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Organochlorine Pesticide Residues in Mothers' Milk in Uganda

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Since 1951 several investigators especially from developed countries have detected organochlorine residues in human breast milk (Slorach and Vaz 1983). However, in developing countries where mother's milk is the primary source of infant nutrition and where these pesticides are still used, few investigations have been carried out (Kanja et al. 1986). Investigations on the levels of organochlorine residues in human milk elucidate the potential risk of these contaminants to the health of breast-fed infants who could be regarded as the terminal link in the human food chain. Furthermore, such investigations illustrate the exposure of the mother which subsequently reflects the magnitude of environmental contamination by these chemicals (Jensen 1983).

Although organochlorine pesticides have been banned or severely restricted in developed countries mainly because of their persistence in the environment and bioaccumulation along the food chains, they are still used in Uganda. About 80 tonnes per year of DDT is used against cotton pests and for controlling mosquitoes. Approximately 392 tonnes per year of dieldrin is used for controlling banana weevils and termites, and an additional 30 tonnes per year of dieldrin is used for ground spraying and selective treatment of tree trunks for tsetse control in the country (Aryamanya-Mugisha 1993). Other organochlorine pesticides used in the country include lindane, aldrin, hexachlorobenzene, camphechlor, chlordane, and heptachlor (Bazirake 1993). In spite of their use in the country, no studies had previously been carried out to monitor their levels in mothers' breast milk. The main objective of the present study was to identify and quantify the levels of organochlorine residues in mothers' milk, and subsequently assess the toxicological implications to the health of the breast-fed infants.

MATERIALS AND METHODS

A total of 143 human milk samples were collected between October 1992 and January 1993. Sixty of the samples were collected from mothers living in Kampala city, and 83 samples were collected from mothers living in the rural

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areas of Iganga District. The criteria outlined by Slorach and Vaz (1983) was used for selecting breast-feeding mothers from whom milk samples were obtained. Following these criteria, milk samples were obtained from mothers who fulfilled all the following conditions: the mothers who had been resident in the study areas for at least five years; the mothers between 18 and 30 years of age; healthy mothers nursing their first or second children and whose last pregnancies were normal; the mothers breast-feeding one child only; and the mothers who were one week to four months post-partum.

The samples were obtained by manual expression and collected in pre-washed universal bottles with aluminum lined caps. About 15 ml of milk sample were obtained from each mother. The samples were kept in a cool box containing ice parks and frozen at -20°C on the same day of collection. The samples were later transported in a cool box with dry ice to the Centre for Environmental Research, Institute for Biogeography, University of Saarland, Saarbrucken, Germany where the laboratory chemical analysis was done.

Extraction and clean-up of the samples was carried out according to the United States Environmental Protection Agency Method 608 (US-EPA 1980). Briefly, the method involved grinding 10 to 15 g of milk sample with anhydrous sodium sulphate to yield a dry free-flowing powder which was then transferred into a glass extraction column of length 30 cm and internal diameter 2 cm. The dry column was then eluted with 80 ml of dichloromethane with the first 40 ml allowed to stay in contact with the powder for 20 to 30 minutes. The eluate was collected in a pre-weighed round bottom flask. Dichloromethane in the eluate was removed using a rotary evaporator at about 35°C and under reduced pressure. The flask was weighed until a constant weight was obtained. The difference between this weight and the original weight of the flask constituted the fat content of the sample. The raw fat extract was cleaned-up in a glass column packed with florisil (3% water) using elution mixture of petroleum ether/dichloromethane at the ratio of 4:1. The flow rate of the elution mixture was controlled so as not to exceed 5 ml/min. The eluate was concentrated to approximately 5 ml using a rotary evaporator at about 35°C under reduced pressure. This was then transferred to a 10 ml flask and evaporated to dryness. One millilitre of iso-octane was added to the flask, mixed thoroughly with a whirl mixer and then transferred to autosampler vials ready for gas chromatography.

A gas chromatograph (GC) SIGMA 1 (Perkin Elmer) with integrator LCI 100 (Perkin Elmer) and autosampler AS-100 (Perkin Elmer) was used for the residues analysis. The GC parameters and operating conditions were as follows: detector, ^{63}Ni electron capture; carrier gas, hydrogen; make-up gas, Ar/CH_4; column: length 25 m, internal diameter 0.25 mm, film thickness 0.25 µm, stationary phase S-54(5% phenyl-, 1% vinyl-, 94% methylsilicon); temperatures, detector 300°C, injector 250°C, oven 90-250°C; injection volume, 1 µl splitless 1 min; detection limit 1 pg. Sum DDT was calculated as p,p'DDT + o,p'DDT + 1.11(p,p'DDE + p,p'DDD), 1.11 being the conversion factor for the low molecular weight of

the DDT metabolites. Confirmation of the identity of the residues was done using a combined Gas Chromatograph/Mass Spectrometer (GC/MS) system; GC Series 8500 (Perkin Elmer) coupled with mass specific detector ITD-800 and Epson PC-AX with NBS/EPA mass spectra library.

The intakes of the various organochlorine residues by the breast-fed infants were calculated assuming that; (a) the child consumed 130 g of mother's milk per kg body weight per day (Slorach and Vaz 1983); (b) that the milk contained 3.5% (w/w) fat. It was felt more appropriate to use this figure than the fat content actually found in the samples in the present study. The latter is affected by the way in which the samples were collected and does not necessarily reflect the fat level in the all milk ingested on a breast-feeding occasion (Slorach and Vaz 1983); and (c) the mean weight of the infant is 5 kg.

RESULTS AND DISCUSSION

Residues of p,p'DDT and p,p'DDE were detected in all 143 samples analyzed. In addition, the following residues were detected in varying proportions: dieldrin (83.2%), o,p'DDT (74.8%), β-HCH(9.1%), α-HCH (3.5%) p,p'DDD (3.5%), and lindane (1.4 %). The number of samples positive for each residue, the mean and median levels in mg/kg milk fat of the residues detected are presented in Tables 1 and 2. The residue levels presented are not corrected for percent recoveries.

Individual differences in the residue levels were recorded. The overall mean level of sum DDT was 3.24 mg/kg milk fat, with a range of 0.26 to 18.72 mg/kg milk fat. The median level of sum DDT 2.54 mg/kg milk fat. The mean ratio of p,p'DDT to p,p'DDE was 0.25. The mean percent extractable fat was 2.9% and ranged from 0.5 to 9.2%.

The overall average estimated daily intake of sum DDT was 14.74 µg/kg body weight which was below the maximum acceptable daily intake (ADI) of 20 µg/kg body weight recommended by FAO/WHO (1985). However, in 28 (19.6%) individual samples, the estimated daily intake of sum DDT exceeded the recommended maximum ADI. The overall average estimated daily intake of dieldrin was 0.32 µg/kg body weight which exceeded by about 3.2 times the maximum AD1 of 0.1 µg/kg body weight recommended by FAO/WHO (1978), and in 108 (75.5%) individual samples, dieldrin levels were above the recommended maximum ADI. The overall average estimated intake of lindane was 2.0 µg/kg body weight which was below the maximum ADI of 8 µg/kg body weight recommended by FAO/WHO (1990). All the samples analyzed had lindane levels below the recommended maximum ADI.

The mean sum DDT of 3.24 mg/kg milk fat in Ugandan mothers' milk was generally lower than those reported from some developing countries, for example, Nigeria 3.83 mg/kg milk fat (Atuma and Okor 1987), India 6.55 mg/kg milk fat

Slorach and Vaz 1983), Kenya 6.99 mg/kg milk fat (Kanja et al. 1986), South Africa 20.1 mg/kg milk fat (Bouwman et al. 1990) and Ethiopia 7.75 mg/kg milk fat (Regassa 1995). In comparison with those reported from some developed countries, the mean sum DDT of 3.24 mg/kg milk fat in Ugandan mothers' milk was higher, for example, Japan 1.88 mg/kg fat (Slorach and Vaz 1983), Sweden 1.00 mg/kg milk fat (Slorach and Vaz 1983) and U.S.A 1.88 mg/kg milk fat (Slorach and Vaz 1983). These low levels of DDT in human milk reported from developed countries was because its use has been banned or severely restricted in these countries.

Table 1. Mean, median and range of α-HCH, β-HCH, lindane and dieldrin detected in mothers' milk from Kampala City and Iganga District, Uganda. The pesticide residues levels are expressed in mg/kg milk fat.

Compound	Kampala City (n=60)	Iganga District (n=83)	Overall (n= 143)
α-HCH			
no. positive[a] (%)	1 (1.7%)	4 (4.8%)	5 (3.5%)
mean[b]±S.E.M[c]	0.46	0.01±0.001	0.10±0.005
median	0.46	0.01	0.01
range	-	0.006-0.012	0.006-0.46
ß-HCH			
no. positive (%)	7 (11.7%)	6 (7.2%)	13 (9.1%)
mean±S.E.M	0.06±0.017	0.07±0.036	0.07±0.004
median	0.04	0.04	0.04
range	0.005-0.13	0.008-0.25	0.005-0.25
lindane			
no. positive (%)	1 (1.7%)	1 (1.2%)	2 (1.4%)
mean±S.E.M	0.87	0.01	0.44±0.018
median	0.87	0.01	0.44
range	-	-	0.01-0.87
dieldrin			
no. positive (%)	50 (83.3%)	69 (83.1%)	119 (83.2%)
mean±S.E.M	0.06±0.006	0.07±0.0009	0.07±0.008
median	0.04	0.05	0.04
range	0.01-0.19	0.007-0.37	0.007-0.37

[a] no. positive = number of samples with quantifiable residue levels
[b] mean was calculated from positive quantifiable samples only
[c] S.E.M = standard error of the mean
residue levels are not corrected for percent recoveries

Table 2. Mean, median and range of the DDT compounds in mothers' milk from Kampala City and Iganga District, Uganda. The pesticide residues levels are expressed in mg/kg milk fat.

Compound	Kampala City (n=60)	Iganga District (n=83)	Overall (n= 143)
p,p'DDE			
no.positive[a] (%)	60 (100%)	83 (100%)	143 (100%)
mean[b]±S.E.M[c]	2.84±0.255	2.00±0.211	2.35±0.201
median	2.55	1.44	1.86
range	0.63-13.58	0.20-13.93	0.20-13.93
p,p'DDD			
no. positive (%)	1 (1.7%)	4 (4.8%)	5 (3.5%)
mean±S.E.M	0.04	0.09±0.027	0.08±0.038
median	0.04	0.08	0.05
range	-	0.04-0.15	0.04-0.15
o,p'DDT			
no.positive (%)	56 (93.3%)	51 (61.4%)	107 (74.8%)
mean±S.E.M	0.07±0.006	0.06±0.011	0.06±0.022
median	0.06	0.03	0.05
range	0.01-0.22	0.01-0.49	0.01-0.49
p,p'DDT			
no.positive (%)	60 (100%)	83 (100%)	143 (100%)
mean±S.E.M	0.76±0.079	0.44±0.051	0.57±0.024
median	0.60	0.26	0.37
range	0.07-3.23	0.03-2.77	0.03-3.23
sum DDT			
mean±S.E.M	3.97±0.353	2.71±0.284	3.24±0.264
median	3.59	1.87	2.55
range	0.88-18.52	0.26-18.72	0.26-18.72
p,p'DDT/p,p'DDE			
mean±S.E.M	0.27±0.017	0.24±0.015	0.25±0.012
median	0.23	0.21	0.22
range	0.09-0.86	0.04-0.82	0.04-0.86

[a] no. positive = number of samples with quantifiable residue levels
[b] mean was calculated from positive quantifiable samples only
[c] S.E.M = standard error of the mean
residue levels are not corrected for percent recoveries

Considering results obtained from the rural areas of Iganga district, the only comparable study from Africa on mothers milk from rural areas was reported by Kanja et al. (1986). The criteria of selection of mothers was the same in both studies. Overall, the mean sum DDT of 2.71 mg/kg milk fat observed in this study in mothers milk samples in rural areas of Iganga District was about three times lower than the mean level of 6.99 mg/kg milk fat found in all the rural mothers in Kenya (Kanja et al. 1986). The present findings were only comparable with those obtained from Meru district of Kenya in which the mean sum DDT was 2.20 mg/kg milk fat. Meru district is one of the rich agricultural areas in Kenya. The highest mean level of sum DDT in rural areas of Kenya was reported from Rusinga Islands with a mean level of 18.73 mg/kg milk fat (Kanja et al. 1986). This level was about seven times higher than that observed in the present study. The high sum-DDT observed in Rusinga Islands was due to the intensive use of DDT in agriculture up to 1981, and it was used also for tsetse control from 1965 to 1967 (Kanja et al. 1986).

Among the other organochlorines, dieldrin residues were the most frequently detected in the samples analyzed. Although dieldrin was extensively used for tsetse control in Iganga district from early 1970s to 1981, no statistically significant difference was found in its mean levels between Iganga and Kampala districts. This finding suggests that the use of dieldrin for tsetse control in this area did not contribute significantly to environmental contamination. Although the use of dieldrin has been banned in most industrialized countries, its still used in most developing nations. Dieldrin which is an oxygenated metabolite of aldrin is more persistent in the environment. Its presence in mothers milk reflects exposure to either aldrin or dieldrin (Hayes 1982). Although aldrin is used in Uganda for control of termites and soil pests, no quantifiable levels were detected in this study.

Technical HCH is used an insecticide in Uganda, but few samples contained (a-HCH (3.5%), β-HCH (9.1%) and lindane (1.4%). Of these isomers, β-HCH is the most environmentally persistent and has 10-30 times higher ability to accumulate in fat tissues than lindane. In addition, α-HCH and lindane may isomerize into β-HCH in living organisms. From the present findings, HCH contamination so far does not seem to be a serious pollution problem in Uganda.

In this study, no quantifiable levels of polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) were detected in mothers milk. PCBs have mainly been detected in mothers milk in industrialized countries (Jensen 1983). Although endosulfan was used for tsetse control in Iganga district, no quantifiable levels were detected in all the samples analyzed.

The maximum ADI is developed on the basis of lifetime exposure, while intake of these contaminants via mothers' milk by the infant is limited to a few months during breast feeding. Hence, evaluation of the exact possible health implications of the present findings that the intake of sum DDT in 19.6% and dieldrin

in 75.5% of the breast-fed infants in the study exceeded the recommended maximum ADIs is difficult. Since there was no firm evidence that the levels of the organochlorine residues detected in mothers milk in this study have very serious harmful effects on the health of the breast-fed infants, breast-feeding should be encouraged and promoted because of its nutritive and immunological advantages, and its importance for the child-mother relationship. Rather, the present findings should be applied by the government and other relevant agencies to educate farmers on good agricultural practice (GAP) in the use of pesticides as set up by FAO International Code of Conduct on the distribution and use of pesticides. Periodic biomonitoring of organochlorine residues should be carried out to assess the trends of environmental contamination by these chemicals in the country.

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