Concentration of DDT compounds in breast milk from African women (Manhiça, Mozambique) at the early stages of domestic indoor spraying with this insecticide

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Abstract

Breast milk concentrations of 4,4'-DDT and its related compounds were studied in samples collected in 2002 and 2006 from two populations of mothers in Manhiça, Mozambique. The 2006 samples were obtained several months after implementation of indoor residual spraying (IRS) with DDT for malaria vector control in dwellings and those from 2002 were taken as reference prior to DDT use. A significant increase in 4,4'-DDT and its main metabolite, 4,4'-DDE, was observed between the 2002 (median values 2.4 and 0.9 ng/ml, respectively) and the 2006 samples (7.3 and 2.6 ng/ml, respectively, p < 0.001 and 0.019, respectively). This observation identifies higher body burden intakes of these compounds in pregnant women already in these initial stages of the IRS program. The increase in both 4,4'-DDT and 4,4'-DDE suggest a rapid transformation of DDT into DDE after incorporation of the insecticide residues. The median baseline concentrations in breast milk in 2002 were low, and the median concentrations in 2006 (280 ng/g lipid) were still lower than in other world populations. However, the observed increases were not uniform and in some individuals high values (5100 ng/g lipid) were determined. Significant differences were found between the concentrations of DDT and related compounds in breast milk according to parity, with higher concentrations in primiparae than multiparae women. These differences overcome the age effect in DDT accumulation between the two groups and evidence that women transfer a significant proportion of their body burden of DDT and its metabolites to their infants.

Key words: DDT, in-door spraying, malaria vector control, parity, breast milk.
1. Introduction

Technical grade DDT is generally composed of 4,4’-DDT (~80%), 2,4’-DDT (~15%) and 4,4’-DDE (~4%). This product started to be widely used as insecticide in the 40s, leading to the accumulation of 4,4’-DDT and its metabolites in many organisms due to their lipophilicity and high resistance to degradation. Humans are at the apex of the food chain and tend to bioaccumulate DDT compounds through diet, but in some cases direct exposure may also be a significant intake mechanism. DDT and its metabolites have also shown to generate adverse effects on the human health, such as on the cognitive development in children during their first years of life (Ribas-Fitó et al., 2006; Morales et al., 2008), alterations of thyroid hormone concentrations (Ouyang et al., 2005, Aneck-Hahn et al., 2007; Alvarez-Pedrerol et al., 2008a,b) or DNA damage (Yanez et al., 2004). Exposure to DDE, its main metabolite, has been related to increase of asthma incidence in infants (Sunyer et al., 2005; 2006) and increases in urinary coproporphyrins (Sunyer et al., 2008).

Evidence of the adverse effects of this insecticide in the environment and humans led to ban DDT for agricultural practices in the 70s. Later, the Stockholm agreement led to a general ban of an important number of persistent organic pollutants, including DDT. These restrictions involved gradual reductions in DDT levels in large areas of the world, e.g. from breast milk contents of 5000–10,000 ng/g lipid in 1951 to ~1000 ng/g lipid at present (Smith, 1999), or in Sweden between 2000 and 1300 ng/g lipid of 4,4’-DDE and 4,4’-DDT, respectively, to 130 and 14 ng/g lipid, respectively, in 1997 (Noren and Meironyte, 2000).

In Africa, where malaria killed 750,000 million people in 2009, insecticides have still been used to eliminate the malaria vector. The Stockholm Convention encouraged reducing reliance on DDT and promoting research and development on safer alternative pesticides and strategies. Many of these efforts have been concentrated on the use of pyrethroids as an alternative to combat the vector, which has been successful in some countries. However, the malaria mosquito has become resistant to pyrethroids (Hargreaves et al., 2003). Accordingly, more than two dozen countries, most of them from sub-Saharan Africa, requested exemptions on the ban of DDT for malaria vector control on the evidence that DDT was the most effective insecticide due to its
persistence (it is sufficient to spray it just once a year in the houses), relatively low cost (about $5 per average five-person household) and efficiency. DDT either kills mosquitoes resting on the walls, or repels them from the dwellings (WHO, 2006; 2007).

The World Health Organization recommended the continued use of DDT in limited quantities for public health purposes in situations where alternatives were not available and where potential loss of human life associated with unstable malaria transmission and epidemics is greatest (WHO, 2006; 2007). One of the principal vector control interventions for reducing malaria transmission is indoor residual spraying (IRS). Reintroduction of DDT for IRS in some African countries like South Africa, Swaziland and Zimbabwe showed a rapid decline in the number of malaria cases in the areas treated with DDT (Mabasso et al., 2004, Maharaj et al., 2005).

DDT was introduced in 1946 in Mozambique where it was used widely in agriculture and health programs until 1988. IRS programs with DDT were introduced in 1946 in the southern part. In 1950 all target areas were covered. As part of the malaria eradication initiative, IRS with DDT was carried out in the Maputo province between 1960 and 1969, but this program had a complete breakdown in the late 1970s due to the civil war (Mabasso et al., 2004). After the war, in 1993 there was a change in policy and the National Malaria Control Program (NMCP) decided to restart the IRS programs with pyrethroids (deltamethrin and lambda-cyhalothrin) in the major towns. In addition to this effort, the Lubombo Spatial Development Initiative (LSDI), an inter-country cross-border malaria control program (including IRS) jointly implemented by Mozambique, South Africa and Swaziland, commenced operations in southern Mozambique in 1999 (WHO 2007). In 1999 initial baseline resistance to pyrethroids began to be detected in malaria vector mosquitoes (Casimiro 2006a,b; Sharp 2007) which led to the implementation of changes from pyrethroids to carbamates (bendiocarb) in November 2000 as part of the LSDI (LSDI 2006).

House spraying with DDT in Mozambique was introduced again at the end of 2005 in public health programs and has now become the main insecticide used for malaria vector control as no resistance to this product was detected in Mozambique (Casimiro 2006a,b; 2007, Cuamba et al 2010 ). The houses and structures were sprayed once a year at an application rate of 3 g per m². Currently the use of DDT in agriculture is still
banned being restricted to mosquito control. To prevent illegal uses, DDT is exclusively
distributed through the Ministry of Health (MISAU). However, misuses as consequence
of poor management in rural areas cannot be excluded (MISAU 2005, 2006). Since
2005, MISAU has imported about 1300 tons of DDT (from India and China) in wettable
powder which are still in use.

In others districts of the Maputo province (Matutuine, Namaacha, Boane, Moamba,
Marracuene and Magude) spraying with DDT began between November 2005 and June
2006. In the Manhiça district, DDT was reintroduced for IRS in 2006 and was used
together with bendiocarb. Between 2006 and 2008 there were three complete rounds of
spraying. A global assessment of the benefits and drawbacks of the DDT reintroduction
for malaria vector control is needed. Mozambique is a good case to study since this
compound was banned for twelve years and then reintroduced with restrictions.

The past and present uses of DDT in Mozambique provide an example of the
accumulation patterns of this compound and its metabolites at the early stages of
reintroduction for public health policies. Breastfeeding is the primary source of early
life infant nutrition. Infants are pre- and post-natally exposed to DDT compounds
through the placenta and primarily breastfeeding. Due to the lipophilicity of DDT and
its metabolites and the relatively high amount of fat in breast milk, it is common to find
these compounds in human milk. Their concentrations in human milk may reflect the
mothers’ body burden and can be used to estimate the dose transferred to infants. The
IRS activities for malaria control can be a route for DDT uptake. Several reports have
described significant concentrations of DDT in breast milk from women from African
countries (Ejobi et al., 1996; Chikuni et al., 1997; Okonkwo et al., 1999; Sereda, 2005;
Bouwman et al., 2006). However, no studies had previously been carried out to monitor
the levels of organochlorine compounds (OCs) in Mozambique. The present study
focuses on establishing the levels of DDT and its metabolites DDE and DDD in human
milk in a rural area located south of the country. Samples were collected in 2002 (before
IRS) and 2006 (after IRS reintroduction), therefore these two groups provide
representative examples of DDT accumulation prior and at the early stages of IRS.

2. Materials and methods
2.1. Study area

The Manhiça district is a rural area located in the north of the Maputo province, limiting with the Indian Ocean in the east. The climate is subtropical with two distinct seasons, one warm and rainy between November and April and another dry and cold from May to October. Most of the inhabitants are farmers who grow sugar cane, bananas and rice, and some of them work in two big sugar cane factories nearby.

The first round of IRS occurred between September 2006 and March 2007, using about 1600 kg of DDT and 700 kg of bendiocarb. The second round was between September 2007 and March 2008, using about 3000 kg of DDT and 1400 kg of bendiocarb. The third round was between May and December 2008, and the amount used was about 4500 kg of DDT and 1000 kg of bendiocarb.

2.2. Sample collection

Mature breast milk samples were collected in 2002 (n = 45) and 2006 (n = 50) in the context of studies conducted at the Centro de Investigação em Saúde da Manhiça (CISM). The research protocol was approved by the Ethics Committees of Mozambique and Hospital Clinic in Barcelona, Spain. All women signed an approved consent form before they were enrolled in the study. Mothers received breast milk containers for self-expression and questionnaires were administered as soon as they had given their informed consent. The information obtained included ages of the mother and children, parity, spraying date and occupational exposure to other pesticides. Samples were stored at –80°C in CISM and at -20°C in IDÆA-CSIC until analysis, which was performed in this Institute.

2.3. Chemical products

Standards of tetrabromobenzene (TBB), PCB 209 and DDT compounds were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standard solutions were prepared in iso-octane for organic trace analysis (Merck, Darmstadt, Germany). Analytical grade
concentrated sulfuric acid, dichloromethane (DCM), methanol, cyclohexane, and \( n \)-hexane were also from Merck.

2.4. Extraction procedures

Volumes of 0.5–1 ml of breast milk were spiked with TBB and PCB 209 as surrogate standards and the mixture was vortex stirred for 60 s at 2000 rpm. \( n \)-Hexane (3 ml) was added, followed by concentrated sulfuric acid (2 ml). After the reaction, the mixture was vortex stirred for 30 s and the supernatant \( n \)-hexane phase was separated by centrifugation. The remaining sulfuric acid solution was re-extracted two times with 2 ml of \( n \)-hexane (stirring 30 s). The combined \( n \)-hexane extracts (7 ml) were additionally cleaned with 2 ml of sulfuric acid (stirring 90 s). Then the \( n \)-hexane phase was separated and reduced to dryness under a gentle nitrogen stream. The extract was transferred to gas chromatography (GC) vials with four rinses of isooctane (25 \( \mu l \) each). Then, it was re-evaporated under the nitrogen stream and 100 \( \mu l \) of PCB142 were added as internal standards before injection.

Total milk lipid concentrations were determined by the crematocrit method (Mayans and Martell, 1994). Lipid content could not be calculated in the samples collected in 2002 due to the low breast milk volumes available.

2.5. GC analysis

The concentrations of 2,4'-DDT, 4,4'-DDT, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD and 4,4'-DDD were determined by GC with electron capture detection (Hewlett Packard 6890N GC-ECD). Samples were injected (2 \( \mu l \)) in splitless mode onto a 60 m DB-5 column protected with a retention gap (J&W Scientific, Folsom, CA, USA). The temperature program started at 90°C (held for 2 min) and increased to 140°C at 20°C/ min, then to 200°C (held for 13 min) at 4°C/ min and finally to 310°C (held for 10 min) at 4°C/ min. Injector, ion source and transfer line temperatures were 250°C, 176°C and 280°C, respectively.
The quantification procedure is described in detail elsewhere (Gari and Grimalt, 2010). OCs identification was based on retention time. Selected samples were analyzed by GC coupled to mass spectrometry for structural confirmation. Calibration straight lines were obtained for all analytes. These standard solutions also contained the injection standards. Quantification was performed by the external standard method using these calibration lines and recovery (TBB and PCB-209) and injection (PCB-142) standards. The use of PCB-142 to correct for volume allows differentiating between corrections due to analyte losses by sample handling and volume variations in the final solvent rinsings for sample introduction into the chromatographic vials. Thus, the recovery standards are also corrected by the injection standard. Limits of detection (LOD) and quantification (LOQ) were calculated from blanks. One blank was included in each sample batch.

2.6. Data analysis

The results were reported by reference to milk volume (2002 and 2006 groups, Table 1) and by reference to fat content (2006 group, Table 2). Concentrations below LOQ were substituted by half of the LOD. \( \Sigma \)DDT were calculated by sum of 4,4’-DDE, 4,4’-DDD and 4,4’-DDT. Univariate statistics were calculated as customary (Rothman et al., 2008). Differences on concentrations of DDT compounds between distinct population groups were assessed with the Mann-Whitney’s U-test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for windows version 15). The statistical significance was set at \( p < 0.05 \) (two sided).

3. Results

3.1. Participant profiles

Maternal age in the 2002 group ranged between 15 and 44 years, with mean and median of 26.1 and 25 years, respectively (Table 1). In the 2006 group, maternal age ranged between 15 and 47 years, with mean and median of 24.2 and 22 years, respectively (Table 1). In this last group there were 16 primiparae and 32 multiparae women (Table 3). These two groups showed significant age differences (\( p < 0.05 \); Table 3), the median
differences being 6 years. The age of the oldest breastfed infant was 325 days. There
were no significant differences between the infant ages of primiparae and multiparae
mothers (Table 3).

3.2. OCs concentrations in breast milk

4,4’-DDT was found in all samples of the 2002 group except one. Breast milk of all
mothers from this group contained 4,4’-DDE above the limit of detection. 4,4’-DDD
was only found at detectable levels in six samples. The 2,4’- isomers of DDE, DDD and
DDT were of lower concentration than the 4,4’- isomers. They were found above the
limit of detection in 20%, 5% and 87.5% of the samples, respectively. All mothers in
the 2006 group had detectable 4,4’-DDT levels and all had detectable 4,4’-DDE levels
except one multiparae. More than half of the mothers from this group had detectable
4,4’-DDD levels. In this group, the concentration of the 2,4’- isomers for DDE, DDD
and DDT were also lower than the concentrations of the 4,4’- isomers. The percentage
of samples with detectable levels of 2,4’-DDE, 2,4’-DDD and 2,4’-DDT were 42%,
15% and 94%, respectively.

The median concentrations of 4,4’-DDE, 4,4’-DDT and 4,4’-DDD were higher in the
2006 than in the 2002 group (Table 1) and according to the U Mann-Whitney test the
differences were statistically significant (p < 0.05) for 4,4’-DDE and 4,4’-DDT (Fig. 1).
4,4’-DDD was not tested due the small number of samples with detectable amounts in
year 2002. The median of $\sum$DDT increased significantly from 3.9 ng/$\mu$l in 2002 to 11
ng/$\mu$l in 2006 (p = 0.002) (Table 1; Fig 1). The highest observed value was 200 ng/$\mu$l in
2006.

The median concentration of 4,4’-DDE in the 2002 group was higher than the median
concentration of 4,4’-DDT (Table 1). The 4,4’-DDE/4,4’-DDT ratio was 2.7. In the
2006 group, the median 4,4’-DDE was also higher than the median 4,4’-DDT and the
difference between the two was about the same, with a 4,4’-DDE/4,4’-DDT ratio of 2.8.
In the 90th percentile of the 2002 group, the 4,4’-DDE/4,4’-DDT ratio was still the same
(2.6). In the 2006 group this ratio was smaller (2.3) indicating higher relative abundance
of 4,4’-DDT. The 90th percentile of the 2006 group was composed of five samples and
only the three with highest $\sum$DDT concentration had higher 4,4’-DDT content than 4,4’-
DDE. In contrast, all the samples from the 90th percentile of the 2002 group were dominated by 4,4′-DDE.

The primiparae had higher levels of all 4,4′- isomers and $\sum$DDT than the multiparae (Table 3). The Mann-Whitney U test showed significant differences for 4,4′-DDT, 4,4′-DDE and $\sum$DDT between these two maternal groups (Table 3). The Mann-Whitney U test showed the same significant differences for 2,4′-DDT and 2,4′-DDE but not for 2,4′-DDD (Table3).

Table 4 shows the comparison of the concentrations of OCs in human breast milk found in this study with other studies reported in the literature.

4. Discussion

4.1. DDT concentrations and indoor residual spraying

The present study describes for the first time the DDT concentrations of breast milk samples in a rural population from Mozambique where DDT was reintroduced by IRS. Breast milk concentrations of OCs in 2006 were significantly higher than in 2002, the median of $\sum$DDT in 2006 being 2.9 times higher than in 2002. IRS with DDT is the main likely cause for the increase of this insecticide in maternal milk samples. These results are consistent with observations in other areas. Bouwman et al. (1990) compared two different groups of breast milk samples, one from an area where DDT was used for malaria vector control in Kwa-Zulu, South Africa, and another from an area without malaria transmission which was used as reference. The observed values of $\sum$DDT from the exposed group were higher than those of the non exposed group and the increase ranged between 1.1-1.5 times. This lower increase compared to Manhiça may be due to the high $\sum$DDT concentrations that were already observed in the former location before this IRS episode, e.g. 12,000 ng/g lipid (Bouwman et al., 1990). Obviously, if the baseline concentrations of this compound are low, the relative DDT increases by IRS are noticed to a higher extent.
The maternal insecticide increase between 2002 and 2006 in Manhiça is about the same when considering either single 4,4’-DDE (3 times), 4,4’-DDT (2.8 times) or 4,4’-DDD (2.8 times) as $\Sigma$DDT. Thus, IRS with a mixture largely predominated by 4,4’-DDT leads to a uniform increase of both the active compound and its main metabolite (4,4’-DDE) showing an important degree of transformation of the former into the latter at the initial stages of insecticide incorporation.

Furthermore, comparison of the concentrations defining the 90th percentile of $\Sigma$DDT, 4,4’-DDE and 4,4’-DDT in the 2002 and 2006 groups show increases of 1.6, 1.7 and 1.9 times, indicating a lower relative rate of DDT transformation among the individuals that received the highest DDT doses. As mentioned above, in the 90th percentile of the 2006 group, only the three samples with the highest $\Sigma$DDT concentration had higher concentration of 4,4’-DDT than 4,4’-DDE and this latter compound predominated in all the samples from the equivalent percentile of the 2002 group.

Besides the general median increase in DDT compounds between 2002 and 2006 (from 2.8 to 3 times), comparison of the median in 2002 and the 90th percentile in 2006 shows increases of 11, 12, 14 and 11 times for $\Sigma$DDT, 4,4’-DDE and 4,4’-DDT and 4,4’-DDD, respectively. These increments show that in the context of this general increment of DDT concentrations in breast milk during the first campaigns of IRS, women were unevenly exposed showing significant differences between DDT intakes.

Nevertheless, comparison of the median DDT concentrations of the 2006 group (280 ng/g lipid) with previous studies reported in the literature (Table 4) shows that those from Manhiça are comparable to those of populations with low exposure. In fact, the median $\Sigma$DDT concentrations of the 2002 group in Manhiça samples is very low when compared to other populations. Thus, assuming a similar average milk fat content as in 2006, the median concentration of $\Sigma$DDT (Table 1) corresponds to 99 ng/g lipid which ranges among the cases with lowest median $\Sigma$DDT in the countries reported. Diverse socio-economic aspects may explain these low DDT levels in the studied population, such as commercial trading difficulties due to the war and the implementation of programs using insecticides other than DDT soon after this conflict was ended.
Accordingly, despite the general median increase in DDT compounds at the onset of IRS, the values observed in Manhiça are still low in comparison with other populations. Only the sample exhibiting highest $\sum$DDT concentrations (5100 ng/g lipid), has concentrations in the range of the median values found in populations routinely using DDT for malaria vector control. The sample with second highest $\sum$DDT concentrations (2000 ng/g lipid) had lower values. These results illustrate that in this context of initial low DDT exposure, the general increase of DDT in breast milk due to IRS does not involve average increments leading to concentrations that are characteristic of highly exposed populations, except in few cases.

4.2. DDT concentrations and parity

Significant differences were found between primiparae and multiparae mothers for the concentrations of 4,4’-DDE, 4,4’-DDT, 2,4’-DDE, 2,4’-DDT and $\sum$DDT, with higher concentrations in the former than the latter. The highest value of $\sum$DDT was obtained from a primiparae mother aged 20 years. These results suggest that the concentrations of $\sum$DDT tend to decrease in human breast milk with increasing number of children nursed. Significant differences in the accumulation of $\sum$DDT with parity were also found in a series of breast milk samples collected in diverse cities of Tunisia between 2003 and 2005 (n = 231; Ennaceur et al., 2008). Lower $\sum$DDT concentrations were continuously observed at increasing number of children between 1 and 4. Similarly, $\sum$DDT were observed to be significantly lower in breast milk from multiparae than primiparae women of Hochiminh (Vietnam; n = 44; Minh et al., 2004), and the same difference was observed in Hanoi (Vietnam) but the difference was not statistically significant (n = 42; Minh et al., 2004). Significant differences (p < 0.01) have also been found for the concentrations of 4,4’-DDE in Norwegian mothers (n = 377; Polder et al., 2009), and breast milk of primiparae which contained this compound in higher concentrations than multiparae. 4,4’-DDE concentrations were also higher in breast milk of primiparae than multiparae women from Buryatia (Russia) but the differences were not statistically significant (Tsydenova et al., 2007). However, in this population concentrations of 4,4’-DDT and 4,4’-DDD were higher among multiparae than primiparae women (n = 33; Tsydenova et al., 2007). In contrast, higher concentrations of 4,4’-DDE and $\sum$DDT have been observed in primiparae than multiparae women from Zimbabwe (n = 68; Chikuni et al., 1997).
In the present study higher concentrations of $\sum$DDT, 4,4’-DDT and several metabolites were found in primiparae than multiparae women and the differences were statistically significant. The age differences between the two maternal groups were also statistically significant. As expected, primiparae were younger than multiparae, with median ages 19 and 25 years, respectively. There is an age dependence on the accumulation of OCs. Older women have higher body burden of these compounds than younger women (Rhainds et al., 1999; Sala et al., 2001; Carrizo et al., 2006). This significant age difference between the two maternal groups should be reflected in higher concentrations of DDT compounds and metabolites in the multiparae group. The observation of higher concentrations of these chemical species in the primiparae group indicates that parity overcomes the difference due to age in the studied population of women from Manhiça. The lipophilic nature of the DDT compounds, the lipid mobilization from fat depot in adipose tissue to breast milk, and the excretion through breast feeding may explain the observed decrease of DDT compounds with parity. Upon child birth and breast feeding these compounds are transferred outside the maternal body and this is reflected in lower levels of them in breast milk (Ejobi et al., 1998; Harris 2001).

As mentioned above, in utero exposure to 4,4’-DDT at low concentrations may lead to infant decreases of cognitive skills (Ribas-Fito et al., 2006; Morales et al., 2008) and alterations of thyroid hormones (Alvarez-Pedrerol et al., 2008a,b). In utero exposure to 4,4’-DDE has been observed to be related with increases of asthma (Sunyer et al., 2005; 2006) and increases in urinary coproporphyrins (Sunyer et al., 2008). These antecedents recommend the implementation of monitoring surveys for assessment of the health effects from the use of DDT in Manhiça population already at the initial period of IRS.

5. Conclusion

The use of DDT for IRS increased the general DDT breast milk concentrations of 4,4’-DDT and its main metabolite, 4,4’-DDE, in mothers from the Manhiça district already in the first stages of implementation of an IRS program. However, the observed median for 2006 is still low in comparison to breast milk concentrations in many world populations. The observed increases were not uniform and in some individuals the
measured breast milk concentrations were high with respect to levels previously described in other sites. These individuals with highest $\sum$DDT concentrations had higher 4,4’-DDT than 4,4’-DDE suggesting some degree of saturation of the metabolic transformation mechanisms. Breast milk concentrations of most DDT compounds were significantly higher in primiparae than multiparae women. The difference is consistent with the transfer of maternal body burden of DDT and its metabolites to infants, either through placenta or by breastfeeding. These results and previous studies on deleterious health effects of in utero low doses of DDT in infants recommend the implementation of monitoring surveys for assessment of the health effects from the use of DDT already at the initial period of use. These studies and surveys of the changes in malaria incidence due to DDT application will provide a fair balance of the advantages and drawbacks of the use of this insecticide for protection of human health.

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