Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa*

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ABSTRACT

Exemption was granted by the Stockholm Convention in 2004 for use of DDT by indoor residual spraying (IRS) as a malaria vector control. South Africa endorsed the use of DDT in its Malaria Control Programmes in malaria endemic regions and IRS remains a primary method of controlling malaria transmitting mosquitoes in this country. This study examines the impact of IRS on the levels of DDT and its metabolites in maternal blood of delivering women as a measure of prenatal exposure.

We report on the concentrations of DDT and its metabolites (*p,p'*- DDE*, p,p'*-DDT, *o,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, and *o,p'*-DDT) in maternal plasma of 255 delivering women residing in three sites along Indian Ocean, namely in malaria endemic where IRS takes place, low risk and non-malaria sites.

Concentrations of measured compounds were found to be significantly higher in the malaria endemic site (p =0.0001): the geometric mean concentration (95% confidence intervals; n=91) for *o,p'*-DDE was 9 ng/g lipids (7-10); for *p,p'*-DDE, 3840 ng/g lipids (3008-4902); for *o,p'*-DDD, 8 ng/g lipids (6-9); for *p,p'*-DDD, 26 ng/g lipids (20-32); for *o,p'*-DDT, 168 ng/g lipids (127-221) and for *p,p'*-DDT, 2194 ng/g lipids (1706-2823). These compounds were also detected in women residing in other sites but in lower concentrations.

The maternal characteristics, age, IRS, number of children and breastfeeding were significantly associated for both *p,p'*-DDE and *p,p'*-DDT levels in the malaria area where exposure through IRS is predominant. There was no association between maternal characteristics and DDT levels in the low risk and non-malaria area.

Results presented are of particular value to the policy decision makers and regulatory toxicology organizations as they characterise the extent of controlled exposure to DDT used exclusively for IRS purposes.

Furthermore, findings of this study will form a base for further investigation of foetal exposure to pollutants.

Key words: DDT, Malaria, Indoor Residual Spraying, prenatal exposure

1. Introduction

Use of DDT [1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane] for malaria vector control, remains a subject of intense scientific and public debate due to its persistence in the environment, its ability to accumulate in the food chain and travel long distances, its affinity for bioaccumulation but also its potential negative health impacts to animals and humans (AMAP, 2004; Porta et al., 1999).

Malaria accounts for 247 million annual cases globally and remains the second highest cause of mortality and morbidity on the African continent with 91% of reported global deaths from malaria occurring in the sub-Saharan Africa region (Jaga and Dharmani, 2003; WHO, 2008).

Available epidemiological data on human health effects of DDT and other pesticides show that they are similar to those found in animals, affecting neurodevelopment, causing endocrine disruptions, as well as producing immune related conditions and asthma (Arndt et al., 1999; Eskenazi et al., 2006; Longnecker et al., 2007; Narita et al., 2007; Sunyer et al., 2005; van Wendel de Joode et al., 2001). Other studies indicate that DDT may be implicated in liver and pancreatic cancer aetiologies (Rogan and Chen, 2005). There is also emerging evidence associating exposure to DDT with leukaemia, lymphoma and testicular cancers (Eskenazi et al., 2009). It has been reported that pre-puberty exposure to DDT increases the risk of developing breast cancer in adulthood (Cohn et al., 2007). A number of studies also showed dose-response relationships between serum concentrations of *p,p'*-DDT and the prevalence of diabetes (Everett et al., 2007; Lee et al., 2006; Rignell-Hydbom et al., 2007).

The most vulnerable window for toxic impact of environmental pollutants is the embryonic and foetal stages. Therefore, of major concern is the possible impact of exposure to DDT on reproductive health including birth outcomes (Eskenazi et al., 2009; Fernandez et al., 2007). It has been reported that *in utero* exposure to DDT can negatively affect the neurodevelopment in childhood stage, resulting in behavioural problems, decreased attention span at infancy, retarded psychomotor development and decreased cognitive function (Ribas-Fito et al., 2006; Sagiv et al., 2008; Torres-Sanchez et al., 2007). There is evidence that exposure to DDT *in utero* may also affect thyroid hormone levels (Alvarez-Pedrerol et al., 2008; Nagayama et al., 2007; Schell et al., 2008). Inconsistent evidence is emerging that high blood concentration of DDTs may affect height, body mass index and other measures of growth in children (Eskenazi et al., 2009). Furthermore, after birth women pass DDT to their babies via lactation (Bouwman et al., 2006).

Based on past and recent research findings confirming the detrimental effects of DDT exposure on human health, the governments and regulatory agencies banned its use and production. In the year 2004, the Stockholm Convention on Persistent Organic Pollutants (POPs) outlawed the use of 12 industrial organic chemicals including DDT (UNEP, 2005).

However, limited exemption was granted for use of DDT to control malaria vector through indoor residual wall spraying (IRS) in malaria endemic regions only, because of the

resurgence of malaria in many countries and lack of similarly effective alternatives and methods. It is estimated that about 25 countries including South Africa, are currently using DDT for IRS which remains a primary means of controlling malaria-transmitting mosquitoes (Kapp, 2004).

In South Africa, in 1996, partial replacement of DDT use for IRS with pyrethroids and other compounds considered to be less toxic resulted in the reappearance of severe malaria outbreaks and by the year 2000, almost 65 000 cases of malaria were diagnosed and 458 deaths occurred countrywide (Figure 1) (DOH, 2010). After the reintroduction of DDT use for IRS in 2001, reported malaria cases in South Africa began to decrease almost immediately with malaria admissions and deaths decreasing by 89% and outpatient malaria cases by 85% (O'Meara et al., 2010). At present, approximately 10% (5 million) of the South African population is at risk of contracting malaria by residing in malaria endemic areas (Rogan and Chen, 2005).

As expected, the re-introduction of DDT for vector control has again intensified public and scientific debate about its toxicity. Of special concern is the exposure of children *in utero* and after birth through breastfeeding and through living in a contaminated environment (Bouwman and Kylin, 2009; Ostrea et al., 2009; Sapbamrer et al., 2008).

In response to the paucity of comprehensive data on levels of DDT and its metabolites in populations residing in malaria endemic region where the IRS with DDT programme is on-going, a study was designed and carried out under the auspices of Norway-South Africa Bilateral Research Collaboration, the South African Medical Research Council, the Norwegian Institute for Air Research and the University of Tromsø, Norway during the summer of 2008.

The main objective of this study was to assess the exposure to DDT as a result of the IRS programme. The study investigated the extent of prenatal exposure to DDT and its metabolites by measuring its concentrations in blood plasma of delivering women residing in three Indian Ocean coastal regions of KwaZulu-Natal Province of South Africa, namely: the malaria endemic region where IRS is actively taking place; low risk malaria region and non-malaria region.

Data obtained will also inform policy decision makers in South Africa and elsewhere as well as the Stockholm Convention, WHO and other global initiatives involved in elimination of the malaria burden.

2. Materials and methods

2.1 Study sites and population

The study took place along the coastal line of the Indian Ocean in the KwaZulu-Natal Province in South Africa. Three sites were selected, namely: malaria endemic where regular IRS is taking place; low risk malaria site and non - malaria site.

The malaria endemic site is situated on the north eastern area of the KwaZulu-Natal Province, on the border of Mozambique. The low risk malaria area is situated about 100 km south of the endemic malaria site and about 160km north of the city of Durban. The non - malaria site is situated on the Southern Coast of KwaZulu-Natal (about 300 km away from low risk and 400 km away from malaria endemic sites). Figure 2 shows the exact locations of each study site within South Africa. Sample collection took place at all study sites during summer months of 2008.

2.2 Recruitment of participants and informed consent

Potential participants were women who presented and were admitted for delivery at the local hospitals in three study sites by a health worker on duty and a trained research assistant. The only exclusion factor was for women who resided less than 10 years in the areas studied. In total 255 participants were studied: 91 subjects resided in malaria endemic region where IRS is taking place, 47 subjects resided in low risk malaria region and 117 Subjects resided in non-malaria region.

Women who volunteered to participate signed an informed consent form and agreed to donate a blood sample before delivery and answer a socioeconomic questionnaire by interview in the language of their choice and allow access to their post-partum records (delivery outcomes and eventual complications). All participants understood that their confidentiality was assured and that overall results would be published to contribute towards national and international data. Study subjects were also informed that if their individual results were found to be of medical concern, they would be informed and referred to an appropriate medical facility for consultation. Participants were also informed that they could withdraw their participation at any time.

2.3 Sampling procedure

From each mother, 10 ml of blood was drawn by venous puncture into EDTA containing Vacutainer tubes before delivery process started. The bloods were centrifuged and the plasma transferred into solvent pre-washed tubes, and immediately frozen at -20^{o}C and stored until shipped frozen on dried ice to the Norwegian Institute for Air Research (NILU) laboratory in the Centre, Tromsø, Norway for analyses.

2.4 Analytical methods

2.4.1 Sample preparation

Plasma samples were extracted using an Oasis® HLB Extraction Cartridge (3cc, 540 mg; Waters Corp., Milford, MA, USA) according to the method by Sandanger et al., (Sandanger et al., 2007). In short, internal standards (C-13 labelled *p,p´*- DDE and *p,p´*- DDT), formic acid (2 ml) and water (2 ml) were added to the plasma samples (2 ml), vortexed and left overnight in a refrigerator. The HLB column was conditioned with methanol (3ml), dichloromethane (3 ml), methanol (3 ml), followed by 5% methanol in 0.1M hydrochloric acid (3 ml). The samples were added to the column and dried with N_2 and extracted using dichloromethane (14 ml). Following evaporation, the samples were re-suspended in hexane. The extracts were subsequently eluted through a column containing 1g deactivated silica (0,063-0,2 mm; Merck, Darmstadt, Germany) and eluted with hexane/dichloromethane (9/1; 6ml) and dichloromethane (6 ml). The extraction and cleanup procedures were automated using a Rapidtrace Automated SPE workstation (Zymark Corp., Hopkinton, MA, USA), and evaporation was performed using a heated vacuum evaporator (Rapidvap; Labconco Corp.,Kansas City, MO, USA).The samples were concentrated and octachloronaphthalene was added as a recovery standard.

2.4.2 Instrumental measurements

The extracts (30 µl) were analysed on an Agilent 7890A gas chromatograph (Agilent Technologies, Böblingen, Germany) equipped with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester, UK). Separation was performed on a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness; J&W, Folsom, USA) with a programmed-temperature vaporisation PTV injector in splitless mode. An injection volume of 1 µL of the extract was injected using a PTV: Agilent 7683 Series, Agilent Technologies, Böblingen, Germany injector in splitless mode. An injection volume of 1µl of the extract was injected. The initial PTV temperatures were initially 70°C and increased by 120°C min^{-1} to a final temperature of 275°C that was held for 3 min. The GC temperature programme

consisted of an initial temperature of 70°C with a hold time of 3 min; the temperature was then ramped at 15°C min^{-1} to 180°C, followed by a temperature ramp of 5°C min^{-1} to 280°C with a hold time of 5 min. Helium (6.0 quality, Hydrogas, Porsgrunn, Norway) was the carrier gas at 1 $ml.min^{-1}$ under constant flow conditions. The MS operated in MRM mode with an EI source set at 220°C. Collision gas was argon at a pressure of approximately 2.3×10-3 mbar. Dwell times for specific ion transitions were 0.05 seconds. Information regarding specific transitions has been previously published in the literature (Pitarch et al., 2007). Peaks with differences in isotopic mass ratios greater than 20% compared to the quantification standard were rejected and not quantified.

2.5 Quality assurance and quality control

The accuracy of the analysis was assured through inclusion of certified reference materials and an internal QAQC pool in the analyses. The NILU laboratory participates in international inter-laboratory comparison programmes (AMAP Human Ringtest for plasma samples with +/- 20% deviation from result as best performance, according to AMAP Ringtest protocol).

The limits of detection (LODs) were calculated using the signal to noise ratio calculations in serum samples, and corresponded to 3 times the area of the noise or 3 times the average concentrations found in blank samples (28 samples).

The coefficient of variation was 13 and 12% within assays for *p,p'-DDE* and *p,p'-DDT,* respectively, and 14% between assays for both metabolites in spiked bovine serum samples (30 spiked samples). Limits of detection for each compound measured are included in results tables.

2.6 Total lipids enzymatic method

Lipids were determined enzymatically and the total lipids were calculated according to a formula by Sandanger et al., (Sandanger et al., 2003).

2.7 Questionnaire data

Socio-economic, demographic, occupational activity, self-reported health status, diet and life style (smoking, alcohol consumption and leisure activities) data were obtained from each participant by interview.

From delivery records, the researchers extracted following information: weight and length of the newborns, head circumference, Naegele term, Abgar score, gestational age, as well as congenital malformations, birth complications and outcomes as per comments of the doctor or sister present at delivery.

2.8 Statistical analyses

All data analyses were performed using STATA package, version 11 for Windows. (STATA 11.1, 2009) Descriptive statistics were calculated for socioeconomic and birth outcomes data. Given that the distributions of the concentration of the compounds measured were not normally distributed, the concentrations were log-transformed and geometric means were used for statistical analysis. The criteria of significance were set at a p value of less than 0.05. The Spearman's Rank Correlation Coefficient Test for comparison for not normally distributed data were used, and the Kruskal-Wallis Rank Sum Test to compare the rank sum of means of more than two groups.

2.9 Ethical considerations

The study protocol was submitted to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and unconditional approval was obtained (Protocol: M040314). The study was also approved by the Provincial Health Research Committee, KwaZulu-Natal Department of Health (Reference: HRKM001/08).

3. Results

3.1 Socio-economic, demographic and lifestyle characteristics

Socioeconomic characteristics for participants in each study site are summarized in Table 1. Questionnaire data confirmed a similar socioeconomic status of participants at all sites. The majority of participants in the three sites were African Blacks and 2% in low risk and non-malaria areas were of Asian origin. Participants in all three regions were South African citizens but in malaria region 1% reported to be foreigners. Zulu was the most spoken home language by 99%, 98% and 87% of women from malaria, low risk and non-malaria sites respectively. About 11% of women in non-malaria region spoke Xhosa and 2% in low risk and non-malaria spoke English. Majority of the women studied were not married but single. Secondary education was completed by 35%, 40% and 55%; and 39%, 52% and 33%

completed tertiary education by women from malaria, low risk and non-malaria sites, respectively. The differences in educational status of participants by site were statistically significant, with woman from the low risk area having higher tertiary education levels. Very few reported to be employed in all sites, reaching statistical significance between sites. They relied on social grants or the financial support of their partner and/or family members. Women reported not to smoke themselves, however about 46% in non- malaria, 30% in low risk malaria and 17% in malaria sites reported to have somebody smoking regularly in their homes.

The most common source of potable water was from the communal tap that was situated outside the houses. A number of participants from the malaria site also relied on either boreholes (25%) or a river (8%) as a source of drinking water; whereas in low risk and non-malaria approximately 9% relied on a river as a source of drinking water. Participants tended not to move homes; mean number of years at the current residence or current address ranged between 16.7 and 18 years, with no significant differences between three study sites. Most of the participants lived in rural settings; highest (99%) in the malaria area, followed by the non- malaria area (90%) and low risk area (68%), the differences were statistically significant. When asked about incidents of malaria in the past, 32% of women from the malaria site reported that they or a family member had contracted malaria in previous years, with highest incidents reported in years 1998, 1999 and in 2004.

Each subject was asked if their home was regularly sprayed by the Malaria Vector Control Programme and 96% in the malaria area answered yes. Intermittent spraying in the low risk site was reported by 20% of participants.

3.2 Maternal data and birth outcomes by site

The maternal data and the birth outcomes are summarised in Table 2. The mean age and mean weight of the mothers at delivery was not significantly different between sites. Mean body mass index (BMI) available only from malaria and non-malaria sites indicate that women from non-malaria site were significantly heavier with a BMI of 30(SD 9). Parity ranged between 0 and 8, with 51%, 20% and 58% of mothers in the malaria site, low risk and non- malaria site, respectively, being nulliparous. The baby birth weight ranged from 1300g to 5150 g with mean birth weights being significantly different across the three study sites. Low birth weight (LBW below 2500g) accounted for 6%, 6% and 11% of babies born in malaria, low risk and non-malaria sites, respectively. In most cases, the LBW was associated with gestational age and preterm emergency deliveries. Mean birth length and head circumference of newborns did not statistically differ between sites. There was a

significant difference between the mean gestational age between the 3 sites, with lowest (37 weeks in non- malaria site) and the highest (39 weeks in low risk site). In all study sites majority of deliveries were vaginal. Highest number of caesarean deliveries (40%) took place in the low risk malaria area, followed by the non- malaria site (23%) and 15% in malaria site. In all sites, number of delivery complications and congenital malformations were also recorded at birth (see Table 2). Gender ratio was not similar at three sites ranging between 47 to 51% frequency for girls.

3.3 DDT levels in maternal plasma at delivery

Concentrations of DDT and metabolites in maternal blood plasma are summarized in Tables 3 and Table 4. For easy comparisons with non-lipid adjusted published data, Table 3 shows wet weight concentrations in pg/ml. For each site GM, first and third quartile and LOD for each compound are presented. The limit of detection for *p,p'*-DDT was 42 pg/ml and for *p,p'*-DDE was 71 pg/ml. Percentages of results above LOD were 99% and 100% in the malaria region, 64% and 87% in low risk region and 3% and 80% and in non-malaria region, respectively.

Table 4 reports comparisons of lipid adjusted in ng/g lipids by site. As expected, all the geometric mean concentrations of compounds measured were highest in the malaria area where IRS is taking place and the differences between sites were significant for all compounds (p=0.0001). The concentrations of metabolites show large variation, especially in the malaria areas, with extreme outliers. At this site, three mothers had very high levels of *p,p'*-DDE (above 45000 ng/g lipid) and *p,p'*-DDT (above 15000 ng/g lipid).

Of the metabolites measured, *p,p'*-DDE was the most abundant. Geometric mean of *p,p'*-DDE in the malaria site was 3840 ng/g lipids, ranging from 37 to 92559 ng/g lipids. *p,p'*-DDE was strongly and positively ($r > 0.7$) associated with *p,p'*-DDT and *o,p'*-DDT, and positively associated with the other metabolites but to a lesser extent (r range 0.3-0.5).

Similarly, levels of *p,p'*-DDT were found to be highest in the malaria site with a median concentration of 2194 ng/g lipids, ranging from 8 to 21856 ng/g lipids. The median (range) concentration of its isomer *o,p'*-DDT was found to be 168(5-1744) ng/g lipids and showed little variation across individuals. For both the malaria endemic area and the low-risk area, *p,p'*-DDE and *p,p'*-DDT levels were highly correlated ($r >0.7$), but not in the non-malarial site ($r = 0.08$). The body mass index and maternal age was negatively correlated with *p,p'*-DDT and *p,p'*-DDE in the malaria area only ($r >0.4$).

The lowest geometric mean ratio (95% CI) for *p,p'*-DDE/*p,p'*-DDT of 2 (1.4 – 2.1) was found in the malaria endemic site, indicating recent exposure. Geometric mean ratios (95% CI) of 5 (3.6 – 7.1) and 4 (3.8 – 4.9) were found for the low-risk and non-malaria site, respectively, also indicating fairly recent exposure or fresh sources of exposure.

The relationship between some important maternal characteristics reported in the literature and the levels of *p,p'*-DDE and *p,p'*-DDT are shown in Table 5. Only *p,p'*-DDE and *p,p'*-DDT which are the main metabolites and most persistent, are presented. These compounds also had acceptable percentage (90%) of results above LOD in the malaria area. Between the three study sites, age (p=0.0064: p=0.0023), level of education (p=0.0311; p=0.0029), permanent employment of the mothers (p=0.0181; p=0.0059) and IRS spraying in the home (p=0.0001; p=0.0001) were significant for both *p,p'*-DDE and *p,p'*-DDT, respectively. In the malaria area age (p=0.0016; p=0.0005), the number of children (p=0.0108; p=0.0114), previous breastfeeding (p=0.0490; p=0.0097) and IRS spraying (0.0316; 0.0095) were significant for both *p,p'*-DDE and *p,p'*-DDT, respectively, and permanent employment of mothers (p=0.0164) was significant for *p,p'*-DDT only. IRS was associated with an increase in *p,p'*-DDE and *p,p'*-DDT, whereas breastfeeding and higher levels of education were associated with a decrease in the pesticide concentrations.

4. Discussion

The use of DDT for IRS to control the malaria vector is a subject of intense debate among governmental parties, regulatory organisations, NGOs and scientists. Although numerous studies show the detrimental effects of DDT to humans and wildlife, there is limited research investigating an association between its controlled use for IRS and health. Prenatal exposure to these compounds is of particular concern.

Our study quantified the concentrations of DDTs and its metabolites in the blood plasma of delivering women to assess exposure in a malaria endemic IRS area and compared these results with low risk and non- malaria areas, all situated along the Indian Ocean coast of South Africa.

This study found that IRS with DDT for malaria vector control in malaria endemic region increases the concentrations of DDT and its metabolites in the blood plasma of delivering women. The levels of DDT and its metabolites were greatly elevated in this group with the *p,p'*- isomers being more abundant than *o,p*'-isomers indicating significant prenatal

exposure. Our findings also suggest that IRS may result in high DDT exposure in the general population. The study by Aneck-Hahn et al., 2007 on South African men residing in IRS houses found even higher concentrations of DDE in their blood (239 ±215 µg/g lipid) (Aneck-Hahn et al., 2007). Of concern, is also the fact that our study detected high levels of DDT in some of delivering women residing outside of the malaria endemic region along the coast of the KwaZulu Natal Province, who had no history of residing in an IRS region.

Highly elevated levels of *p,p'*-DDE and *p,p'*- DDT isomers measured in the plasma of delivering women in the IRS study site is of great concern. Bulk of the reported research identifies DDT and its metabolites as having multiple detrimental health effects of IRS on human health. However, data on levels of the exposure and health effects of IRS on pregnant women, neonates and young children and other susceptible population groups are still lacking. The study by Torres-Sanchez et al., 2007 on a perinatal cohort in Mexico reported on association between *in utero* exposure to *p,p'*-DDE and neurodevelopment in infants during the first year of life at a concentration of 7.8 ng/ml in the 3rd trimester, which is magnitudes lower than our results (Torres-Sanchez et al., 2007). They also identified the first trimester of the pregnancy to be a critical window of exposure to DDE. Recently, Eskenazi et al., 2009 reviewed 494 studies and concluded that there is a growing body of evidence that exposure to DDT and DDE may be associated with breast cancer, diabetes, decrease of semen quality and modulation of immune response. Some studies associate exposure to DDT with spontaneous abortions, short gestational length, low birth weight, short duration of lactation, urogenital birth defects and impaired neurodevelopment in children (Eskenazi et al., 2009). Thus, not all studies are consistent in their findings; concern is particularly evident in the relation to long term and chronic effects. This review also showed that recent studies of DDT/DDE levels in pregnant women and women of reproductive age differ by geographical regions.

The ratios of *p,p*'-DDE/*p,p*'-DDT of 5 and 4 in the low-risk malaria area and non-malaria areas reflects continuous recent exposure, similar to the malaria area. These ratios cannot only be attributed to food source and further research is required to identify the source of exposure. The correlation between *p,p*'-DDT and *p,p*'-DDE indicate a similar source of exposure for high and low risk malarial sites, but not in the non-malarial site. The low ratios in the non-malaria and low risk areas suggest geographical dispersal of DDT.

The maternal characteristics where the overall *p* value was significant, but not within each area, is due to the difference in the pesticide levels in the three sites that influences the results. The majority of the significant p-values for maternal characteristics were found in

the malaria area, because the majority of exposure occurred in that area. The results have shown that the younger mothers had the highest DDT levels. This is partly influenced by the lack of previous breastfeeding for nulliparous women and/or fewer children, as breastfeeding reduces maternal levels as also shown by Lopez-Carillo et al., (Lopez-Carrillo et al., 2001). In the present study we found that both *p,p'*-DDE and *p,p'*-DDT metabolites were lowered by previous breastfeeding.

A high level of education in the low risk area was associated with lower concentration of *p,p'*-DDT. In addition, maternal permanent employment in the malaria area decreased *p,p'*-DDT levels. This was not the same for *p,p'*-DDE, most likely because of the low number (n=4) of mothers employed and the fluctuation of the employment status in SA.

Research by Bornman et al., 2010 performed in Limpopo Province of South Africa (bordering to Zimbabwe) where IRS also takes place, found an association between DDE concentration in pregnant women and external urogenital birth defects, such as cryptorchidism, hypospadias, micropenis, and chlordee (Bornman et al., 2010).

Exposure to DDT and other pesticides during different stages of gestation is a subject of many studies worldwide. However, it is difficult to directly compare our results with those studies as a source of exposure in malaria areas is IRS with DDT (Wang et al., 2009; Weldon et al., 2010).

In 2006, we performed a pilot study in seven different regions of South Africa, with one site being the same malaria endemic site of the present study and measured the large spectrum of POPs. Concentrations of *p,p'*-DDE and *p,p'*-DDT were 5178 ng/g lipid and 1797 ng/g lipid respectively, which are similar to current results (Rollin et al., 2009) . Direct comparisons are not possible due to different sample sizes but cautiously we can state that 2006 results compared with the current study performed in 2008 indicate standardised procedures and consistent concentrations of DDT used for IRS.

Of concern is the persistency of DDT compounds in the environment, especially in soil and dust, foods and water but also its accumulation in human milk. Continuous intake of these contaminants is prolonged even after cessation of spraying through environmental contamination. Recent South African studies have reported on concentrations of DDT in breast milk, diet and environment in a region situated close to our current malaria site. Sereda et al reported mean sum of DDT in maternal breast milk fat (mf) of 10 µg/g ranging from 0.1 to 22 µg/g mf (Sereda et al., 2009; Bouwman et al., 2006). These concentrations

are higher than median concentrations of 0.80 µg/g mf reported for *p,p'*- DDE by Ribas-Fitó et al., in milk of breastfeeding Spainish mothers (Ribas-Fito et al., 2005).

Another study by Van Dyk et al., 2010 performed in another IRS region of South Africa, namely Limpopo Province, found detectable levels of DDT in the indoor air, soil, floor dust, portable water, vegetables and chickens (Van Dyk et al., 2010).

Further research is required to establish health effects on the levels of exposure and possible means of reducing exposure in pregnant woman residing in IRS regions.

Most of the reported data on IRS and DDT concentration in humans are mainly associated with occupational exposure of male personnel directly engaged in spraying operations of the Malaria Control Programmes (Bimenya et al., 2010; Bouwman et al., 1991a; Bouwman et al., 1991b; Dalvie et al., 2004; de Jager et al., 2009).

In South Africa and bordering countries, a systematic program of insecticide resistance monitoring and surveillance, including collection of data on vector species and behaviour; ecology and transmission patterns (entomological inoculation rates) is taking place (Casimiro et al., 2006). This research is contributing to the understanding of vector changes and is communicated to malaria control programmes.

5. Conclusions

Findings of our study reaffirm the need for further research into the use of DDT and other insecticides to control malaria vector that are applied selectively at controlled doses to minimize the risks to the environment and the health of the population. Additional studies are also needed to further the understanding of potential health consequences to the foetus from DDT and other insecticides. Synergistic and additive risks of concomitant use of DDT with other alternatives and/or newer currently used insecticides need to be investigated as these are poorly defined. Furthermore, follow up studies of infant health and development in IRS regions should be initiated.

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Figure 1: Probable and confirmed malaria cases per year in South Africa 1990-2009
Source DOH, SA (DOH 2010)

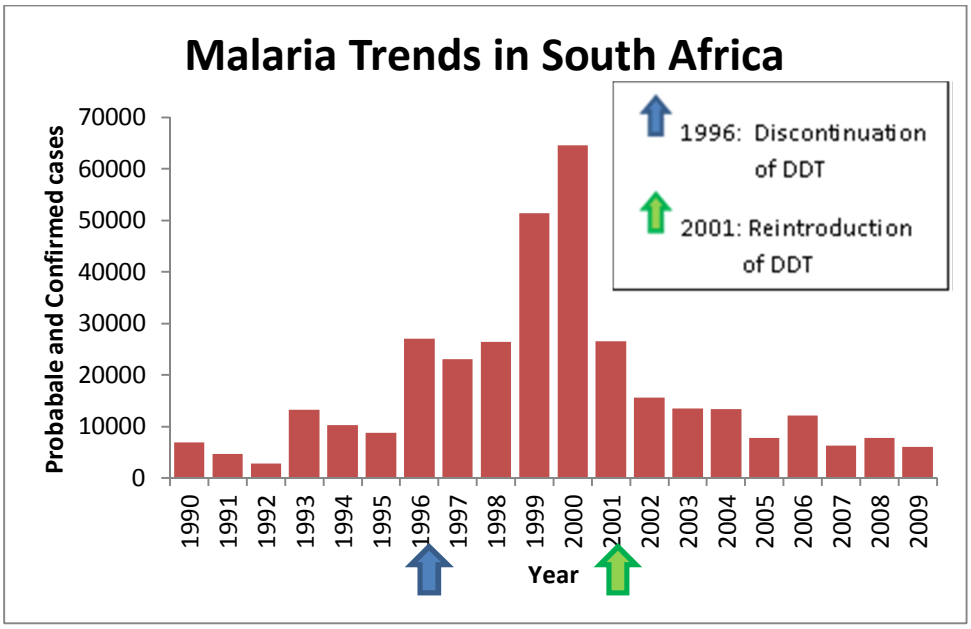

Figure 2: Geographical positions of study sites within KwaZulu- Natal, South Africa.

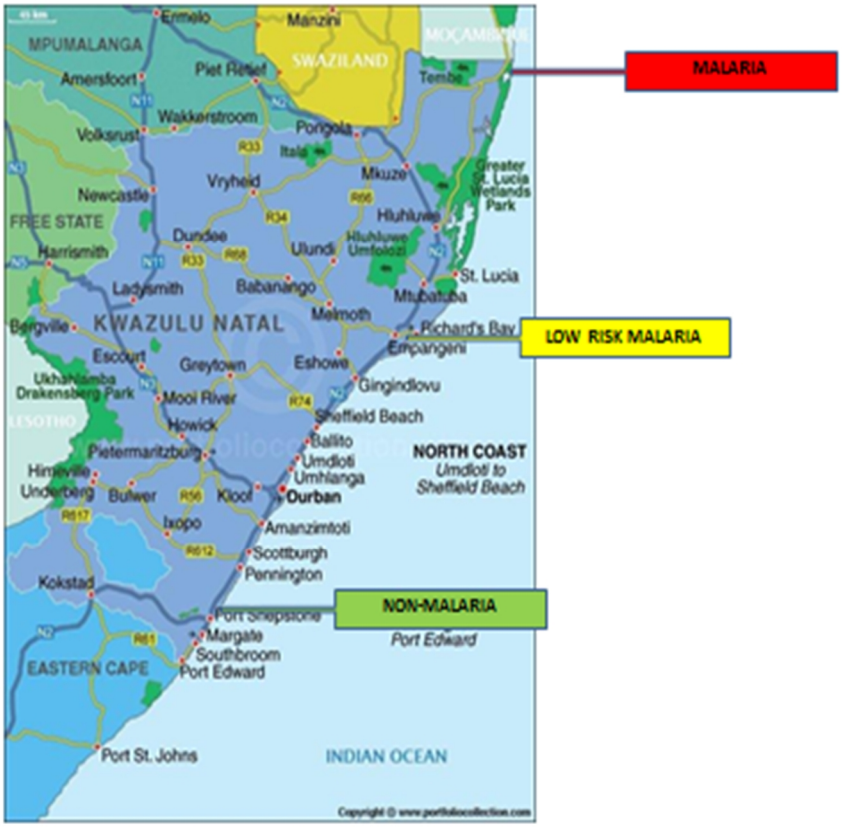

Table 1: Socioeconomic characteristic of participants by site

	Malaria n=91	**Low risk n=47**	**Non-malaria n=117**	***P value***
Population group (%)				
B= Black	B 100	B 98	B 98	ns
A= Asian	-	A 2	A 2	
Nationality (%)				
South African	99	100	100	ns
Other	1	-	-	
Language (%)				
Zulu	99	98	87	
Xhosa	-	-	11	<0.05
English	-	2	2	
Other	1	-	-	
Marital Status (%)				
Married	5	13	16	
Single	62	85	76	<0.05
Living together	33	2	7	
Widowed	-	-	1	
Maternal education (%)				
Primary school	26	8	11	<0.05
Secondary school	35	40	55	
Tertiary	39	52	33	
Employment mothers (%)				<0.05
Yes	4	11	16	
Somebody smoking at home (%)	17	30	46	<0.05
Drinking water source (%)				
Indoor Tap	3	20	14	
Outdoor Tap	60	62	78	<0.05
Rainwater	2	6	0	
Borehole	25	2	0	
River	8	10	8	
Length of residence at current home				ns
Years (SD)	18.0(9.7)	17.0(11.0)	16.7(9.7)	
Place of residence (%)				
Urban	0	4	6	
Rural	99	68	90	<0.05
Peri-urban	1	21	4	
Others	0	7	0	

Table 2: Maternal data and birth outcomes

	Malaria n=91	**Low risk n=47**	**Non-malaria n=117**	***P value***
Maternal mean age Years (SD*) Range	 24(7) (14-42)	 25(6) (15-45)	 24(5) (16-37)	ns
Maternal weight prior to delivery Mean Kg (SD)	 71(11)	 74(13)	 76(13)	ns
BMI Mean at delivery (kg/m^2)	 24 (4)	 Not available	 30 (9)	<0.05
Parity mean (SD) Range	1.0(1.5) (0 – 6)	1.2(1.6) (0 – 8)	0.8(1.0) (0 – 5)	<0.05
Birth weight(g) Mean (SD) Range Underweight (%) ≤2500g	 3147(464) 2100 – 4250 6	 3250(426) 2300 – 4400 6	 2990(531) 1300 – 5150 11	<0.05
Birth length (cm) Mean (SD)	 49(3)	 50(2)	 49(3)	ns
Head circumference (cm) Mean (SD)	 35(1)	 35(2)	 35(2)	ns
Gestation age (weeks) Mean (SD)	 38(2)	 39(1)	 37(2)	<0.05
Delivery mode (%) Vaginal Caesarean section	 85 15	 60 40	 77 23	
Gender (%) girls	49	47	51	
Maternal delivery complications	death 1	postpartum 1 haemorrhage 1 asphyxia 1 epilepsy 1 anaemia 2	placenta retention 2 hypertension 11 preterm 2 preeclampsia 1 anaemia 2 epilepsy 1 tumour 1	
Congenital malformations detected at birth	none	Down Syndrome 1	hand or foot extra digits 3	

*SD = standard deviation

Table 3: DDT metabolites in maternal plasma at delivery (pg/ml) by site

Compounds	Statistics	Malaria N=91	Low Risk N=47	Non-malaria N=117
o,p' -DDE	GM[a]	47	36	
	Median	35	35	
	Range	35 - 1105	35 - 94	
	IQR[b]	35 – 52	35 - 35	
	LOD[c]	35	35	0
	% detected >LOD	27	2	
p,p' -DDE	GM	20279	1167	189
	Median	22613	1064	179
	Range	290 - 444782	71 - 24889	70 - 2783
	IQR	11217-37523	372 – 5661	113 – 315
	LOD	71	71	71
	% detected >LOD	100	87	80
o,p' -DDD	GM	41	29	
	Median	28	28	
	Range	28 - 3149	28 - 102	
	IQR	28 – 47	28 -28	
	LOD	28	28	0
	% detected >LOD	33	4	
p,p' -DDD	GM	137	57	
	Median	150	42	
	Range	42 - 1478	42 - 583	
	IQR	42 – 370	42 – 43	
	LOD	42	42	0
	% detected >LOD	66	15	
o,p' -DDT	GM	851	35	
	Median	1287	35	
	Range	35 - 11186	35 - 2127	
	IQR	652 – 2062	35 – 35	
	LOD	35	35	0
	% detected >LOD	87	21	
p,p' -DDT	GM	11841	227	44
	Median	13666	123	42
	Range	42 - 110271	42 - 13260	42 - 280
	IQR	7951 - 23668	42 – 1093	42 – 42
	LOD	42	42	42
	% detected >LOD	99	64	3

[a] geometric mean
[b] inter-quartile range
[c] limit of detection

Table 4: Lipid corrected (ng/g lipid) of DDT and metabolites in mothers at delivery at the three sites

Compounds	**Statistics**	**Malaria N=91**	**Low Risk N=47**	**Non-malaria N=117**
o,p'- DDE	GM[a] (95% CI) Median Range IQR[b]	9 (7-10) 7 4 - 172 6 - 7	6(5-6) 6 4 - 25 5 - 6	5(5-6) 6 3 - 8 5 - 6
p,p'- DDE	GM (95% CI) Median Range IQR	3840(3008-4902) 4092 37 - 92559 1986 - 7341	191(116-315) 184 11 - 4739 54 - 908	29(25-33) 26 8 -343 18 - 49
o,p'- DDD	GM (95% CI) Median Range IQR	8(6-9) 6 3 - 456 5 - 10	5(4-5) 4 3 - 20 4 - 5	4(4-5) 4 2 - 7 4 - 5
p,p' -DDD	GM (95% CI) Median Range IQR	26(20-32) 26 5 - 230 8 - 66	9(7-11) 7 5 – 111 6 - 8	7(6-7) 7 4 - 10 6 - 8
o,p -'DDT	GM (95% CI) Median Range IQR	168(127-221) 226 5 – 1744 108 - 376	9(7-12) 6 4 - 405 5 - 8.5	5(5-6) 6 3 - 8 5 - 6
p,p' -DDT	GM (95% CI) Median Range IQR	2194(1706-2823) 2788 8 - 21856 1279 - 4525	38(22-65) 27 5 - 2425 7 - 165	7(6-7) 7 4 – 37 6 - 8

[a] geometric mean

[b] inter-quartile range

For all the DDT metabolites p=0.0001 between the three areas.

Table 5: Relationship between maternal characteristics and *p,p'*-DDE and *p,p'*-DDT concentrations (ng/g lipids) by study site

Maternal characteristics	*p,p'*-DDE GM (95%CI)							*p,p'*-DDT GM(95%CI)						
Number of subjects (n)	n	Malaria	n	Low risk	n	Non-malaria	*P-value overall*	n	Malaria	n	Low risk	n	Non-malaria	*P-value overall*
Age (years)														
≤20	31	6638(4948-8904)	8	508(90-2874)	28	31(22-43)		31	4075(3147-5276)	8	189(27-1330)	28	7(6-8)	
20-29	39	2862(1872-4374)	26	167(95-296)	66	27(23-32)	0.0064	39	1682(1077-2626)	26	28(15-51)	66	7(6-7)	0.0023
≥30	21	2957(1773-4930)	13	138(44-432)	18	35(25-49)		21	1442(851-2443)	13	27(9-81)	18	7(6-8)	
P-value		0.0016		0.2013		0.3614			0.0005		0.0794		0.3476	
Level of Education														
Primary school	21	5425(3026-9725)	4	247(7-9018)	12	29(17-48)		21	2838(1803-4467)	4	80(1-5690)	12	8(6-9)	
Secondary school	29	4860(3456-6834)	19	294(127-677)	61	32(27-38)	0.0311	29	2551(1471-4425)	19	95(39-232)	61	7(6-7)	0.0029
Tertiary	30	3007(1859-4864)	24	131(68-252)	36	28(21-36)		30	1571(988-2499)	24	16(9-29)	36	7(6-7)	
P-value		0.2690		0.2795		0.6117			0.0684		0.0049		0.3905	
Employment mothers														
Yes	4	1765(73-42468)	5	417(64-2723)	18	26(19-35)		4	482(29-8089)	5	55(6-466)	18	6(6-7)	
No	87	3980(3130-5060)	42	174(102-297)	93	30(26-36)	0.0181	87	2353(1843-3003)	42	36(20-66)	93	7(6-7)	0.0059
P-value		0.5229		0.2848		0.5274			0.0164		0.4478		0.3873	
IRS Spraying														
Yes	87	4117(3249-5218)	9	319(83-1230)	1	13*		87	2406(1898-3049)	9	60(12-307)	1	8*	
No	4	845(64-11099)	37	162(92-283)	111	30(26-34)	0.0001	4	297(20-4398)	37	34(18-63)	111	7(6-7)	0.0001
P-value		0.0316		0.2505		0.2218			0.0095		0.4547		0.2587	
Number of children														
0	50	4960(3546-6937)	21	311(146-664)	58	31(26-39)		50	2787(1933-4017)	21	49(19-127)	58	7(6-7)	
1	19	3256(1916-5535)	10	98(30-315)	32	25(20-33)		19	1631(1011-2632)	10	26(8-87)	32	7(6-7)	
2	8	2021(1231-3319)	8	214(52-888)	13	29(19-44)	0.1256	8	1451(839-2509)	8	60(10-343)	13	6(5-7)	0.2770
≥3	12	2204(978-4966)	8	110(26-463)	8	28(14-57)		12	1484(620-3551)	8	20(8-52)	8	7(5-10)	
P-value		0.0108		0.2671		0.6004			0.0114		0.7261		0.8905	
Cumulative Breastfeeding														
Never	55	4415(3110-6266)	23	281(139-569)	72	31(26-37)	0.2319	55	2544(1758-3682)	23	43(18-104)	72	7(6-7)	0.5300
1-48 months	36	3103(2264-	24	132(64-	45	26(21-		36	1751(1296-	24	34(17-70)	45	7(6-7)	

P-value		4254) 0.0490		273) 0.1363		32) 0.2439			2366) 0.0097		0.9745		0.3521	

n = no of subjects

* =only 1 observation in this group.