

DDT, DDE and DDD in Human Milk from South Africa

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Received: 25 January 2008 / Accepted: 24 June 2008 / Published online: 29 July 2008
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Abstract Human breast milk samples ($n = 30$) were collected from mothers within the age range of 19–40 years from Thohoyandou area, South Africa. DDT and its metabolites were extracted from the milk samples using diethyl ether. The crude extracts were subjected to column chromatography. The eluates were then evaporated on a stream of nitrogen up to 0.5 mL. One microliter of the cleaned extracts were injected into GC-ECD for selected organochlorine compounds. The sum total of DDT and its metabolites from each village ranged from not detectable for DMS, GNN and THN to 1,930 ng g⁻¹ fat wt for BDL while Σ DDE ranged from 1.32 ng g⁻¹ fat wt for GNN to 2,570 ng g⁻¹ fat wt for TKD. Σ DDD ranged from not detectable for GNN to 4,060 ng g⁻¹ fat wt for MNN. Σ DDE was the most predominant followed by Σ DDD and finally Σ DDT. This was an indication of breakdown of the parent compound, DDT. Some villages namely, Lufule and Budeli, and Maniini and Makhuvha exhibited similar DDT occurrence of 89% in their areas. Other villages, DMS, TLM, and MND and TKD showed a similarity percentage of 82% while others showed similarities of 75% for GNN and MTT, 69% and 65% for MPG and THN respectively. A significant cluster of DDT and its metabolites between the infants' weight range of 2.5–3.9 kg/body wt was observed. Increase in lipid content was followed by a decrease in the sum DDT in the older mothers (27–30). The estimated daily intake varied from 260 to 4,696 ng/g, nd-10,551 ng/g and nd-4,237 ng/g for DDE, DDD and DDT

respectively. These values are significantly ($p < 0.05$) higher than the FAO/WHO acceptable daily intake (ADI) of 20 ng/g. The Σ DDT was found to decrease with increasing age of the mothers. The observed high levels of DDE compared to DDT indicated chronic exposure of the mothers to DDT, which is metabolised to DDE and retained in the body.

Keywords DDT · DDE · DDD · Human milk · South Africa

Human breast milk is unique as a matrix for biomonitoring because it also serves as a food source for a segment of the human population. Thus, monitoring of environmental contaminants such as DDT and its metabolites in breast milk is important in order to assess the body burden of potentially harmful chemicals. Also surveys of persistent organic pollutants (POPs) in breast milk are recognised as a relevant, simple and non-invasive method in case of nursing mothers and their breast-feeding babies.

Analysis of human milk for organochlorine contaminants from South African mothers has been conducted since the early 1990s (Bouwman et al. 1990). The main objective has been to monitor the mothers' body burden and the infants' intake of these toxic pollutants. Similar monitoring programmes have also been reported by other researchers (Norén 1988; Skaare and Polder 1990; Dewailly et al. 1996; Duarte-Davidson et al. 1992; Atuma and Aune 1999; Aune et al. 1999; Okonkwo et al. 1999; Okonkwo and Kampira 2002; Burke et al. 2003; Minh et al. 2004; Bouwman et al. 1992, 2006). DDT and its metabolites have been among the major contaminants monitored. The determination of these compounds in various environmental matrices has been of major concern because of

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the potential adverse effects on the mothers as well as the breastfed infants. DDT has been linked to the cause of intellectual quotient (IQ) and hormonal interference in humans. Various DDT metabolites have been reported to have hormone-like activities which include blocking of the action of male hormones (Colborn et al. 1996; Kelce 1995; Cheek et al. 1999; Vos et al. 2000).

The analytical data generated from the aforementioned studies have been used in various risk assessment of organochlorine for this risk group. In order to update and expand the data base regarding the exposure of pregnant women and infants to organochlorines, particularly DDT (since it is still used for public health programmes) in South Africa, a project was embarked on in 2004 in which DDT and its metabolites were determined in mother's milk.

In this paper we present the results from a study of 30 samples of breast milk from mothers in Thohoyandou, South Africa.

Materials and Methods

The study was conducted in Thohoyandou area in Limpopo Province, South Africa. The following villages were selected for the study: Tshilungoma, Mphego, Dumasi, Tshikhudini, Tshisele, Mulenzhe, Mutoti, Gondeni, Hamukoma, Maniini, Makwarela, Thohoyandou, Makhuvha, Dididi and Lufule. Figure 1 shows a map representing study area. A government clinic in Dumasi was used for

sample collection. Breast-feeding mothers between the ages of 19–40 and with normal deliveries were selected for the study. Mothers expressed 5–50 mL of breast milk into thoroughly washed and oven dried glass bottles under the supervision of qualified nurses. A total of 30 milk samples were collected and stored in the freezer until ready for use. The World Health Organisation questionnaires were used to collect information on mothers' age, diet, number of children, longevity in the area and frequency of spray.

All glassware used (Pyrex brand) were soaked in dilute HNO_3 overnight, thoroughly washed and rinsed properly with distilled water, and finally with acetone. They were then dried in an oven at 80°C for 24 h before use. The 100 mL sample glass bottles used for milk collection underwent the same washing procedure as indicated above.

DDT, DDE and DDD (Sigma Aldrich) standard solutions were used to establish the calibration curve with pentachloronitrobenzene (PCNB) as the internal standard. PCNB was selected as an internal standard due to its high sensitivity, and can also be analysed using GC-ECD. Analytical grade reagent chemicals (diethyl ether AAR, 99% Set Point Instruments, SA; acetone HPLC Reagent Grade, ACS, ISO, UV-VIS Spectroscopy 99.99% Sharlan; *n*-Hexane GR, ACS, ISO 99%; iso-propanol HOLPRO Analytics PTY Ltd, SA and methanol AR of 99.99% purity Bio-zone Chemicals, SA) were used for extraction. Florisil[®] 60–100 mesh (Aldrich); sodium oxalate (99% Hopkins and Williams USA); anhydrous sodium sulphate (anhydrous powder), and sulphuric acid 98% (Rochelle

Fig. 1 Study area showing map of South Africa (top left) and Limpopo Province and Thohoyandou area (bottom right)



Chemicals SA) used for column chromatography were all of analytical reagent quality.

The extraction method employed in this study was as described by Okonkwo et al. (1999) and Mutshatshi et al. (2008), modified by Cok et al. (1997) and Burke et al. (2003). Briefly, the frozen milk samples were thawed to room temperature and homogenised by shaking manually for about 10 min. Thereafter, 5 mL methanol and 0.1 g of sodium oxalate were added to 10–20 mL samples in a 100 mL beaker with further shaking for 5 min and left for 2 h. Extraction was performed with 15 mL of hexane/diethyl ether (1:1 v/v) in 100 mL separating funnel. The organic upper layer was collected in a weighed test tube and extraction repeated thrice. The solvents from the combined organic extracts were removed using a gentle stream of nitrogen and the fat weight determined gravimetrically. In order to remove the fat and other polar materials, the extracted fat was re-dissolved in 2 mL hexane and 5.0 mL of concentrated H₂SO₄ added. This was centrifuged for 15 min at 3,000 rpm. The acid residues were then extracted twice with additional 2 mL of hexane. The organic phase was collected and dried in a flow of nitrogen. Dried residues were dissolved in 2 mL of hexane and then passed through column chromatography for clean up.

For the column chromatography a glass column was packed with 1–2% (v/m) florisil and anhydrous Na₂SO₄ placed at the top. The extracts were placed into the column and thereafter eluted with 10 mL of hexane followed by 10 mL of hexane:methanol:iso-propanol (45:40:15, v/v/v). Both elutes were then collected and dried in a stream of nitrogen. The dry residues were dissolved in 0.5 mL hexane and 0.2 mL of pentachloronitrobenzene internal standard added.

The chromatographic apparatus used was GC17A (SHIMADZU) coupled with electron capture detector (ECD). The chromatographic column used was ZB-5, 5% Phenol, 95% – Dimethylpolysiloxane (30 m × 0.25 id nm, 0.25 film thickness in µm-Separations, SA). The carrier gas flow rate was 1.8 mL/min and the make-up gases used was nitrogen (AFROX, SA) with minimum purity of 99.999% with the total flow of about 42 mL/min. The carrier gas flow rate was measured using a soap bubble flow meter connected to the detector exit. The injector port and detector temperatures were 250°C and 300°C respectively. The column initial temperature was 110°C held for 2 min and programmed to 210°C at a rate of 25°C/min, held for 2 min, and then increased to 270°C at a rate of 5°C/min and held for 5 min and then finally to 290°C at 5°C/min. The final temperature was held for additional 5 min. With a 1 mL analytical syringe (Klebur Chemicals) sample volumes of 1.0 µL were injected into the chromatograph in the splitless mode with the valve closed for about 90 s. Injections were carried out in triplicate and the peaks were well separated under the above conditions. The analyte

species were identified by comparing their retention times with those of the standards.

In order to test the performance of the extraction method, five 5 mL aqueous milk samples were spiked with 1 mL of 50–500 µg/L of standard 2,4-DDE, 2,4-DDD, 4,4-DDE, 4,4-DDD and 4,4-DDT. All the spiked samples were thereafter extracted, subjected to column chromatography, concentrated and analysed as described earlier. A reagent or extraction blank, consisting of deionised water in equal amount to the sample was included in every batch of samples.

The similarity (%) in terms of OCPs occurrences on the sample pool was calculated using Primer Version 5. Correlation ($p < 0.05$) were performed using “one-way spearman test”. One-way ANOVA was used for significant test. Standard deviations and means were calculated using Microsoft Excel.

Results and Discussions

The results of the recovery tests are summarized in Table 1. As can be seen in Table 1, the % recovery ranged from 80% to 130%. The high % recovery values obtained validated the method used. The sum total of DDT and its metabolites from each village are shown in Table 2. ΣDDT ranged from not detectable for DMS, GNN and THN to 1,930 ng g⁻¹ fat wt for BDL while ΣDDE ranged from 1.32 ng g⁻¹ fat wt for GNN to 2,570 ng g⁻¹ fat wt for TKD. ΣDDD ranged from not detectable for GNN to 4,060 ng g⁻¹ fat wt for MNN. A study by Bouwman et al. (2006) found that the mothers from Jozini in KwaZulu Natal had significantly higher levels of DDT (ΣDDT 4,480 ng g⁻¹ milk fat) than women in Kwaliweni (ΣDDT 1,100 ng g⁻¹ milk fat). The DDT levels from Jozini were lower than the previous survey conducted 14 years ago from a comparable town close by, Mseleni, with ΣDDT of 15 800 ng g⁻¹ milk fat (Bouwman et al. 1992). The reduction was ascribed to the 5-year interval with no DDT application, before DDT application was resumed 1 year before sampling.

These values are within the same range as reported by Schecter et al. (1989), lower than the studies by Minh et al.

Table 1 Recoveries of OCs in spiked milk samples and limits of detection

Compound	Added amount ng	Recovery
2,4'-DDD	50–500	80
4,4'-DDD	50–500	130
2,4'-DDE	50–500	120
4,4'-DDE	50–500	90
4,4'-DDT	50–500	94

Table 2 Sum of DDT and its metabolites from each village

Σ of compounds of interest (ng/g)	Villages where collected samples were analysed									
	LFL	MND	MNN	DMS	BDL	TKD	TLM	GNN	MKV	THN
ΣDDT	540	8,540	1,250	nd	1,930	1,880	730	nd	1,090	nd
ΣDDE	1,130	14,580	850	2,490	1,290	2,570	2,010	1.32	2,570	560
ΣDDD	1,130	1,240	4,060	340	480	1,520	2,660	nd	5,910	260

LFL – Lufule, MND – Mangondi, MNN – Maniini, DMS – Dumasi, BDL – Budeli, TKD – Tshikhudini, TLM – Tshilungoma, GNN – Gondeni, MKV – Makhuvha, THN – Thohoyandou, nd – not detected

(2004), Chikuni et al. (1991), Bouwman et al. (1992) and higher than the report by Okonkwo et al. (1999). A closer examination showed that ΣDDE was the most predominant followed by ΣDDD and finally ΣDDT. This was an indication of breakdown of the parent compound, DDT. Considering the fact that the study area is known to be extremely hot, especially during the summer months, the breakdown of DDT is expected to be fairly rapid.

Figure 2 compares the occurrence of OCs in human milk within the villages. As can be seen from Fig. 2, Lufule and Budeli, and Maniini and Makhuvha exhibited similar DDT occurrence of 89% in these areas. Those villages were then followed by the DMS, TLM, and MND and TKD with a similarity percentage of 82% and the rest of the villages showed similarities of 75% for GNN and MTT, 69% and 65% for MPG and THN respectively. The percentage fat contents with respect to the detected OCPs are shown in Fig. 3. From Fig. 3, 2,4'DDE and 4,4'DDT exhibited similar fat contents of 65%. The other metabolites showed lower similarities.

Figure 4 attempted to establish whether there was any relationship between DDT and children’s weight at birth. As can be seen from the figure, there is a significant cluster of DDT and its metabolites between the weight range of 2.5–3.9 kg/body wt. However, Bouwman et al. (1992); Farhand et al. (2005) examined the association of serum levels of DDT and DDE with birth weight and gestational age of male infants in a population of women during a

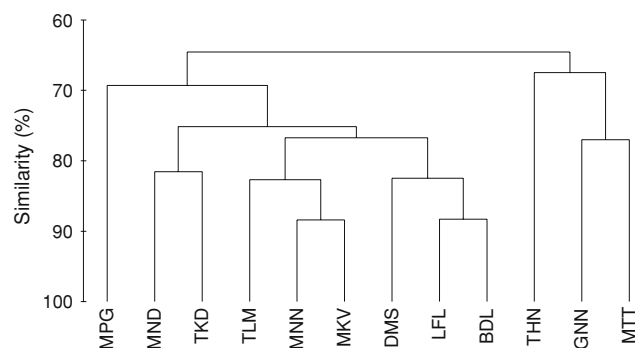


Fig. 2 Comparison of villages’ in terms of pesticides occurrence in human breast milk

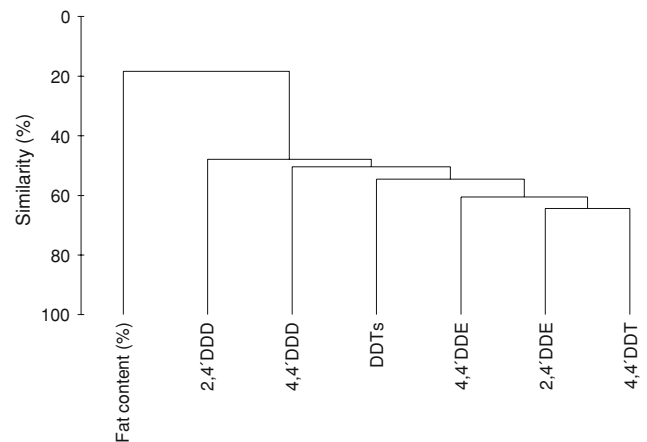


Fig. 3 Percentage fat contents of OCs in human milk

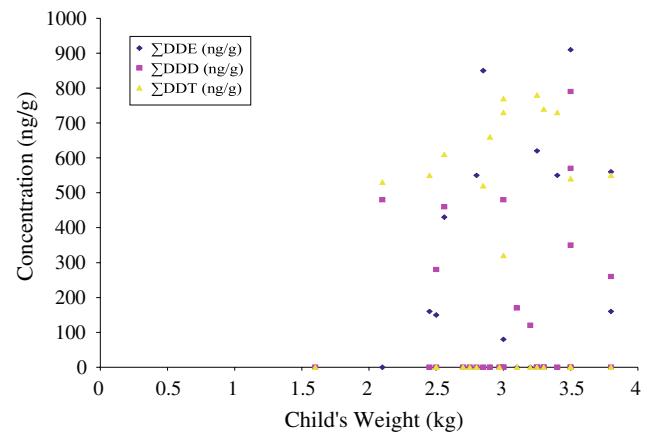


Fig. 4 Relationship between DDT and children’s weight at birth

period of nationwide organochlorine usage. The overall results showed no significant relations or trend between serum measurements of DDT or DDE and birth weight.

It is known that adult females excrete lipophilic contaminants such as OCs via lactation and thus reduce the body burden of such contaminants. In this study, an attempt was made to find out whether there was any correlation between maternal age and fat content of the milk samples with the concentrations of DDTs obtained. The summarised results of this investigation are shown in Fig. 5.

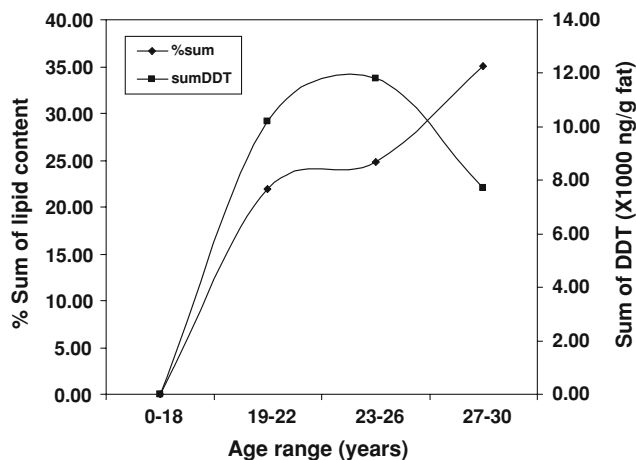


Fig. 5 Maternal age, sum % lipid contents and sum DDT (ng/g fat wt) of milk samples studied

Mothers were classified arbitrarily according to their age into three groups: 19–22, 23–26 and 27–30 since most of the mothers' ages were within 19–30 except one 40 year old mother. The figure shows that as the % sum lipid content increased, there was a corresponding increase and thereafter a drop in the sum DDT in the older mothers (27–30). The drop in sum DDT with age is in agreement with the report by Saxena and Siddiqui (1982), although other

studies have shown no correlation between maternal age and DDT levels (Spicer and Kereu 1993; Hernández et al. 1993; Okonkwo et al. 1999). It is possible that other variables such as diet, number of pregnancies and duration of residence may also contribute to the level of DDTs in mothers.

The results of the estimated daily intake (ADI) are shown in Fig. 6. The results were calculated based on the assumption that the average milk consumption by the infants (3–7 kg) was 700 g/day. The mean values for daily intake of organochlorines were estimated using Eq. 1

$$DI = \frac{C_{\text{milk}} \times 700 \text{ g} \times C_{\text{lipid}}}{3 - 7 \text{ kg}} \quad (1)$$

where DI is daily intake (ng/g body wt/day); C_{milk} = concentration of the chemical in milk (ng/g lipid wt) and C_{lipid} = lipid content in milk (%).

The DI levels obtained differed from one village to another. This is not surprising since the weights of the infants varied from 3 to 7 kg as observed during sample collection. It was observed that the levels of DI for DDE, DDD and DDT significantly exceeded the FAO/WHO guideline of 20 ng/g. This fact calls for concern since children are highly susceptible to effects from environmental contaminants. Differences in dietary habits, socio-economic status, metabolic rates of the mothers

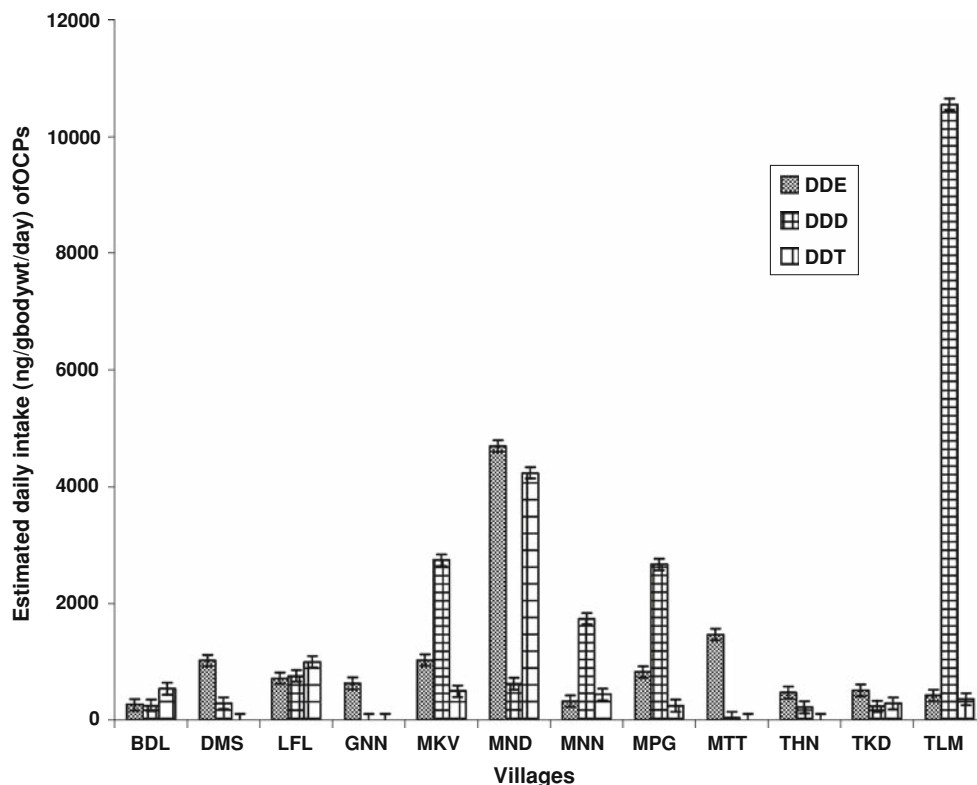


Fig. 6 Estimated daily intake (ng/g body wt/day) of OCPs by infants in Thohoyandou

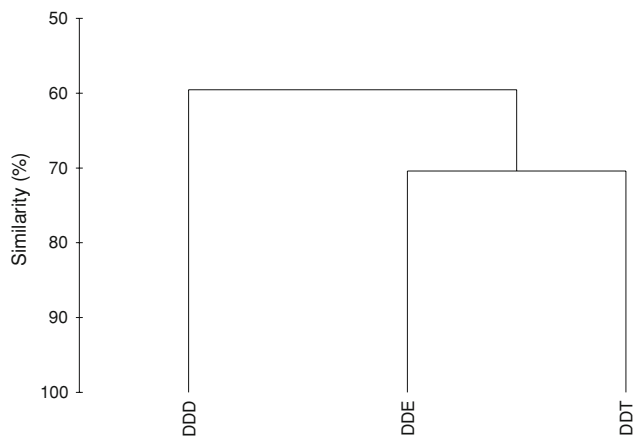


Fig. 7 Similarity (%) of OCPs (DDT, DDE and DD) from villages in Thohoyandou

and the frequency of spray of DDT in the area may have contributed to the wide concentrations in DI levels obtained from the various villages. However, because of the well-recognised advantages of breast-feeding, this practice should not be discouraged. The present study should be applied to introduce better practice in the use of DDT.

Figure 7 indicates the percentage similarities of the DDT and its metabolites when considering the estimated daily intakes in Fig. 6. The analysis indicated that the estimated daily intakes of two compounds (DDT and DDE) peaked at 70% with respect to their presence within the samples collected from the villages. This meant that whenever there was DDT, there was 70% chance of detecting DDE, while DDD had 60% chance of being present. The study also showed that the estimated daily intakes of DDT and its metabolites within the villages in Thohoyandou followed the same pattern as presented in Fig. 6. The estimated daily intake of villages like MPG and MKV, BDL and LFL, and DMS and THN were all at about 94% similarities.

The concentrations of OCs determined in the current study are significantly higher than the values reported from other countries. The obvious risk associated with breast-feeding is the possible contamination of mothers' milk and the transfer of the contaminant from mother to child. However, human breast milk is well endowed with lots of resistant factors which offer the infant an immunological protection. Breastfeeding also offers emotional bonding between mother and child. The high levels of OCs obtained in the present study raises cause for concern. But because of the well-recognised advantages of breast-feeding, this practice should not be discouraged. Instead the results of the present study should be applied to introduce better practice in the use of DDT within the study area.

Acknowledgments The authors are indebted to the National Research Foundation of South Africa and Tshwane University of Technology for financial support. The mothers who donated their precious food, breast milk, and the nurses who participated in this project are indebted to.

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