Population characteristics of young African women influencing prenatal exposure to DDT (Manhiça, Mozambique)

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Abstract The concentrations of DDT compounds in cord blood of 214 children born between 2003 and 2006 in Manhiça (Mozambique) have been determined. In this time interval corresponding to the period before DDT reintroduction for indoor residual spraying the observed values averaged 0.8 and 0.4 ng/ml for 4,4'-DDE and 4,4'-DDT, respectively, and were similar to those found in western countries. However, the 4,4'-DDT/4,4'-DDE ratio was high indicating that the inputs of these compounds arriving to children *in utero* originated from recent uses of the insecticide. The strongest factor affecting DDT concentration was parity. A well-defined decreasing concentration trend was observed for the cord blood concentrations in the period of study. The trend was also observed for multiparae and primiparae mothers independently. Children from multiparae women showed much lower concentrations than primiparae women. Children from mothers with secondary school level exhibited lower concentrations of these pesticides than mothers with lower degree of education.

Key words DDT, cord blood concentrations, malaria vector control, parity, gender, temporal DDT trends.

Introduction

DDT started to be widely used as insecticide in the 40s, leading to the accumulation of 4,4'-DDT and its metabolites in many organisms. Evidence of the lipophilicity, high resistance to degradation and toxic effects of this insecticide for the environment and humans led to ban it for agricultural practices in the 70s. Later, it was included in the Stockholm agreement which banned an important number of persistent organochlorine pollutants.

In Africa, where malaria killed 750,000 million people in 2009, more than two dozen countries requested exemptions on the ban of DDT for malaria vector control on the evidence that this compound was the most effective insecticide due to its persistence, relatively low cost 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 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 (Mabasso et al. 2004; Maharaj et al. 2005).

However, DDT monitoring in the early human age is important. During pregnancy these compounds are transferred from mother to fetus through the placenta (Sala et al. 2001). Fetuses are more vulnerable than adults as their immune systems and detoxification mechanisms are not fully developed. DDT may therefore cause damage and may predispose to prospective health problems (Gladen et al. 1988), such as delays on the cognitive development in children during their first years of life (Ribas-Fito 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; 2008) and increases in urinary coproporphyrins (Sunyer et al. 2006).

A global assessment of the benefits and drawbacks of the DDT reintroduction for malaria vector control is needed. Besides frequencies and modes of application, population features may also be relevant for the transfer of this insecticide into

newborns. These aspects have been considered in cohorts from western countries but information from populations with other characteristics is scarce. Mozambique provides a representative case of young African populations. DDT was banned in this country 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 prior to reintroduction of IRS for public health policies.

The present study focuses on establishing the levels of DDT and its metabolites in human cord blood in Manhiça, a rural area located south of the country. Samples were collected between 2003 and 2006 before IRS. This information will be useful for assessment of the population features influencing on the intake of this compound in newborns. The results will contribute to obtaining balanced cost-benefit estimations of the use of DDT for malaria vector control or other applications.

Materials and methods

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.

Sample collection

This is a cross-sectional study based on 214 umbilical cord samples from infants born between 2003 and 2006 at the Manhica Health Centre in the context of several birth cohorts conducted at the Centro de Investigação em Saúde da Manhiça (CISM). In the case of multiparous women, only samples corresponding to one delivery were collected.

All mothers signed an approved consent before they were enrolled in the study. Information on maternal age, parity, educational level, and infant sex was obtained from the study data base. For the specific objective of the present study, mothers were revisited in their households and asked for a new consent. Each participant signed a new informed approval form allowing to use the remaining samples for the present study.

The research protocol was approved by the Ethics Committees of Mozambique and Hospital Clinic in Barcelona, Catalonia, Spain.

Samples were stored at –20°C at CISM and later sent to the Institute of Environmental Assessment and Water Research (IDÆA-CSIC) for analysis.

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.

Extraction procedures

Volumes of 0.5–1 ml of cord blood 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 twice 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 μl each). Finally, it was re-evaporated under the nitrogen stream and 100 μl of PCB142 were added as internal standards before injection.

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 μl) 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^{o}C (held for 2 min) and increased to 140^{o}C at 20^{o}C/min, then to 200^{o}C (held for 13 min) at 4^{o}C/min and finally to 310^{o}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). Identification of organochlorine compounds (OCs) 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.

Data analysis

The results were reported by reference to fresh weight (ng/ml). Concentrations below LOQ were substituted by half of the LOD. ∑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). The parity was defined as primiparous (women who had a first child) and multiparous (women who had more than one child). The concentration values were $\log_{10}$ transformed for normalization. Student's *T-test* was used for comparison of DDT levels for variables with two categories while *F test* for one way ANOVA was carried out when the variable was divided into three categories. 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).

Results

Participant profile

The characteristics of the maternal and newborn participants are described in Table 1. Maternal ages ranged between 14 and 43 years (median 22.8 years). Maternal education level was mostly primary school (74%), 16% were illiterate and 10% had

secondary studies. This information was missing in 18% of the cases. The proportion of multiparous mothers was higher than primiparous mothers, 74% and 26%, respectively. On an annual basis, the recorded annual percentages were 77% and 23% in 2004, 65% and 35% in 2005, and 75% and 25% in 2006. This information was missing in 15% of the cases. The missing data were due to absence of appropriate records in the interviews of the original cohort studies. There was no overlap between individuals lacking educational or parity data. Obviously, association analyses between these characteristics and concentration levels excluded the newborns with missing data which were different in the two studies.

DDT concentrations in cord blood

4,4'-DDE, 4,4'-DDT and 4,4'-DDD and their 2,4'- isomers were analyzed in 214 cord blood samples. The 4,4'- isomers were found in 96% (DDE), 95% (DDT) and 28% (DDD) of the samples. Due to the low occurrence of 4,4'-DDD and 2,4'- isomers, these were not included in the statistical analysis. As shown in Table 2, means ± standard deviations of 4,4'-DDE and 4,4'-DDT were 0.8 ng/ml ± 0.9 ng/ml and 0.4 ng/ml ± 0.6 ng/ml, respectively. Median 4,4'-DDE and 4,4'-DDT values were 0.5 and 0.2 ng/ml, respectively. These concentrations are similar to those found in newborns from cohorts of western countries in which DDT has not been used as insecticide for agricultural applications in the last 25 years, e.g. mean 4,4'-DDE values of 0.82 ng/ml in Valencia (Vizcaino et al. 2010) (Table 3) and they are higher than those reported in newborns from remote sites in which, presumably, this insecticide has rarely been used, e.g. 0.53 and 0.41 ng/ml in Artic (Butler et al. 2003) and Quebec (Canada) (Rhainds et al. 1999), respectively (Table 3). Mean cord blood concentrations in newborns from cohorts recruited in areas of regular use of DDT for control of the Malaria vector such as Oaxaca (Barraza-Vazquez et al. 2008), Veracruz (Mexico) (Waliszewski et al. 2001) or New Delhi (Pathak e al. 2009) (India) exhibit much higher values than those found in the Manhiça cohort considered in the present study (Table 3).

These results are consistent with the history of the use of DDT in Mozambique which started with an extensive application for agriculture and health in the 1940s and ended in 1988. However, the mean concentrations of 4,4'-DDE and 4,4'-DDT in the cohort of Manhiça exhibit a proportion of 4,4'-DDT (33%) that is higher than those found in remote sites (0-0.05%), western countries (0-10%) or areas with regular use of DDT for control of malaria vectors (12-32%) (Table 3). This high proportion of 4,4'-

DDT indicates that despite its generally low concentration values the contribution of the insecticide arriving to children *in utero* was recent, e.g. from applications within a few years before cord blood collection. The origin of these applications is not related to the use of this insecticide for malaria control since the official IRS program in the region started by the end of 2006.

Discussion

Gender differences

The average cord blood 4,4'-DDE and 4,4'-DDT concentrations in male and female newborns are shown in Table 4. In both cases, female newborns (0.9 ng/ml and 0.5 ng/ml, respectively) had higher concentrations than males (0.7 ng/ml and 0.4 ng/ml, respectively) and the differences were statistically significant for 4,4'-DDE ($p = 0.01$). Conversely, in a previous study of a cohort from Menorca ($n = 410$, 49% male), gender comparison of cord blood levels showed higher 4,4'-DDE mean concentrations in male (1.8 ng/ml) than female (1.6 ng/ml) and higher mean 4,4'-DDT concentrations in female (0.2 ng/ml) than male (0.1 ng/ml) but the results were not statistically significant (Grimalt et al. 2010). The population number in the present study is about half (Table 1) and the results for 4,4'-DDE are statistically significant.

DDT concentrations and maternal characteristics

Cord blood concentrations were averaged by the three maternal education level groups (Table 1) and the significance of the differences was compared with the F test. The group of mothers who received secondary education showed lower 4,4'-DDE and 4,4'-DDT cord blood concentrations than the groups with lower degree of education. The difference was significant ($p = 0.02$) for 4,4'-DDT. No difference was observed between the groups of mothers having primary school and those illiterate. These results differ from other observations. In Valencia ($n = 499$; Spain), the mothers with higher education level (university degrees) had children with higher concentrations of OCs in cord blood, including DDT and its metabolites (Vizcaino et al. 2010). In this case, the trend was attributed to diet, namely fish consumption (Vizcaino et al. 2010). The reverse trend in Mozambique may reflect different dietary habits or life style than in Spain.

Primiparous mothers had significantly higher concentrations of 4,4'-DDE in cord blood than multiparous mothers ($p = 0.001$; Table 4). 4,4'-DDT also showed the same trend but the difference between the two groups was not statistically significant ($p = 0.07$; Table 4). These results are consistent with one previous study in Oaxaca (Mexico) in which multiparous mothers delivered children with lower 4,4'-DDE concentrations than primiparous mothers (Barraza-Vazquez et al. 2008). Lower concentrations of 2,4'-DDT, 4,4'-DDT, 4,4'-DDD, 2,4'-DDE and 4,4'-DDT in multiparous than in primiparous women were observed in a cohort study from Hokkaido (Japan, n = 186) but the differences were not statistically significant (Kanazawa et al., 2012). The same differences between multiparous and primiparous mothers were also observed in breast milk concentrations of DDT compounds in Tunisia (2003-2005; n = 231) (Ennaceur et al. 2008), Hochiminh and Hanoi (Vietnam; n = 42 in each city) (Minh et al. 2004), Norway (2002-2006; n = 377) (Polder et al. 2009), and for 4,4'-DDE in Buryatia (Russia) (Tsydenova et al. 2007). 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. Furthermore, during pregnancy lipids and lipoproteins are transferred from maternal tissues to fetus through the placenta. This process results in carry-over of organochlorine compounds through the placenta and their presence in lipid-rich tissues of the fetus (Waliszewski et al., 2000). Upon child birth and breast feeding these compounds are therefore transferred outside the maternal body and this is likely reflected in lower concentrations in cord blood of forthcoming newborns.

No relationship was observed between maternal age and cord blood concentrations of DDT species. Older women had higher body burden of these compounds than younger women (Rhainds et al. 1999; Sala et al. 2001; Vizcaino et al. 2010;) and, in some cases, brought to live children with higher OC cord blood levels (Rhainds et al. 1999; Sala et al. 2001; Carrizo et al. 2007; Vizcaino et al. 2010; Kanazawa et al., 2012). The lack of age dependence observed in Manhiça is likely due to the higher proportion of multiparae women in the older age group (average ages in primiparae and multiparae 18.7 and 26.0 years, respectively).

Age distribution and parity of the cohort mothers involves main differences between western countries and the population considered in the present study. For instance, in Valencia, where a strong maternal age dependence of the OC concentrations in cord blood was observed, the median age was 30 years (age range 16-43 years) and

55% mothers were primiparae and 45% multiparae. In Manhiça, mothers were much younger, median 22.8 years (age range 15.5-43.4 years), and the proportion of multiparae women (68%) was much higher than primiparae women (32%). In comparison, age was having less weight in this last cohort than in Valencia whereas the relative weight of parity was the opposite.

Temporal trends

Examination of the average 4,4'-DDT and 4,4'-DDE cord blood concentrations of the newborns included in the study shows a well defined decreasing trend for both compounds (Table 2). 4,4'-DDE concentrations declined steadily all years, from 1.6 ng/ml in 2003 to 0.6 ng/ml in 2006. 4,4'-DDT concentrations also decreased, from 0.7 ng/ml in 2003 down to 0.3 ng/ml in 2006, although the mean concentrations in 2004 (0.8 ng/ml) were a bit higher than in 2003. This small increase between these two years was not significant considering the low number of participants in 2003 (n = 5). The medians of these concentration values showed the same trends (Table 2). The ratio between 4,4'-DDT and 4,4'-DDE also showed a decreasing trend between 2004 and 2006. These trends are similar to those observed in Hokkaido from whole blood samples collected between 2002 and 2005 (Kanazawa et al., 2012). All these data reflect higher exposure to DDT in earlier dates than in the more recent period.

Examination of the temporal trends between 2004 and 2006 for the groups of primiparae and multiparae mothers shows the same decreasing pattern (Fig. 1). In all years, primiparae mothers exhibited higher concentrations of 4,4'-DDE and 4,4'-DDT than multiparae mothers. However, the temporal evolution was towards decreasing concentration differences in both groups.

In contrast, the study of the 4,4'-DDT/4,4'-DDE ratio showed significant differences between primiparae and multiparae mothers in 2004 and 2005 being considerably higher in the multiparous group (although the differences were not significant at $p < 0.05$; Fig. 1). Considering that the group of multiparae mothers was older than the group of primiparae mothers (24-27 years and 18-19 years, respectively; $p < 0.05$), the difference may reflect higher exposure to 4,4'-DDT in the past which was probably more relevant in older women. This group, despite their high detoxification rate as consequence of multiple delivers and previous breast feeding periods, still had a body burden of DDT species that reflects higher exposure to the original insecticide compound. Then, in 2006, the average 4,4'-DDT/4,4'-DDE ratio of primiparous and

multiparous mothers showed no significant difference between the two groups which may reflect the increased detoxification trend of the latter.

Limitations

The main limitation of this study concerned sample availability. Samples were obtained from already existing cohort samples organized by CISM. This limited the number of cases for some year periods.

Conclusions

The average 4,4'-DDE and 4,4'-DDT cord blood concentrations in the population of Manhiça (Mozambique) measured before IRS were similar to those found in newborn cohorts of western countries. However, the ratio between 4,4'-DDT and 4,4'-DDE was high indicating that despite these generally low concentrations the inputs of these compounds arriving to children *in utero* were recent. Parity was the strongest factor affecting DDT concentrations. Children from multiparae women showed significantly lower concentrations than primiparae women. In other cohorts a direct dependence between maternal age and cord blood concentrations of these compounds is observed but in the young community of mothers from Manhiça parity overcomes the age effect. Children from mothers with higher degree of education (secondary school) had lower concentrations of these pesticides than illiterate mothers or those having primary studies.

A well defined decreasing concentration trend is observed for the cord blood concentrations between 2003 and 2006. This trend is also observed for multiparae and primiparae mothers separately. However, the 4,4'-DDT/4,4'-DDE ratio is higher in the former than in the latter group. The higher ratios in multiparae women, who are generally older than primiparae women, are consistent with a higher exposure to the insecticide further back in time in mothers having other children as consequence of the detoxification processes involved in maternal activities (deliver and breastfeeding).

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Table 1: Characteristics of the participants (N=214)

	n	%
Maternal age		
Median	22.8	
Range	14-43	
Newborn sex		
Male	104	49
Female	110	51
Maternal education level		
Illiterate	28	16
Primary	129	74
Secondary	18	10
Missing data	39	
Pregnancy		
Primiparous	48	26
Multiparous	134	74
Missing data	32	

Table 2: Cord blood plasma DDE and DDT concentrations (ng/ml) in newborns from Manhiça.

	4,4'-DDE	4,4'-DDT	totalDDT	4,4'-DDT/4,4'-DDE
2003 (n = 5)				
Mean (SD)	1.5 (0.7)	0.7 (0.8)	2.3 (1.1)	0.5 (0.5)
Median	1.4	0.3	2.3	0.3
Range	0.5 - 2.2	0.1 - 2.0	0.7 - 3.4	0.2 - 1.5
*p95	2.2	2.0	3.4	1.5
2004 (n = 37)				
Mean (SD)	1.2 (1.3)	0.8 (0.7)	2.0 (1.9)	0.7 (0.4)
Median	0.8	0.5	1.3	0.6
Range	0.1 - 5.6	0.08 - 2.4	0.2 - 7.4	0.2 - 2.0
p95	4.6	2.2	7.2	1.6
2005 (n = 54)				
Mean (SD)	1 (1.1)	0.6 (0.7)	1.6 (1.8)	0.6 (0.4)
Median	0.6	0.3	1	0.4
Range	0.09 - 6.3	0.02 - 3.4	0.1 - 8.5	0.08 - 1.9
p95	4	2.5	7.1	1.3
2006 (n = 118)				
Mean (SD)	0.6 (0.5)	0.3 (0.4)	0.8 (0.8)	0.4 (0.4)
Median	0.4	0.2	0.6	0.3
Range	0.04 - 4.0	0.02 - 2.8	0.08 - 4.5	0.04 -1.6
p95	1.3	0.9	2.4	1.1
Total (n = 214)				
Mean (SD)	0.8 (0.9)	0.4 (0.6)	1.3 (1.4)	0.5 (0.4)
Median	0.5	0.2	0.8	0.4
Range	0.04 - 6.3	0.02 - 3.4	0.08 - 8.5	0.04 – 2.0
p95	2.6	1.8	4.5	1.4

*p95: percentil 95

Table 3. Comparison of the arithmetic mean concentrations of OCs in umbilical cord blood plasma from Manhiça (Mozambique) and those reported in previous studies.

Area of study	N	Period of delivery	4,4'-DDE[a] ng/ml	4,4´-DDT[a] ng/ml	Reference
Oaxaca (Mexico)	86	2000	7540[a]	2370[a]	Barraza-Vazquez et al. 2008
Veracruz (Mexico)	60	1997-1998	6.0	0.8	Waliszewski et al. 2001
New Delhi (India)	23	2006-2008	1.98	0.93	Pathak et al. 2009
Menorca (Balearic Islands, Spain)	410	1997-1998	1.6	0.18	Carrizo et al. 2006
Ribera d'Ebre (Catalonia, Spain)	73	1997-1999	1.2	0.13	Carrizo et al. 2006
Chukotka (Russia)	48	2001-2002	0.89	na	Eik Anda et al. 2007
Valencia (Spain)	499	2004-2006	0.82	0.08	Vizcaino et al. 2010
Manhiça (Mozambique)	214	2003-2006	0.80	0.4	this study
Rio de Janeiro (Brasil)	10	1997-1998	0.76	nd	Sarcinelli et al. 2003
Artic (Canada)	407	1994-1999	0.53	0.03	Butler Walker et al. 2003
Québec (Canada)	656	1993-1995	0.41[a]	na	Rhainds et al. 1999
Brescia (Italy)	70	2006	0.25[a]	nd	Bergonzi et al. 2009

[a] geometric mean; na: not analyzed; nd: not detected

Table 4: Mean organochlorine compounds in cord blood grouped by newborn gender, parity and maternal education level.

	Category (n)	4,4'-DDE (ng/ml)	4,4'-DDT (ng/ml)	totalDDT (ng/ml)
Gender*	male (104)	0.7	0.4	1.1
	female (110)	0.9	0.5	1.4
	p value	0.01	0.19	0.02
	IC	-0.23 – -0. 03	-0.24–0 .04	-0.23 – 0.02
Parity*	primiparous (50)	1.12	0.5	1.7
	multiparous (149)	0.7	0.4	1.2
	p value	0.001	0.073	0.003
	IC	0.09– 0.33	-0.01– 0.3	0.07– 0.32
Maternal education level**	illiterate (28)	0.8	0.3	1.1
	primary (129)	0.8	0.4	1.2
	secondary (18)	0.5	0.2	0.7
	F	1.05	4.29	1.86
	p value	0.35	0.02	0.16

*t student test; **Multiple comparisons using the F test; IC: confidence interval

FIGURE CAPTION

Figure 1. Concentrations of 4,4’-DDE, 4,4’-DDT, total DDT compounds and 4,4’-DDT/4,4’-DDE ratios in cord blood of children born between 2004 and 2006 in Manhiça (Mozambique). Vertical bars describe standard error.