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Organochlorine Insecticides Residues in Human Milk: A Study of Lactating Mothers in Siphofaneni, Swaziland

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The early spectacular successes achieved by 1,1,1-Trichloro-2.2-bis-(p-chlorophenyl)ethane, DDT, in malaria eradication in some countries, significant reduction in others and its low cost has seen the continuous use of this insecticide in the developing countries. DDT and its analogues, DDE, are the archetype of fat soluble, non-degradable and bioaccumulating compounds. The appearance of DDT in human tissues and its effect on wildlife especially reproduction in pelagic birds (US EPA 1975) triggered its determination in agricultural produce, soil, adipose tissue, water and human milk. Of particular interest is the danger posed by these compounds to the infant whose indispensable and the most important food is the human milk. The presence of organochlorine insecticides in human milk has been reported in studies conducted in many parts of the world such as Papua New Guinea (Spicer & Kereu 1993), Spain (Hernandez 1993), Iraq (Al Omar 1985) and Yugoslavia (Krauthacker 1991) and a host of other countries. The results of these studies indicated DDT levels higher than FAO/WHO recommended level.

In Swaziland, the department of Public Health applied about 12 tons 75%(wettable powder) on the inner walls of homes in the lowveld region in the 1994/95 season (*Dlamini 1995*). A follow up study on possible DDT contamination in the Kingdom is scarce. Considering the danger the continuous use of DDT poses to humans especially the infant, there was an urgent need, therefore, to conduct a study especially in those subjects that are highly vulnerable. The present study focuses on establishing the level of DDT and its metabolite, DDE, in human milk in Siphofaneni area of Swaziland, and to establish if the residue levels exceed the FAO/WHO recommended limits.

MATERIALS AND METHODS

A total of 103 human milk samples were collected from breast-feeding mothers residing in the village around Siphofaneni area in Swaziland. The donors were between 18-36 years of age and were healthy and had had normal deliveries. A Government clinic provided the collection centre and the mother's milk was manually expressed into a thoroughly clean dry glass container during the early periods of lactation.The milk samples were immediately placed into a refrigerator at 4°C and later transported to our laboratory where they were all frozen until the analysis. A questionnaire was prepared to collect information on the period of stay in the area, occupation, dietary habits, frequency of spray and number of children.

All solvents were of analytical reagent grade. The standard solutions were prepared from

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DDT and DDE stock standard solution (Perkin-Elmer), 100µg/ml, by transferring suitable volumes using a 250 Eppindorf microlitre pipet and making up to the required concentration with n-hexane.

The frozen milk samples were thawed to room temperature and were homogenised by shaking manually. About 10 g of each milk sample was extracted with n-hexane. The crude extracts obtained were concentrated to dryness by passing purified nitrogen without heating into the pre-weighed sample bottles. The bottles were re-weighed to obtain the weight of extractable fat. Thereafter, the dried fat crude extract was re-dissolved in n-hexane and subjected to column chromatography for purification on a florisil and sodium sulphate column. The samples were eluted with petroleum ether in n-hexane. The fractions collected were each concentrated down to suitable volumes for analysis.

In order to evaluate the percentage recovery of the analytes from the extraction procedure, fifteen aqueous milk fractions were divided into three groups(A, B and C) of five. Each group was spiked with 5.0 µg/ml p,p'DDT, o,p'DDT and p,p'DDE respectively. All the spiked samples were thereafter extracted, cleaned and concentrated and analysed as described earlier.

Gas chromatography analysis was conducted using a Varian 3400 gas liquid chromatography equipped with Ni^{63} electron capture detector. A 30 m x 0.53 mm (ID) capillary column coated to a thickness of 1 mm with a non-polar stationary phase, DB 608(J & W Scientific) was used for the analysis. Nitrogen (oxygen free with minimum water content of <5 volumes per million) was used as the carrier gas at a flow rate of 15 ml/min. The carrier gas flow rate was measured using a soap bubble flow meter connected to the detector exist. The injector port, column and detector temperatures were 280°C 270°C and 280°C respectively. The peaks were measured by retention time which were out-put through an integrator (Hewlett Packard). The analyte species were identified by comparing their retention times with those of the standards. A program, OCS-MAN was used for the generation of results and data storage. Using a 1ml analytical syringe (Klebur Chemicals), 0.2µl of the analyte solution was injected into the chromatograph. Injections were carried out in triplicate and the peaks were well separated under the above conditions.

RESULTS AND DISCUSSION

The results of the recovery test of aqueous milk fractions spiked with standard p,p'DDT, o,p'DDT and p,p'DDE are summarised in Table 1. The mean values of 4.69 ± 0.14, 4.64 ± 0.16 and 4.73 ± 0.12 µg/ml were recovered for p,p'DDT, o,p'DDT and p,p'DDE respectively; and the percentage recovery constituted 93.8 ± 2.8%, 92.8 ± 3.2% and 94.6 ± 2.4% for p,p'DDT, o,p'DDT and p,p'DDE respectively. The high percentage recovery indicated the reliability of the extraction procedure.

The residue range values, mean levels with standard deviations, ΣDDT and the percentage extractable fat of DDT and DDE compounds in mothers' milk are shown in Table 2. From Table 2, all the contaminants showed a minimum detection concentration of 0.01 mg/kg. The highest range, 0.01-6.47 mg/kg, and mean level, 1.13 ± 0.18 mg/kg, was shown by p,p'DDT..

Table 1 Recovery of DDT and DDE compounds in fortified milk extracts.

n	Compound	added	extracted	Mean levels ±SD(µg/ml)	% recovery ±SD
5	p,p'DDT	5	4.62 4.51 4.86 4.70 4.74	4.69±0.14	93.8 ±2.8
5	o,p'DDT	5	4.77 4.61 4.40 4.63 4.79	4.64±0.16	92.8±3.2
5	p,p'DDE	5	4.65 4.57 4.72 4.83 4.86	4.73±0.12	94.6±2.4

The least range, 0.01-0.61 mg/kg, and mean level, 0.2 ± 0.01 mg/kg, was observed for p,p,DDE, while the range and mean levels of 0.02-3.90 mg/kg and 0.48 ± 0.07 mg/kg respectively were obtained for o,p'DDT. As can be seen from Table 2, the ΣDDT-R ranged from 0.01-8.39 mg/kg with a mean level of 1.66 ± 0.24.These values are by far higher than those reported by (Spicer and Kereu. 1993), (Hernandez 1993) and (Ramakrishnan et al. 1985). It can also been seen in Table 2, that 83.5%, 76.7% and 47.6% of p,p'DDT, o,p'DDT and p,p'DDE respectively occurred in the samples. This indicated that the most predominant contaminant in the milk samples was p,p'DDT. If the FAO/WHO acceptable daily intake (ADI) of 0.02 mg/kg body weight for total DDT is adopted for nursed infants in order to elucidate their burden from lactation; and assuming that the mean daily intake of milk by an infant of mean weight of 5kg is 0.6kg, then the mean concentration expected not to be exceeded should be 0.167 mg/kg. From Table 2, 75.7%, 57.3% and 5.0% of p,p'DDT, o,p'DDT and p,p'DDE respectively exceeded the FAO/WHO acceptable daily intake(ADI).

Significant differences were observed between the levels of DDT and DDE. From Table 2, the levels of DDE were lower with a mean level of 0.02 mg/kg, and only 5% of the samples had their levels above the FAO/WHO acceptable daily intake (ADI). According to Kanja (1986), the presence of high levels of DDE in the breast milk is an indication of chronic exposure of the mother to DDT, which is subsequently metabolised to DDE and retained in the body. The low level of p,p'DDE observed in the present study indicated a relatively recent exposure of the mothers to DDT. The major source of this contamination may have come from the indoor spraying of DDT to combat malaria-carrying mosquitoes in the area studied.

p,p'DDT(1.66), o,p'DDT(0.69) and p,p'DDE(0.08) were higher than their respective mean concentrations of 1.13 mg/kg for p,p'DDT, 0.48 mg/kg for o,p'DDT and 0.02 mg/kg for p,p'DDE. This was an indication of a big variability among the sample results. From the mean sum DDT residues in Table 3, it was observed that differences existed in the levels of the residues from one sampling site to another. However, all the sites had mean sum DDT residues that exceeded the acceptable limit. Mphaphati showed the highest mean level of 3.22 mg/kg, and Siphofaneni with the lowest mean level of 0.36 mg/kg. The observed differences may be attributed to the amount of DDT sprayed in the homes and also due to the net assimilation and metabolism rates of the pesticide residues.

No correlation was observed between maternal age and the levels of DDT and DDE in the samples studied. This is in agreement with the work of Spicer and Kereu (1993) and Hernandez *et al.* (1993); although other studies have shown some correlation between age and DDT levels (Spicer 1987; Siddiqui and Saxena 1985). Also no significant difference was found between DDT and DDE levels and mothers' breast-feeding their first children as compared with the others. This may be attributed to the fact that intake of residue was still significant at the time of sampling. This agrees with the work of Krauthacker (1991), Drijver (1988) and Hernandez (1993) who found no significant difference between primigravidas and multigravidas with the level of DDT.
However, Spicer and Kereu (1993) found significant difference between total DDT in breast milk and mothers feeding their first babies compared with their other children.
The levels of DDT and DDE found in this study are high compared with other countries. Since these compounds are generally neurotoxic, there are several reasons for anxiety. A continuous monitoring protocol is therefore required for these compounds in Swaziland.

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