gases. Analysis is performed on cross bond DB-35 5 % biphenyl, 65 % dimethyl polysilicone capillary column having 30 meter length, 0.35 mm ID and 0.50 Pm stream of nitrogen gas. For best recovery a known concentration of standard mixture was spiked for a set of samples before

extraction. Each pesticides peak was identified on basis of specific retention time in comparison with those of the standards. The quantitative concentration of pesticide isomers in the samples were calculated by peak area of isomers, final volume and weight of the sample in significant of the peak area of known concentration of the respective isomers in standard mixture. Peak identification was performed by the GC software (Total Chrome) calibration table set up with absolute retention time. A 2.0 μL of the final extract (1 mL) was injected at a temperature of 250 ˚C. Initial oven temperature was kept at 100 °C which was increased with the rate of 4 °C/ min up to 300 °C. Holding time at 100 °C was 5 min and at 300 °C was 10 min respectively. Injector temperature was 250 °C and detector temperature was 270 °C. The total run length was 50 minutes.

Standardization of Methods for Analysis

Quantification was carried out by comparison with standard mixture as internal standards, as well as with an external standard. A spiked milk sample of known concentration (1.00 μg L^{-1}) was analyzed according to this procedure; the results were within the confidence intervals. Recovery was determined by spiking the samples as mentioned above. The recoveries of spiked OCs standards ranged from 90 % to 99 % (average value 89 %); the detection limits ranged from 0.5 ng L^{-1} to 10.0 ng L^{-1} respectively. The average extraction recovery was 92.8 (± 4.0) % for the OCs fractions. The % recovery, %RSD, LOD and LOR calculated during the analysis and values are given in Table 1.

Analytical Quality Control / Quality Assurance

The quality of data was assured through analysis of the appropriate Certified ReferenceMaterials. Deuterated surrogates and internal standards were used to compensate for loss during sample extraction and work-up. The surrogate standard was used to monitor method performance for each sample, while the internal standard was used to compensate for variation in the final extract volume, injection volume, and instrument sensitivity. Analysis of blank samples was carried out with each batch. All compounds were identified by retention times.

Statistical Methods

The analyses were obtained with the help of SPSS statistical software after transformation of the raw data to obtain symmetrical distributions. Results are calculated on basis of mean values with standard deviation. Comparisons were made with the Kruskal-Wallis test or the Mann-Whitney test when comparing two groups. Correlations were calculated with Pearson correlation.

Results

Forty samples were collected and analyzed for pesticide residues. Present result reported on the basis of whole milk. Each sample was accompanied by a case history sheet identifying the mother with relevant particulars such as age, profession, number of deliveries etc. Each sample which when subjected to GC-ECD has unique pattern of pesticide concentrations. The mean, median, range and SD of agricultural pesticide residues are given in Table II. The mean value for the total amount of ΣOCPs in milk samples was calculated as 0.351 mg L^{-1}.

The mean levels of α β γ isomers of HCH were calculated as 0.004, 0.090, 0.015 mg L^{-1} respectively. On the other hand their calculated ranges are from 0 - 0.048, 0 - 0.900, 0 - 0.110 mg L^{-1} respectively. The total content of HCH (sum of α, β and γ HCH) in milk samples was calculated as 0.109 mg L^{-1} (Table 2).

4.4 DDT is detected at mean levels of 0.015 mg L^{-1} and in the range of 0-0.425 mg L^{-1} . One of the major metabolites of DDT is 4,4 DDE which was found in the range from 0 - 0.160 mg L^{-1} and its mean was calculated as 0.016 mg L^{-1} . The mean level of 4,4 DDD was detected as 0.090 ppm at the range of 0 – 1.806 mg L^{-1} . The mean of total DDT (sum of 4,4 DDT; 4,4 DDE; and 4,4 DDD) was detected at mean level of 0.121 mg L^{-1} . The mean concentration of Methoxychlor was calculated as 0.121 of the total samples of milk (Table 2).

Table 3 shows the result in colostrum and mature milk. This table indicates frequencies that express samples with levels above the determination limit, mean, standard deviation, standard error and range (min-max). Among organochlorine compounds the sum of all HCH isomers was found in greater content in colostrum milk (1.086 mg L^{-1}) while in mature milk it was calculated as 0.143 mg L^{-1} . The sum of DDT in colostrum revealed 0.203 mg L^{-1} and decreased in mature milk as 0.143 mg L^{-1} . In the case of Methoxychlor the highest mean concentrations was found to 0.261 mg L^{-1} in colostrum milk. On the other hand mean values of Σ OCPs were significantly higher in colostrum (0.650 mg L^{-1}) than mature milk (0.440 mg L^{-1}).

Table 4 shows the concentration of OCPs in all milk samples with respect to multipara and primipara women. Considerable differences were observed in primipara versus multipara samples. Among organochlorine compounds HCH isomers (α, β, γ) revealed ∑0.151 mg L^{-1} in primipara ∑0.189 mg L^{-1} in multipara donors. On the other hand DDT is found to have decreasing (% > LOD) trends from primipara to multipara samples.

The concentration of pesticide residues according to the mother's age are represented in Table 5. Among HCH compound β-HCH shows highest accumulation rate in all age groups. The highest mean concentration of total HCH is calculated as 0.179 mg L^{-1} in women between age group of 33-40. Highest mean value i.e. 0.116 of DDT metabolites was observed in women whose age

group lies between 33-40 years. In short these results of pesticide accumulation in milk samples indicate highest concentrations in the age group of women between 33-40 years. According to pesticide deposition with reference to various age groups it can be written as in following order 17 – 24 < 25 – 32 < 33 – 40 years. In short we can say that in milk samples of women of 33-40 years of age heavy pesticide accumulation was observed as compared to samples of younger women. Table 6 shows the t-test, coefficient correlation, correlation determination of paired samples group.

Discussion

The chief aim of this research was to determine the environmental health hazards due to pesticides contamination in breast milk. In Pakistan the usage and production of most of these OCPs, particulary DDT is forbidden like other countries due to serious health hazards but illegal use of these pesticides may be expected (14).

Despite the ban on many organochlorine chemicals, HCH is still used in many developing countries because of its very low cost. In the present investigation of human milk samples HCH and its isomer α, β and γ were detected in noticeable quantities. According to Jense (15) β- HCH is more stable than its isomers. In the present finding above 50% of samples of milk contained β HCH in the highest concentration this is in line with the present investigation. The elimination rate of β HCH is slower than that of other isomer and it thus might be predominantly detected in the breast milk samples. HCH isomers were also reported to be predominant in Mexican mother's milk (16). This trend was strengthened by the present investigation. Breast milk samples when tested in the women of two Russian areas i.e., Murmansk and Monchegorsk indicated β HCH was commonly detected compound. Its mean value was reported to be higher than α and γ HCH (17). Similar findings were observed in milk samples collected from primiparae living in Dalian and Shenyang, (China) by Kunsiue (18). Likewise in the present report β HCH was remained on the top of the list among the HCH isomers. A previous report by Kumar indicated total level of HCH from four villages of India i.e. Bakhoti (0.123 ppm), Chiraigaon (0.129 ppm), Ghodhakhas (0.131 ppm) and Minahas (0.127 ppm) (19). Surprisingly this was the different pattern of HCH accumulation in milk which is higher than the current status of ΣHCH (0.109 mg L^{-1}).The value reported in above research finding was higher than present estimation. It may be due to different extent of the exposure and the ability of the donors to metabolize these compounds.

The *p'p* DDE concentration (2620 ng g^{-1}) was reported to be higher then *p'p* DDT (127 ng g^{-1}) in milk samples of mothers living in Hong Kong (20). A further report on the DDE which was conducted in Veracruz shows p'p DDE in lactating women found in greatest persistence. Researchers measured the mean value of *p'p* DDE (4.00 ppm), *p'p* DDD (0.01 ppm) and *p'p* DDT (0.65 ppm) in Mexican mother (16). Similar trends were observed in the present findings of milk samples. In current study 4-4DDE was found at high frequency i.e., 39 % then 4-4 DDT, 4-4 DDD In contrast to this report 4-4 DDT and 4-4 DDE were decreased while 4-4 DDD concentration was increased in milk samples of current study.

The heavy accumulation of organochlorines in milk samples of Karachi women can be a result of past usage of these chemicals since the mean life of these compounds is several years however their possible route of entry into female bodies may be their diet. As far as the mature milk samples are concerned, the OC pesticides were detected in high concentration. Upon comparison with the other researchers it shows the dramatic differences in OCs levels in breast milk when collected at different times. According to a report significant reduction in the levels of OCs in breast milk samples was found during lactation and these levels later on decline between the first and fifth weeks after delivery (12). Various organochlorine chemicals like HCB, β-HCH, *p-p'*-DDE and *p,p'*-DDT were detected in different body compartment of 60 lactating women including colostrum drawn within fifth day and mature which was collected on the 30th day postpartum (16). Likewise in the present investigation we observed decreasing trend in the level of β-HCH and the sum of DDT in mature milk as compared to colostrum. Due to relatively high content of fat in breast milk these lipophillic compounds reside in the milk and eventually may be transferred from the mother to the child via breast milk. Rogan reported that the body burden of organochlorine compounds including DDE can be reduced by lactation (21). The earlier work support the present findings as we found the higher mean concentration of OCPs in colostrum but lower in women having mature milk (Table 3). A general downward trend of OCPs concentration was observed in breast milk of Canadian women during lactation, with sporadic increases, was also reported by Mes and colleagues (22). It is observed that the burden of chlorinated contaminants in human milk is greatest at the onset of breast feeding soon after the birth of the child (23).The OCP levels in the breast milk of Norwegian women reported a significant decline during lactation (24). In present study mean concentration of pesticides were lower in mature than the colostrum. In previous study the most predominant compound was *p'p* DDE with a considerable decline of 26 % from colostrum to mature milk. An average of 36% decrease was reported in the values of HCB, β HCH, *p'p* DDE and the sum of DDT in mature milk as compared to colostrum (16). In the current research a declining trend was also observed in β HCH and sum of DDT from colostrum to mature milk (Table 3). By the present finding it clear shows that human milk is an elimination route for xenobiotics. In the present research report concentration of 4,4-DDE is higher in mature than colostrum. It is our general observation that DDE and many other harmful chemicals in Govt. hospital are still in practice for ward fumigation due to its economical reason. It may accumulate in human body by inhalation particularly in those women who are admitted during their delivery period.

We found significant correlation (Table 6) in the levels of organochlorine compounds between colostrum and mature milk accordance with previous report (16). In contrast with previous report observed significant correlation between colostrum and mature milk only for HCB (25). Zhiwei found very close correlation between organochlorine chemicals in colostrum and mature milk (26).

In lactating women, several factors such as number of previous children, age of mother, food intake preferences and breast feeding parameters can affect concentrations of OCPs in human milk (27). Despite this, concentrations of OCPs decrease with increase in the number of previous children nursed (28). Present findings of ∑OCPs in human breast milk tend to increase with increasing the number of children (Table 4). On the contrary concentrations of OCP in human milk were found to decrease with increase in the number of children (18). This difference in findings may be due to different geographical background and due to different culture and dietary habit. In the current study observed significant relation ($p < 0.05$) between multipara and primipara (Table 6) but in the previous statistic data shows, there was no association ($p > 0.05$) between the concentration of OCPs and number of children, which might be due to enduring exposures of these compounds.

In the present research work categorically the women (donor of the milk) was considered young in the age between 18-24 years, middle age (25-32 and aged one between age 33-40 years. Although the women between 33-40 years of age are not older in fact they are of middle age but traditionally in Pakistan especially in the rural areas women get married and become mother before 20 years.

Most studies show age is an indicator of exposure level because the passage of time allows the continued accumulation of pollutants. Researchers reported association between *p'p* – DDE levels and age (29, 30). In present research we also observed such circumstance. Among elderly women of Karachi, concentration of these pesticides was found to be more as compared to young ones (Table 5). Other report showed the levels of these compounds tended to increase as the age of women increasing in both groups of multiparae and primiparae (31). It means age factor may influence the accumulation of toxic chemicals in human body.

The present data shows the relationship between level of OCPs in breast milk and age of mothers. We performed the Chi square analysis ($p < 0.05$) between the mother's age and OCP concentration. This indicates that OCP concentration in lactating women milk tends to increase with age, also observed this pattern in previous investigation (18, 32).

Last but not the least it can be concluded that it is essential to observe the unlawful and misuses of the organoclorine pesticides in public and health sectors. Moreover, in the view of environmental toxicology increased pesticide levels in mother milk has greater risk to maternal and infant health which deserve strict monitoring in terms of their usage in all sectors.

References

1. Sim M, McNeil J. Monitoring chemical exposure using breast milk: a methodological review. Ameri J of Epidem 1992: 136: 1-11.
2. Longnecker MP, Wolff MS, Gladen BC, Brock JW, Granjean P, Jacobson JL, Korrick SA, Rogan W.J, Weisglas-Kuperus N, Hertz-Picciotto I, Ayotte E, Boersma ER, Altshul L.M, Heinzow B, Pagano JJ, Jensen A.A. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. Environ Health Perpect 2003; 111: 65-70.
3. Ribas-Fito N, Cardo E, Sala M, de Muga, ME, Mazon C, Verdu A, Kogevinas M, Grimalt JO, Sunyer J. Breastfeeding exposure to organochlorine compounds and neurodevelopment in infants. Pediatrics 2003; 111: 580-585.
4. Toft G, Hagmar L, Giwercman A, Bonde, JP. Review Epidemiological evidence on reproductive effects of persistent organochlorines in humans. Reprod Toxicol 2004; 19: 5-26.
5. Newton ER.. Breast milk – the gold standard. Clin Obstet Gynecol 2004; 47; 632-642.
6. American Academy of Pediatrics (AAP). Breastfeeding and the use of human milk. Pediatrics 2005; 115: 496-506.
7. Playford RJ, McDonald CE, ,Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. Am J Clin Nutr 2000; 72: 5-14.
8. Issac C.E. Human milk inactivates pathogens individually, additively and synergistically. J Nutr 2005; 135: 1286-1288.
9. Villalpando SM, del Prado M. Interrelation among dietary energy and fat intakes, maternal body fatness, and milk total lipid in humans. J Mammary Gland Biol 1999; 4: 285-295.
10. Agostoni, C., Marangoni, f., Lammardo, AM., Giovannini, M., Riva, E., Galli, C.. Breastfeeding duratuion, milk fat composition and developmental indices at 1 year of life among breastfed infants. Prostag. Leukotr. Ess. 2001. 64: 105-109.
11. Bopp, M., Lovelady, C., Hunter, C., Kinsella, T. Maternal diet and exercise: effects on long-chain polyunsaturated fatty acid concentrations in breast milk. J. Am. Diet. Assoc. (2005). 105: 1098-1103.
12. Furst, P., Kruger, Ch., Meemken, HA., Groebel, W. PCDD and PCDF levels in human milk-dependence on the period of lactation. *Chemosphere* 1989 18: 439-444.

13. Uehara, R., Peng, G., Nakamura, Y., Mastuura, N., Kondo, N., Tada, H. Human milk survey for dioxins in the general population in Japan. *Chemosphere* 2006: 62: 1135-1141.
14. Smith, D., (1998). Worldwide trends in DDT levels in human breast milk. *Int. J. Epidem*. 28: 179-188.
15. Jensen, A.A. Chemical contamination in human milk. *Residue Review* 1983: 89, 1-128.
16. Waliszewski, SM., Aguirre, AA., Infanzon, RM., Silva, CS. and Siliceo, J. Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum and milk from inhabitants of Veracruz, Mexico. *Arch. Environ. Contam. Toxicol*. 2001: 40 (3): 432-438.
17. Polder, A., Becher, G., Savinova, TN., Skaare, J.U. Dioxins, PCBs and some chlorinated pesticides in human milk from the Kola Peninsula, Russia. *Chemosphere* 1998: 37 (9-12): 1795- 1806.
18. Kunisue, T., Someya, M., Kayama, F., Jin, Y., Tanabe, S.. Persistent organochlotines in human breast milk collected from primiparae in Dalian and Shenyang, China. *Environ. Pollut*. 2004: 131: 381-392.
19. Kumar, A., Dayal, P., Shukla, G., Singh, G., Joseph, P.E.. DDT and HCH residue load in mother's breast milk: A survey of Lactating mother's from remote villages in Agra region. *Environ. Int*. 2006: 32: 248-251.
20. Poon, BH.T., Leung, C K.M., Wong, CK.C., Wong, MH.. Polychlorinated biphenyls and organochlorine pesticides in human adipose tissue and breast milk collected in Hong Kong. *Arch. Environ. Cotam. Toxicol*. 2005: 49: 274-282.
21. Rogan, W.J., Bagniewska, A. and Damstra T. (1980). Pollutants in breast milk. *New Eng. J. Med*. 302: 1450-1453.
22. Mes, J., Doyle, J.A., Adams, B.R., Davies, D.J. and Turton, D. (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. *Arch. Environ. Contam. Toxicol*. 13: 217-223.
23. Yakushiji, T. (1988). Contamination, clearance and transfer of PCB from human milk. Rev. *Environ. Contam. Toxicol.* 101, 139-162.
24. Skaare, J.U. and Polder, A. (1990). Polychlorinated biphenyls and organochlorine pesticides in milk of Norwegian women during lactation. *Arch*. *Environ. Contam. Toxicol.* 19 (5): 640-645.
25. Ribas-Fito, N., Grimalt, J.O., Marco, E., Sala, M., Mazon, C. and Sunyer, J., (2005). Breast feeding and concentrations of HCB and *p,p'*-DDE at the age of 1 year. *Environ. Res*. 98: 8-13.
26. Zhiwei, Y., Lubica, P., Beta, D., Jan, P., Antón, K., Tomas, T and Irva, H,P. (2007). Comparision of organochlorine compound concentrations in colostrums and mature milk. *Chemosphere.* 1012-1018.
27. Harris, C.A.., Woolridge, M.W., Hay, A.W. (2001). Factors affecting the transfer of organochlorine pesticide residues to breast milk. Chemosphere 43: 243-256.
28. Minh, N.H., Someya, M., Minh, T.B., Kunsisue, T., Iwata, H., Watanabe, M., Tanabe, S., Viet, P.H. and Tuyen, B.C. (2004). Pertiment organochlorine residues in human breast milk from Hanoi and Hochiminh city, Vietnam: Contamination, accumulation kinetics and risk assessment for infants. *Environ. Pollut.* 129: 431-441.
29. Glynn, AW., Granath, F., Aune, M., Atuma, S., Darmerud, PO., Bjerselius, R., et al. Organochlorines in Swedish women determinants in serum concentrations. *Environ. Health Perspect*. 2003: 111:349-355.
30. Zumbado, M., Goethals, M., Alvarez-leon, EE., Luzardo, OP., Cabrera, F., Serra-Majem, L. and Dominguez- Boada, L. Inadvertent exposure to organochlorine pesticides DDT and derivatives in people from the Canary Islands (Spain). *Sci. Total Environ* 2005: 339: 49-62.
31. Todaka, T., Hirakawa, H., Kajivara, J., Hori, T., Tubishi, K., Onozuka, DConcentration of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin like PCBs in blood collected from 195 pregnanat women in Sapporo, city, Japan. *Chemosphere* 2007: 69: 1228-1237.
32. Ntow, WJ., Tagoe, L.M., Drechsel,P., Kelderman, P., Gijzen, H.J. and Nyarko, E. Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. *Environ. Res*. 2008: 106: 17-26

Alpha HCH	Heptachlor
Beta HCH	Aldrin
Gamma HCH	Hepta-exo-epoxide
Delta HCH	Alpha Endosulfan
4,4-DDE	Beta Endosulfan
Dieldrin	Endosulfan Sulphate
Endrin	Methoxychlor
4,4-DDD	4,4-DDT

Name of Standard Pesticides

Table 1 *Recovery of pesticides*

Organochlorine	**Recovery (%)**	**R.S.D (%)**	**LOD ppm**	**LOR**
Alpha-HCH	95.4	4.6	0.002	0.005
Beta-HCH	92.4	5.0	0.002	0.006
Gamma-HCH	98.5	4.1	0.003	0.01
4,4-DDE	97.8	5.2	0.002	0.005
4,4-DDT	92.4	6.0	0.003	0.01
Methoxychlor	95.4	4.7	0.007	0.02
4,4-DDD	95.4	4.7	0.002	0.006

LOD= Limit of detection, R.S.D= Relative standard deviation, LOR= Limit of

Table 2. *Statistical analysis of pesticide residues in 40 whole milk samples.*

Compound	**Positive test (%)**	**Mean**	**SD**	**SE**	**Median (CI 95%)**
α-HCH	40	0.004	0.011	0.001	0 (0-0.048)
β-HCH	52	0.090	0.220	0.034	0.0005 (0-0.900)
γ-HCH	27	0.015	0.037	0.004	0 (0-0.110)
		$\sum$0.109			
4,4-DDT	27	0.015	0.067	0.010	0 (0-0.425)
4,4 DDE	42	0.016	0.038	0.006	0 (0-0.160)
4-4,DDD	32	0.090	0.326	0.051	0 (0-1.806)
		$\sum$0.121			
Methoxychlor	32	0.121	0.347	0.054	0 (0-1.766)

SD= Standard deviation, SE= Standard Error, CI = Confidence limit at 95 %

Table 3. Concentration (mg L^{-1}) of organochlorine compounds in colostrum and mature milk.

Compound	Colostrum (15)				
	Mean	**SD**	**SE**	**Range**	**Median (CI 95%)**
α-HCH	0.007	0.014	0.003	nd-0.048	0(0.0007 – 0.014)
β-HCH	0.161	0.304	0.078	nd-0.948	0.001(0.0007 - 0.329)
γ-HCH	0.018	0.033	0.008	nd-0.110	0(0.0002 – 0.036)
	∑0.186				
4,4-DDT	0.032	0.109	0.028	nd-0.425	0(0.028 - 0.092)
4,4 DDE	0.012	0.041	0.010	nd-0.160	0(0.010 - 0.034)
4-4,DDD	0.159	0.464	0.120	nd-1.806	0(0.097 - 0.415)
	∑0.203				
Methoxychlor	0.261	0.537	0.139	nd-1.766	0(0.036 - 0.558)
	Mature (15)				
	Mean	**SD**	**SE**	**Range**	**Median (CI 95%)**
α-HCH	0.005	0.010	0.002	nd-0.036	0 (0.0005 - 0.010)
β-HCH	0.117	0.229	0.059	nd-0.805	0.001 (0.0098 - 0.243)
γ-HCH	0.021	0.035	0.009	nd-0.109	0 (0.0016 - 0.040)
	∑0.143				
4,4-DDT	0.005	0.010	0.002	nd-0.030	0 (0.0005 – 0.0105)
4,4 DDE	0.051	0.140	0.036	nd-0.549	0 (0.0265-0.0128)
4-4,DDD	0.087	0.321	0.082	nd-1.246	0 (0.090-0.264)
	∑0.143				
Methoxychlor	0.154	0.361	0.093	nd-1.287	0 (0.045-0.353)

SD= Standard deviation, SE= Standard Error, CI = Confidence limit at 95 %

Table 4. Concentration (mg L^{-1}) of organochlorine compounds in primipara and multipara milk

Compounds	Primipara (15)				
	Mean	**SD**	**SE**	**Range**	**Median (CI 95%)**
A-HCH	0.007	0.012	0.003	nd - 0.036	0.0003-0.013
B-HCH	0.115	0.265	0.070	nd - 0.805	0.0317-0.261
γ-HCH	0.029	0.038	0.010	nd - 0.110	0.007-0.050
	∑0.151				
4,4-DDT	0.003	0.008	0.002	nd- 0.030	0.001-0.007
4,4 DDE	0.022	0.047	0.012	nd - 0.160	0.004-0.048
4-4,DDD	0.218	0.565	0.151	nd - 1.806	0.094-0.530
	∑0.261				
Methoxychlor	0.017	0.051	0.013	Nd - 0.188	0.012-0.045
	Multipara (15)				
	Mean	**SD**	**SE**	**Range**	**Median (CI 95%)**
A-HCH	0.006	0.012	0.003	nd - 0.048	0.000-0.012
B-HCH	0.170	0.279	0.072	nd - 0.948	0.015-0.324
γ-HCH	0.013	0.028	0.007	nd - 0.109	0.002-0.028
	∑0.189				
4,4-DDT	0.035	0.109	0.028	nd - 0.425	0.025-0.095
4,4 DDE	0.043	0.140	0.036	nd - 0.549	0.034-0.120
4-4,DDD	0.040	0.086	0.022	nd - 0.280	0.007-0.087
	∑0.118				
Methoxychlor	0.279	0.545	0.141	nd – 1.766	0.022-0.580

SD= Standard deviation, SE= Standard Error, CI = Confidence limit at 95 %

Table 5. Mean values and (standard deviation) of pesticide residues (mg L^{-1}) in 40 whole milk samples with respect to various age groups of donors.

Compounds	Age groups		
	18 – 24 year	**25 – 32 year**	**33-40 year**
α-HCH	0.0005 (0.001)	0.0012 (0.004)	0.0083 (0.014)
β-HCH	0.018 (0.044)	0.070 (0.141)	0.125 (0.283)
γ-HCH	0.011 (0.023)	0.013 (0.032)	0.046 (0.151)
	Σ 0.034	Σ 0.084	Σ 0.179
4,4-DDT	0.003 (0.008)	0.006 (0.014)	0.023 (0.092)
4,4 DDE	0.002 (0.005)	0.014 (0.046)	0.020 (0.039)
4-4,DDD	0.070 (0.121)	0.015 (0.046)	0.093 (0.405)
	Σ 0.075	Σ 0.035	Σ 0.116
Methoxychlor	0.159 (0.272	0.012 (0.024)	0.084 (0.270)

Table 6. Pearson correlation between colostrum and mature milk, primipara and multipara.

Compounds	Colostrum vs.Mature milk			Primipara vs .Multipara		
	r	**r^2**	**t-test**	**r**	**r^2**	**t-test**
α – HCH	0.568	0.323	2.488	- 0.276	0.076	1.035
β – HCH	0.704	0.496	3.574	0.815	0.664	5.021
γ – HCH	0.514	0.227	2.160	0.661	0.437	3.176
4,4-DDT	- 0.127	0.016	0.461	- 0.122	0.015	0.443
4,4-DDE	- 0.113	0.113	0.410	- 0.135	0.018	0.491
4,4-DDD	0.026	0.0006	0.094	0.907	0.822	7.765
Methoxychlor	0.623	0.388	2.872	0.493	0.243	2.040

r = Coefficient Correlation, r^2= Correlation determination of paired sample group

Table 7. Fat content in breast milk (gram)

	Colostrum	Mature	Primipara	Multipara
Mean	3.11	12.0	6.8	8.7
Variance	0.04	1.16	22.11	20.60
SD	0.20	1.07	4.70	4.53
CV	0.06	0.09	0.71	0.53
Range	0.7	3.4	11	10.4
Median	3.1	12.5	3.1	11
Skewness	1.00	0.10	0.46	0.34

SD= Standard Deviation, CV= Coefficient of variation

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