

**ORGANOCHLORINE PESTICIDES AND PCBS IN HUMAN BREAST MILK****O. BAMGBOSE<sup>+</sup> and B.O. OPEOLU**

Department of Environmental Management &amp; Toxicology, University of Agriculture, Abeokuta, Nigeria.

(Received: June, 2007; Accepted: December, 2007)

**Abstract**

One hundred and Fifty (150) samples of human breast milk (colostrums) collected from donors patronizing a postnatal center in Nigeria were analyzed for the levels of lindane, total DDT and total PCBs residues. Donors were stratified with respect to factors that may affect accumulation of these compounds such as age, previous lactation, parity, occupation, income and education status by use of questionnaire.

Analysis was performed by preliminary lipid extraction followed by partitioning on a column and finally by gas liquid chromatography equipped with an electron capture detector.

Lindane, total DDT and PCBs were detected in all samples with a concentration range of 2-16 ng/ml for lindane, 28-750 ng/ml for DDT and 16-1431 ng/ml for PCBs. The mean values were 14.12 ng/ml, 186 ng/ml and 468.62 ng/ml, respectively.

Correlation between levels of pesticides concentrated and the following parameters of age, parity and occupation were not significant. Comparison of the mean results from this study with the levels obtained from other studies in the more industrialized regions of the world showed that the residue levels in human milk in this location in Nigeria is higher than the world's average, while it is several factors less than the average obtained from some African countries.

**1. Introduction**

Organochlorine pesticides (OCPs) with their high persistence in the environment accumulate in fatty foods and human adipose tissues. Contamination of human milk by organochlorine and other related compounds has been extensively reported world-wide (Jensen, 1983, GEMS/FOOD, 1998). Breast milk containing high concentrations of organochlorine compounds is a source of contamination for the breast-fed baby. The child's exposure to these contaminants starts as early as in the uterus due to the long half-life of these compounds.

The intake of such compounds via food (expressed on body weight basis) is much greater by breast-fed infants than by adults, due to the intake of milk fat containing the contaminants. Human milk, at the top of the food chain, represents the major route of elimination of OCPs by lactating women (Rogan *et al.*, 1986; Sim and McNeil, 1992). The excretion of these compounds during lactation is much higher than their intake these with the diet (Jensen and Slorach, 1991), which may imply that the tissue deposits of the organochlorine compounds in women decrease with consecutive lactations.

In the course of lactation, persistent organochlorine compounds dissolved in milk fat are removed from a woman's body, thereby leading to breast-fed children being subjected to higher levels of exposure to certain chemicals than the adults. Exposure to high levels of

these contaminants in the uterus has been related to more frequent infections, suggesting immunological impairment and manifesting as reduced neurological optimality at birth, developmental delays in gross motor function during infancy, slow cognitive processing and attention difficulties, impaired intellectual function in school-age children, as well as lower behaviour and activity rating (Guo *et al.*, 1995, Jacobson and Jacobson, 1996).

It has therefore become necessary in a country like Nigeria where organochlorine compounds are still in use for agricultural crops, unlike in developed countries where a ban has been imposed, to monitor on continuous basis the concentrations of organochlorine pesticide residues and metabolites in human milk.

This study focuses on the analysis of organochlorine pesticides and PCBs in breast milk of selected women in Abeokuta city as a means of assessing the exposure of babies to these compounds from breast milk and with a view to comparing the pesticide levels obtained with those from other parts of the world.

**2. Materials and Method****Sampling Area:**

The sampling area specifically used for this study is the Abeokuta South Local Government area. The location of collection of the breast milk sample is the

postnatal center at Sacred Heart Specialist Hospital, Lantoro, Abeokuta, Ogun State.

#### *Sample Container:*

Polythene sample container of about 10 mls volume which was used for the collection had been previously sterilized.

#### *Sample Collection:*

Breast milk samples were collected by manual expression. The sample collection time/duration lasted from January 2004 to December 2006. One hundred and fifty (150) milk samples were collected from individual mothers who had given their consent after due explanation of the purpose of the study. Samples of breast milk (between 5-10 ml) were deposited directly into the polythene tubes by self-manual expression between the hour of 9.00 am in the morning and 1.00 pm in the afternoon. Samples were cooled in ice, then transferred to the Toxicology laboratory of the Department of Environmental Management and Toxicology in Abeokuta on the same day and stored at -18 °C before being analysed.

#### *Exposure Assessment Questionnaire:*

Modified from the standard WHO questionnaire for pesticide residues in human milk, the questionnaire determined the age, health status and breast-feeding pattern of the infant, food consumption pattern, parity, medication use, residency and occupation of mother.

#### *Extraction Procedure:*

Samples were analysed using a modified method described by Hernandez *et al.* (1993). Aldrin and mirex were used as internal standards and added to 4 ml of a thoroughly mixed aliquot of the milk sample. Following solid-liquid-phase extraction with florisol and iso-hexane and elution with iso-hexane/dichloromethane (80:20, v/v), the eluent was evaporated with a vacuum-rotary evaporator to dryness after which the residues are reconstituted in a final volume of 2 ml of iso-hexane and transferred to auto sampler vial.

#### *Sample Clean-Up:*

Sample extracts of 80 mg fat were dissolved in 200 µl (ethyl acetate/cyclohexane (1:1)). These were then injected into the Gel Permeation Chromatography (GPC) for clean up. For sample extracts containing more than 80 mg fat, the extracts were diluted with solvent to give 80 mg fat before they were injected for clean up. The column was eluted with ethyl acetate/cyclohexane (1:1) at a flow rate of 1-1.5 ml/min. The first 9 ml of the effluent after injections were discarded and the next 20 ml were collected and concentrated. The column was washed with 20 ml solvent mixture after each injection. The concentrates were then dissolved in 3 ml hexane for GC-ECD detection.

#### *Analyses:*

Analyses were performed by high-resolution gas chromatography on a Varian 3500 gas-

chromatograph equipped with an auto sampler, a HP-5.30 m x 0.25 mm capillary column and electron capture detector. Column packing 2% OV (methyl silicone) and 4% QF (mixed in ratio 3:5) ultra pure nitrogen at a flow rate 2.0 ml/min with variable attenuation and pulse, column temperature 185 °C and injector port 270 °C. Pesticide standards were usually injected each day before any run and at the end of the day to check column behaviour. Identification of the component of the sample extracts was based on the comparison of the retention times ( $R_t$ ) of the peaks obtained from their chromatograms with those obtained from known components of standards under identical condition (Hernandez *et al.*, 1993).

#### *Recovery Studies:*

Samples were fortified with 10 ng/ml lindane standard mix at three different instances during the study. The fortified samples were extracted and cleaned up using the method described above. The average recovery value was 81.6%.

### 3. Discussion

#### *Background Data of Donors:*

The donors were aged between 17 and 41 years; with 2% under 18 years, 38% between 18-25 years, 46% between 26-32 years and the remaining 14% were between 33-41 years. The total of 84% obtained for the age range 18-25 and 26-32 was close to the work of Czaja *et al.* (1997), Ecobichon *et al.* (1989) and Hong *et al.* (1994) all of whom reported values of between 70-76% in their studies for similar age range as in this study. 94% of the donors reside in Abeokuta and its environment while 6% of the donors live outside Abeokuta city.

Also, from the information obtained, 48 (32%) out of the 150 donors were primiparae and 102 (68%) were multiparae with the following percentages recorded for number of births: (2nd child 24%, 3rd child 14%, 4th child 20%, 5th child 0.04%, 6th child 0.02%, 7th child 0.04%). 90% of the donors had all their infants surviving compared to 8% who had earlier lost a child and 2% who had lost more than one child. Of great importance is the level of education of the participating donors: Out of the 150 donors, 26% had no formal education, 44% had primary education, 22% had secondary education and only 8% had tertiary education. Educational status could have accounted for 54% of the donors being unskilled workers, with 34% as semiskilled and the remaining 12% skilled workers.

Of the 150 donors, 46% had monthly income of less than N5000, 46% had income between N5000-N10,000 and only 8% had income above N10,000. Also, 44% out of the total donors did not take any medicine during the pregnancy period, 54% took normal drugs

(e.g. vitamins and blood enhancing pills) and only 2% took malaria drug.

*Levels of Lindane, Total DDT and PCBs in Human Milk:*

The pesticide residues, total DDT (i.e. DDE and DDT), lindane and PCBs were found in all the one hundred and fifty (150) milk samples analysed. Residue levels (mean and standard deviation) of the two organochlorines and PCBs detected are shown in Table 1. The level of DDT ranged between 28-750 ng/ml with a mean of 186 ng/ml and median of 147 ng/ml. For PCBs a range of 16-1431 ng/ml, median of 186 ng/ml and mean of 468.62 ng/ml were observed. While for lindane, the concentration ranged from 2-33 ng/ml with a median of 13.5 ng/ml and a mean of 14.12 ng/ml.

Comparison of the mean results obtained with the levels from other studies showed that total DDT levels in human milk from Abeokuta women is much higher than those than from the developed countries but slightly lower than the African average (Table 2).

Fish is one of the major sources of protein in the diet of people in Abeokuta as a result of its availability in terms of cost. However fish skin and muscles have

been shown to concentrate total DDT to level as high as 29 ppm (Longnecker *et al.*, 1997).

The levels of organochlorine detected in the breast milk samples of women in Abeokuta may be due this high due to the high consumption of food and food product such as fish and vegetables that may have been treated with pesticides (Osibanjo and Bamgbose, 1990).

It was also observed that the women with the first child had lindane concentration ranging from 3-33 ng/ml while multiparous mothers had lindane concentration ranging from 2-22 ng/ml, which corroborate the work of Noren and Lunden (1991) in Sweden, Hernandez *et al.* (1993) in Spain and Newsome *et al.* (1995) in Canada, which reported a general decline in residue levels of human milk with parity.

Lowest and highest concentration of PCBs and lindane (2 ng/ml lindane, 16 ng/ml PCB; 33 ng/ml lindane and 1431 ng/ml PCB) were found in the same mothers. Mothers that had PCB concentration above 1000 ng/ml were mainly multiparous and about 50% of the mothers had concentrations of PCBs of 700 ng/ml and above, showing that the concentration of PCBs increase with parity in contrast to that of

**Table 1:** Concentration of detected pesticides and PCBs in analyzed samples (ng / ml) and their ratios.

	Lindane	Total DDT	PCBs
N	150	150	150
Median	13.5	147	186
Mode	13	21	35
Mean	14.12	186	468.62
Range (ng/ml)	2-33	28-750	16-1431
Std deviation	7.43	160.3	431.3
PCB / Lindane		33.19	
PCB / DDT		2.52	
DDT / Lindane		13.17	

**Table 2:** Comparison of pesticides and PCBs levels in human milk from other countries.

Countries	References	Lindane	Total DDT	PCB
Sweden	Noren & Lunden (1991)	0.69	34.40	132.5
Spain	Hernandez <i>et al.</i> (1993)	1.48	26.32	184.6
Canada	Newsome <i>et al.</i> (1995)	0.39	22.1	207.3
Norway	Johansen <i>et al.</i> (1994)	3.44	18.14	286.9
Ghana	Ntow (2001)	17.15	201	518.4
This study		14.12	186	468.2

**Table 3:** Summary of multiple regression analyses

Variable	Lindane	Total DDT	PCB
Age of mother	0.134	0.611	0.178
Parity	0.116	0.241	0.109
Occupation	-0.42	-0.38	-0.27
Level of Income	0.101	0.132	0.223
Lindane	-	0.09	0.114
Total DDT	0.09	-	0.434
PCB	0.114	0.434	-

organochlorines which decrease with parity (Ecobichon *et al.*, 1989 and Hong *et al.*, 1994).

Mothers with low-income of between N1,000-N5,000 per month recorded lindane and PCBs concentration levels of 2-25 ng/ml 16-1431 ng/ml, respectively. For highest earners, the levels range from 4-22 ng/ml for lindane and 35-984 ng/ml for PCBs. There is no significant positive correlation between residue levels and income as indicated by the correlation coefficients of 0.101, 0.132 and 0.223 for lindane, total DDT and PCBs, respectively.

From Table 1, the ratio of the level of concentration of PCBs to lindane (33.19) is very high. This can be attributed to the prevalent use of PCBs (electrical appliances, transformer gaskets) in the environment as compared to lindane. The low level of lindane recorded may be as a result of the restriction of indane to developing countries (Czaja *et al.*, 1997). The ratio of PCBs to DDT (2.52) indicates that the use of DDT in the Nigerian environment is as prevalent as the use of PCBs (Osibanjo and Bamgbose, 1990). No relationship could be established between residue level and occupation of donors. For example the donor with the lowest DDT and PCBs concentration is a farmer with six children, while the donor with the highest PCBs concentration is a livestock trader and the donor with the highest DDT concentration is a semi skilled tradesman working as a beautician.

The correlation coefficient between residue levels and age were 0.611, 0.134 and 0.178 for DDT, lindane and PCBs, respectively, indicating that the two variables (concentration and age) are independent of each other (Table 3). This agrees with the reported work of Dewailly *et al.* (1994) and Mes *et al.* (1993) that age, more than the number of deliveries, seems to affect the concentration of organochlorine compounds in breast milk. This phenomenon may imply that deposits of these compounds replenish rapidly in some women's tissue after lactation. This study also indicates a similar situation in which many of the donor's concentration of DDT and PCBs in the second lactation milk were higher when longer intervals existed between deliveries (Hong *et al.*, 1991). Also, correlation between concentrations as shown in Table 3 did not indicate any significant correlation between the various residues detected. In addition, individual variations in the concentration of lindane, total DDT and PCBs in human milk were not unexpected since they depend on many factors. Some of these factors include exposure age, weight of the mothers, dietary habits and parity of the mothers (Table 2). Of great importance is the fat content of the milk, which depends on the time of sampling (during the meal, the day, the period and seasonal variation), which may also influence the result obtained (Kanja *et al.*, 1992).

#### 4. Conclusion

Determination of residues of organochlorine pesticides and Polychlorinated biphenyls in breast milk samples of selected women in Abeokuta indicates extensive exposure to lindane, total DDT and PCBs. Residue levels in the milk sample were lower than levels reported from other African Countries while the levels were higher than those reported for the developed countries and the WHO levels.

Correlation between levels of pesticides concentrated and parameters such as age, parity, occupation and level of income were not significant.

#### REFERENCES

- Czaja, K., Ludwicki, J.K., Goralczyk, K. and Strucinski P., 1997. Organochlorine Pesticides HCB and PCBs in Human Milk in Poland. *Bull. Environ. Contam. Toxicol.*, 58, 769-755.
- Dewailly, E., Dodi, S., Verreault, R., Ayotte, P., Sauve, L., Morin, J. and Brisson, J., 1994. High Organochlorine Body Burden in Women with Estrogen Receptor - Positive Breast Cancer. *J. Natl cancer Inst.*, 86, 232-234.
- Ecobichon, D.J., Joy, R.M., Dewailly, E. and Weber, J., 1989. High Levels of PCBs in Breastmilk of Women from Quebec. *Bull Environ. Contam. Toxicol.*, 43, 641-646.
- GEMS / FOOD, 1998. International dietary survey WHO / FSF / FOS / 98.4 Geneva, WHO.
- Guo, Y.L., Lambert, G.H. and Hsu, C.C., 1995. Growth Abnormalities in the Population Exposed in Utero and Early Postnatally to Polychlorinated biphenyls and dibenzofurans. *Environ Health Perspect*, 103, 117-122.
- Hernandez, L.M., Fernandez, M. A., Hoyas, E., Gonzalez, M.J. and Garcia, J.F., 1993. Organochlorine Insecticide and Polychlorinated biphenyl Residues in Human Breast Milk in Madrid (Spain). *Bull. Environ. Contam. Toxicol.*, 50, 308-315.
- Hong, C.S., Xiao, J., Casey, A.C. Bush, B., Fitzgerald, E.F. Hwang, S.A., 1994. Mono ortho- and Non-ortho-substituted Polychlorinated biphenyls in Human Milk from Previous Lactation. *Arch. Environ Contam. Toxicol.*, 33, 170-174.
- Jacobson, J.L. and Jacobson, S.W., 1996. Intellectual Impairment in Children Exposed to Polychlorinated biphenyls in-utero. *N. Eng J. Med.*, 335, 783-789.
- Jensen, A.A., 1983. Chemical Contaminants in Human Milk. *Residue Rev.*, 89, 1-128.
- Jensen, A.A and Storach, S.A, 1991. Chemical Contaminants in Human Milk. CRC Press, Boca Raton.
- Johansen, H., Becher, G., Polder, A. and Skaare, J.U., 1994. Congener-Specific Determination of Polychlorinated biphenyls and Organochlorine Pesticides in Human Milk from Norwegian Mothers living in Oslo. *J. Toxicol. Environ. Health.*, 42, 157-171.
- Kanja, L.W., Skaare, J.U., Ojwang, S.B. and Maitai, C.K.A., 1992. Comparison of Organochlorine Pesticides Residue in Maternal Adipose Tissue, Maternal Blood, Cord Blood and Human Milk from Mother / Infant Pair. *Arch. Environ. Contam. Toxicol.*, 22, 21-24.

- Longnecker, M.P., Rogan W.J. and Lucier, G., 1997. The Human Health Effect of DDT and PCBs and an Overview of Organochlorines in Public Health. *Annual. Rev. Public health.*, 18, 211-244.
- Mes, J., Davies, D.J., Doucet, J., Weber, D. and McMullen, E., 1993. Specific Polychlorinated biphenyl congener Distribution in Breast Milk of Canadian Women. *Environmental Technology*, 14, 555-565.
- Newsome, W.H., Davies, D. and Doucet, 1995. PCBs and Organochlorine Pesticides in Canadian Human Milk. *Chemosphere*, 30, 2143-2153.
- Noren, K. and Lunden, A., 1991. Trend Studies of Polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans in Human Milk. *Chemosphere*, 23, 1895-1901.
- Ntow, W.J., 2001. Organochlorine Pesticides in Water, Sediment, Crops and Human Fluids in a Famous Community in Ghana. *Arch Environ. Contam. Toxicol.*, 40(4), 557-63.
- Osibanjo, O. and Bamgbose, O., 1990. Chlorinated Hydrocarbons in Marine Fish and Shell Fish of Nigeria. *Marine Pollution Bulletin*, 21, 581-586.
- Rogan, W.J., Gladen, B.C., McKinney, J.D., Carreras, N., Hardy, P., Thullen, J., Tingelstad, J. and Tully, M.: 1986. Polychlorinated biphenyls and dichlorodiphenyl dichloroethane (DDE) in Human Milk: Effects of maternal factors and previous lactation. *Am.J. Publ. Health.*, 76,172-177.
- Sim, M.A. and Meneil, J.J.: 1992. Monitoring Chemical Exposure using Breast Milk; A Methodological Review. *Am J. Epidermol.* 136. 1-11.
- Spicer, P.E., 1993. Organochlorine Insecticides Residues in Human Breast Milk: A Survey of lactation mothers from a remote area in Papua New Guinea. *Bull Environ. Contam. Toxicol.*