

Exposure to organochlorine compounds at the early stages of DDT use for indoor residual spraying in domestic environments in Manhiça, Mozambique

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Aos meus pais
Aos meus irmãos
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..." Los humanos estamos haciendo un experimento, un experimento en el que el planeta es el objeto del experimento y nosotros vivimos dentro del experimento..."

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SUMMARY

Past and present uses of DDT and pyrethroids have led to their incorporation into humans, mainly through the food chain and sometimes by direct exposure.

The present work focuses on establishing the levels of DDT, its analogous compounds (DDE and DDD), and pyrethroids in humans and the human environment in Manhiça, a rural area where they have been used as insecticides for indoor residual spraying (IRS) and insecticide treated nets (ITN) in malaria control programs. Thatch samples from human dwellings, breast milk from pregnant women and cord blood from newborns were analyzed for assessment of the concentration levels of these compounds.

The results showed that DDT and its analogues were already present in humans and dwellings before reintroduction of this insecticide for IRS. As consequence of these applications DDT concentrations increased significantly. The higher proportion of 4,4'-DDT than 4,4'-DDE evidenced that the observed amounts were due to recent applications of this insecticide. Concerning pyrethroids, their presence has been identified in both breast milk and human dwellings showing that both agricultural applications and use for ITN may be responsible for their occurrence in humans and human environments of Manhiça.

RESUM

L'ús en el passat i en temps actuals del DDT i piretroides ha donat lloc a la seva incorporació en els humans, principalment mitjançant la cadena tròfica i a vegades per exposició directa.

Aquest treball té com objectiu establir els nivells de DDT i els seus compostos anàlegs (DDE i DDD), i piretroides en humans i l'ambient humà a Manhiça, una àrea rural on aquests s'han utilitzat com insecticides per aplicació interna (*indoor residual spraying*, IRS) i tractament de xarxes de protecció (*insecticide treated nets*, ITN) en programes de control de la malària. Per a esbrinar els nivells de concentració d'aquests compostos s'analitzaren mostres de palla de cabanes, de llet materna i de sang de cordó de nou nats.

Els resultats mostraren que el DDT i els seus compostos anàlegs ja eren presents en humans i cabanes abans de la reintroducció d'aquest insecticida per IRS. L'ús del DDT en aquest programa féu augmentar considerablement les concentracions d'aquest insecticida. La major proporció de 4,4'-DDT que 4,4'-DDE posà de manifest que les quantitats observades corresponien a aplicacions recents d'aquest insecticida. Respecte als piretroides, s'han trobat en mostres de llet materna i cabanes tot mostrant que tant les aplicacions agrícoles com el seu ús en ITN poder esser la causa de la seva presència en els humans i els ambients humans de Manhiça.

PREFACE

One of the ways to combat the vector that transmits malaria is by regular fumigation of the houses with pesticides and by using bednets impregnated with pesticides. It is known that some of the pesticides commonly used for this purpose may cross the placenta and reach the fetus. It is also known that they are transferred via breast milk to infants. Both processes may lead to adverse effects on the nervous and immune systems.

Manhiça is a rural village situated 80 Km away from Maputo, the capital of Mozambique. There, malaria is endemic and indoor fumigations were performed first with pyrethroid and recently with DDT. The fumigations with DDT started in the last trimester of 2006 and are currently performed once a year.

The work reported in this PhD thesis has been developed as a consequence of the first part of the project EPSIMA (*Exposição a Pesticidas e Saúde Infantil em Manhiça*) which began in late 2006 with the aim to assess the exposure to DDTs and pyrethroids pesticides in women, newborns and human environment prior and during DDT reintroduction in indoor residual spraying (IRS) for malaria control and, subsequently, to study the exposure effects to these pesticides on children's health in the district of Manhiça.

Specifically, this PhD thesis reports the results of the assessment of the background exposure to pesticides prior the DDT reintroduction in IRS in puerperal women (breast milk samples) and newborns (cord blood samples) and, during and immediately after the fumigations, in breast milk and thatch samples.

The lack of information on pesticide use in developing countries constitutes an important gap in the scientific literature. Reintroduction of DDT for IRS in sub-saharan Africa has reactivated the debate on the advantages and risks of the use of this insecticide, particularly for child health. Progress into these questions first requires the assessment of the pesticide contamination in humans and human environment. This is the aspect addressed in the present PhD work.

This PhD thesis is presented as a compendium of publications, according to normative of the Doctoral Program in Biomedicine of the Department of Experimental and Health Science at UPF. It consists of a summary, an introduction, objectives, methods and results (which include 4 articles already submitted -one of them already accepted-), a general discussion and final conclusions.

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PART I. INTRODUCTION AND OBJECTIVES

Chapter 1. INTRODUCTION

Persistent Organic Pollutants (POPs) are resistant to photolytic, biological and chemical degradation. Once released into the environment, POPs remain intact for long periods of time. They are halogenated, usually chlorinated. The chlorine-carbon bond is very stable against hydrolysis, the higher the number of chlorine substitutions, the higher the resistance to biological and photolytic degradation. **Organochlorine compounds (OCs)** have high biostability, slow biodegradation, accumulate in fatty tissues, have a long half-life and are often referred to as Persistent Bioaccumulative Toxics (PBTs). Their good solubility in lipids facilitates their passage through the phospholipid structure of biological membranes. Their high lipophilicity allows them to bioaccumulate in fat or organic tissue in factor rates up to 70,000 times the water background levels.

POPs can transfer between species through the food chain. Higher organisms in the top of the food chain absorb the greatest concentrations. In addition, POPs are semi-volatile and can be air transported over long distances. Due to these properties and their chemical stability they are distributed worldwide, even reaching regions where they have never been used. Volatilization in warm areas and condensation in cold regions, a process known as global distillation effect, leads to the long-term accumulation of several of these compounds in high latitude areas and high mountain regions.

The Stockholm Convention on POPs was a global treaty aimed to protect the human health and the environment from these compounds. It was signed in 2001 and has been ratified by many countries. These countries committed to implement restrictions to the use and release of POPs and to develop research on alternative compounds, as well as to monitor environmental levels and internal dose in the general population. The twelve initial POPs were chosen for their persistence and bioaccumulation and were referred as “the dirty dozen”. They comprised: aldrin, dieldrin, dichlorodiphenyltrichloroethane (DDT), endrin, chlordane, mirex, toxaphene, heptachlor, hexachlorobenzene (HCB), polychlorobiphenyls (PCBs), polychlorodibenzo-p-dioxins and polychlorodibenzofurans. In the fourth Stockholm Convention on POPs held in 2009, nine chemicals were added to the list:

chlordecone, α -hexachlorocyclohexane (α -HCH), β -hexachlorocyclohexane (β -HCH), lindane (γ -HCH), pentachlorobenzene, hexabromobiphenyl, hexabromodiphenyl ether, heptabromodiphenyl ether, perfluorooctanesulfonic acid and its salts, perfluorooctanesulfonyl fluoride, tetrabromodiphenyl ether and pentabromodiphenyl ether (1).

POPs started to be produced and used in the 1920s. Many of them were widely used during the boom of industrial production after World War II, when thousands of synthetic chemicals were manufactured (1, 2). They are not synthesized by natural processes. Their origin is related to human activity either by direct synthesis or as by-products of other processes. Before 1950 these compounds were not in the environment nor were other compounds of similar structure, e.g. with a high proportion of C-Cl bonds. Thus, human metabolism has never been exposed to these compounds before the above mentioned date.

OCs has been described to have toxic effects and carcinogenic activity in animals and humans as well as activity as hormone disrupters which can alter the normal function of the endocrine and reproductive systems. Some of these compounds such as HCB, HCH and DDT were among the most widely used pesticides in the world during 1970-1980. Due to their toxicity, these compounds have been banned in most countries and their use has decreased considerably.

Humans are constantly exposed to OCs because they are present in food and in the environment. OC exposure through placenta and breastfeeding is associated with poor infant neurodevelopment, intrauterine growth retardation, reduced antibody activity against infant vaccines and immunological disfunction (3-5).

1.1. Dichlorodiphenyltrichloroethane (DDT)

1.1.1 History

DDT was first synthesized in 1874, but its properties were not discovered until the late 1930s when the chemist Paul Muller discovered that it was very effective as contact poison against several arthropods. This discovery gave him the Nobel Prize of medicine in 1948. This compound was used at the beginning to protect military zones from malaria, typhus and other vector-borne infectious diseases. The large-scale industrial production of this insecticide started in 1943, and the trade and wide use in agriculture began in 1945 (6). In the early 1960s, about 400,000 tons/year of DDT were used worldwide, about 75% of them being applied in agriculture.

DDT kills a wide variety of insect pests, such as houseflies, body lice, mosquitoes and beetles. This activity is heightened by its high stability. It can be manufactured by simple and cheap processes (7). DDT was also used in global malaria eradication campaigns by spraying inside and outside houses between 1955 and 1969. It eliminated the risk of malaria for about 700 million people mainly in North America, Europe, Soviet Union, Caribbean Islands and Taiwan. Most nations of sub-Saharan Africa, Papua New Guinea, and Indonesia were left out from the global eradication program. Between the 1940s and 1960s several African countries were involved in pilot projects of Indoor Residual Spraying (IRS) with DDT (8), as a result of which the malaria vectors and prevalence rates were reduced.

In 1962 Rachel Carson published “Silent Spring” warning on the harmful effects of pesticides on the environment. Carson suggested that the wide use of DDT could be the main cause of reduction of bird populations, many of whom were at the top of the food chain, like the peregrine falcon and the bald eagle. The safety of DDT for the human health and the environment was questioned. Based on evidence from its ecological impact, persistence in the environment and toxicity to birds, many countries began to ban DDT

use. Sweden prohibited to use it in 1970, the United States of America in 1972, and England in 1986 (9).

1.1.2. Physical-Chemical properties, analogues and metabolites

DDT as other OC compounds is hydrophobic, has a high chemical stability and tends to accumulate in human tissues. For this reason, it was included in the Stockholm Convention. The term DDT generally refers to 1,1'-(2,2,2-trichloroethylidene)-bis(4-chlorobenzene) or 1-trichloro-2,2-bis-(p-chlorophenyl)-ethane. DDT is produced by condensing chlorobenzene with chloral in the presence of concentrated sulfuric acid which acts as a catalyst (10). It is hydrophobic, colorless, crystalline solid with a weak chemical odor. It is nearly insoluble in water and very soluble in most organic solvents, fats, and oils. DDT has been sold under different trade names: Anofex, Cezarex, Chlorophenothane, Clofenotane, Dicophane, Dinocide, Gesarol, Guesapon, Guesarol, Gyron, Ixodex, Neocid, Neocidol, and Zerdane.

It occurs in mixtures of two isomers, 1-chloro-2[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (2,4'-DDT) and 1-chloro-4[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (4,4'-DDT). Commercial DDT is a mixture of several closely-related compounds. The major component is the 4,4' isomer with some 2,4'-DDT and small amounts of other analogues. In general the DDT commercial insecticide has the following composition: 4,4'-DDT (77.1%), 2,4'-DDT (14.9%), 4,4'-DDD (0.3%), 2,4'-DDD (0.1%), 4,4'-DDE (4.0%), 2,4'-DDE (0.1%) and impurities (3.5%). All DDT isomers are solids, white, crystalline, tasteless and odorless. In the environment, DDT breaks down to 4,4'-DDE which is extremely stable and resists further transformation. Physical and chemical properties of 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 2,4'-DDE, and 2,4'-DDD are listed in Table 1 (10).

DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane] and **DDE** [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene] are the most important DDT analogues, both exist as by-products in commercial DDT formulations and both may be formed by environmental DDT

transformation. DDT is dehydrochlorinated to form DDE and reductively dechlorinated to DDD (Fig. 1) by biological or environmental transformation (11). Other DDT metabolites are: DDMU (1-chloro-2,2-bis(p-chlorophenyl)ethane), DDMS (1-chloro-2,2-bis(p-chlorophenyl)ethane), DDNU (bis(4'-chlorophenyl)ethylene), DDOH (2,2-Bis(4-chlorophenyl)ethanol) and DDA (2,2-bis(p-chlorophenyl) acetic acid). This last one is non lipid soluble and eliminated through the urine of living beings.

Two different pathways for the metabolism of DDT have been proposed in rodents. The major urinary metabolite of DDT, DDA, is produced by a sequence involving reductive dechlorination, dehydrochlorination, reduction, hydroxylation, and oxidation of the aliphatic portion of the molecule. DDT is initially metabolized in the liver to two intermediary metabolites, DDE and DDD. In rats, DDE is slowly converted in the liver to DDMU and then to DDA. In hamsters and mice, the DDD to DDMU pathway seems to be a minor pathway, indicating that the removal of the alpha hydrogen from DDD is a rate limiting step in DDMU formation. The main difference between mice and hamsters is the relative inability of the latter to form DDE.

Figure 1. Degradation of DDT to DDE (left) and DDD (right) (12)

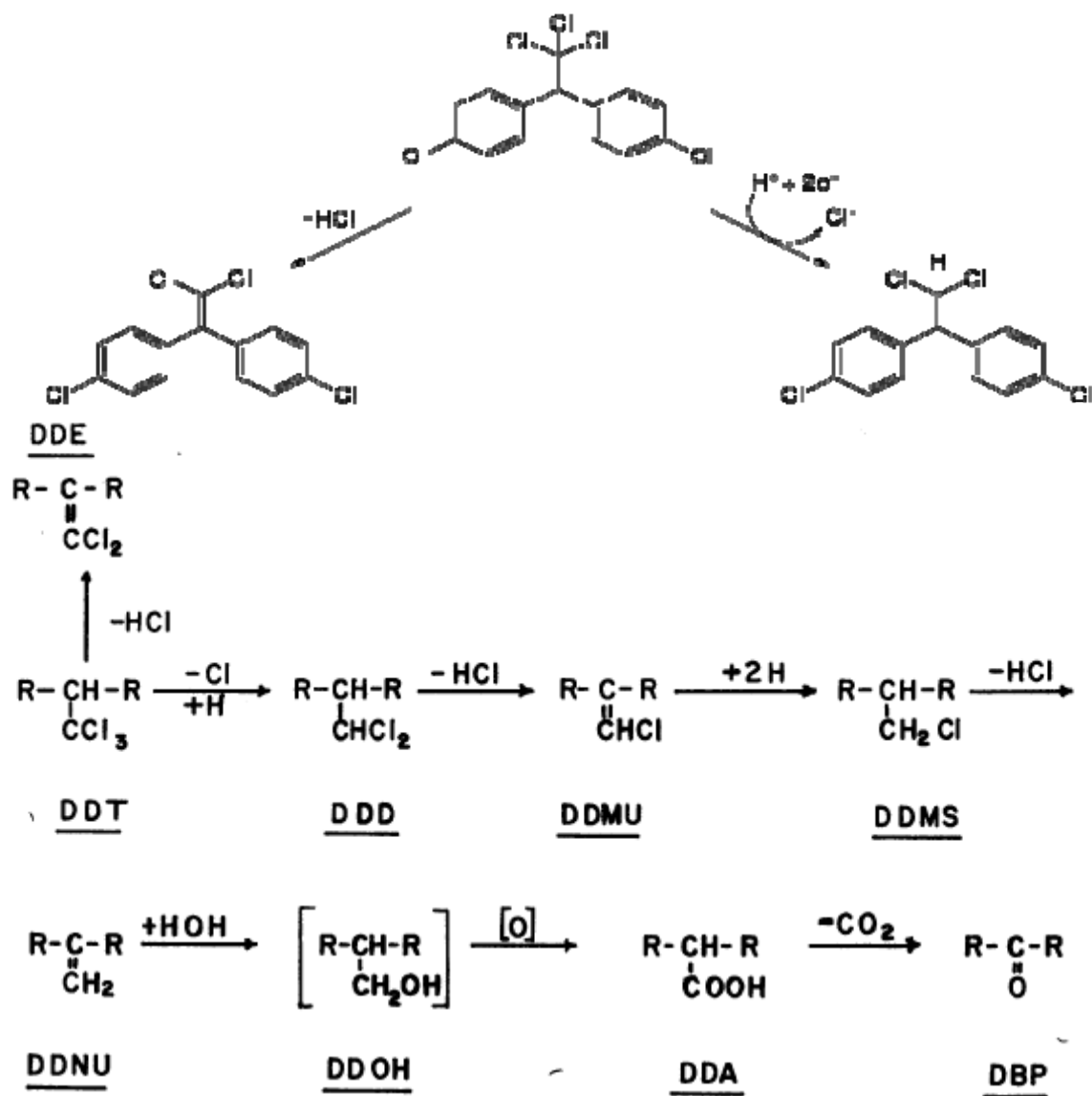


Table 1: Physical and chemical properties of 4,4'- and 2,4'-DDT, 4,4'- and 2,4'- DDE, 4,4'- and 2,4'-DDD

Property	4,4'-DDT	2,4'-DDT	4,4'-DDE	2,4'-DDE	4,4'-DDD	2,4'-DDD
Molecular weight	354.49	354.49	318.03	318.03	320.05	320.05
Physical state	solid	solid	crystalline solid	No data	solid	Solid
Melting point	109°C	74.2°C	89°C	No data	109-110°C	76–78°C
Boiling Point	Decomposes	No data	336°C	No data	350°C	No data
Density	0.98–0.99 g/cm ³	0.98–0.99 g/cm ³		No data	1.4 g/cm ³	No data
Empirical Formula	C ₁₄ H ₉ Cl ₅	C ₁₄ H ₉ Cl ₅	C ₁₄ H ₈ Cl ₄	C ₁₄ H ₈ Cl ₄	C ₁₄ H ₁₀ Cl ₄	C ₁₄ H ₁₀ Cl ₄
Water Solubility	0.025 mg/L; 25°C	0.085 mg/L; 25°C	0.12 mg/L; 25°C	0.14 mg/L; 25°C	0.090 mg/L; 25°C	0.1 mg/L; 25°C
Partition Coefficients						
Log Kow	6.91	6.79	6.51	6	6.02	5.87
Log Koc	5.18	5.35	4.7	5.19	5.18	5.19
Vapor pressure	1.60·10 ⁻⁷ torr; 20°C	1.1·10 ⁻⁷ torr; 20 °C	6.0·10 ⁻⁶ torr; 25 °C	6.2·10 ⁻⁶ torr; 25 °C	1.35·10 ⁻⁶ torr; 25 °C	1.94·10 ⁻⁶ torr; 30 °C
		5.9·10 ⁻⁷ atm-	2.1·10 ⁻⁵ atm-	1.8·10 ⁻⁵ atm-		
Henry's Law	8.3·10 ⁻⁶ atm-m ³ /mol	m ³ /mol	m ³ /mol	m ³ /mol	4.0·10 ⁻⁶ atm-m ³ /mol	8.2·10 ⁻⁶ atm-m ³ /mol

1.1.3. DDT toxicity

DDT is easily incorporated by organisms. Accumulation varies by species, duration and concentration of exposure, and environmental conditions. Their resistance to breakdown enables them to be stored in sediments and soils which can act as long-term depots. DDTs are released into atmosphere by agricultural spray, post-application volatilization, and wind erosions. The concentrations depend on the season and phase, being higher in the summer than in the winter and higher in the gaseous than in the particulate phase (13). Due to its presence in the atmosphere this compound may undergo long-range transport (10, 14) from source areas to the Arctic and Antarctic regions (15). Higher DDT levels than the EPA inhalation unit risk (0.097 ng/m³) have been found in indoor air of sprayed dwellings (16, 17). These indoor residues are also likely to contribute to the contamination of the surrounding environment as DDT disperses (16). This compound has also been found in countries where it has been banned (18). Another possible source is manufacture (19).

Soil constitutes an important compartment in the eco-environmental system for storage and transport of DDT. Its half life can range from 22 days to 30 years depending on the soil conditions. Worldwide, large amounts of DDT were released to soil during spraying operations or from direct or indirect releases during manufacturing, formulation, storage or disposal, favoring its infiltration to the subsoil and involving a risk to the water table (13, 20-22). DDT has also been found in soil, dried hay and wheat straw (23-25), vegetables and chicken (17) in DDT sprayed areas. DDT can be adsorbed by plants (24), (26). Thus, its occurrence in soil could involve potential danger to food safety and healthy life (27).

In lakes, DDT is absorbed by plants and animals living in them. Studies have shown that the concentration of DDT in lake water is only 0.002 ppb (parts per billion), but algae in the lake have concentrations of 2.5 ppm (parts per million). Fish feeding on the water trophic chain have DDT levels of 2 ppm. Piscivorous fish species, e.g. tiger-fish, and piscivorous birds, e.g. cormorants, have concentrations of 5 and 10 ppm, respectively. Crocodiles (who eat both tiger-fish and cormorants) have levels as high as 34 ppm.

By the phenomenon of global distillation, DDT and its breakdown products are transported from warmer regions of the world to Arctic areas, where they accumulate.

DDT use worldwide has benefited agriculture and relieved some of the health problems, but these gains come with price. It has been involved in causing reproductive abnormalities in wildlife (bird, fish, and mammals), hyperactivity, tremor, seizures and cancer in rats and nonhuman primates (28, 29). It can cause eggshell thinning in birds. It is also toxic to invertebrates (30).

Banning of DDT has allowed recovering of populations of birds (31) and many other animal species.

DDT and DDE are highly soluble in lipids. Their concentrations are higher in human adipose tissue (about 65% fat) than breastmilk (about 3% fat) and lower in blood (1% fat) (28). DDE is the most persistent DDT analogue, with a half-life of about 7-11 years. Some ingested DDT is converted to DDA. Non-metabolised DDT and DDE are stored in fat and eliminated via breast milk.

Dose-response studies in rats to assess liver effects were used to obtain information of oral Minimal Risk Levels (MRL) of DDT. MRL is 0.0005 mg/kg/day for oral acute exposure (10). The human Acceptable Daily Intake (ADI) level for DDTs is 0.01 mg/kg-body weight (32). Fatal poisoning or single dose toxicity depends on the solvent used. The Median Lethal Dosage (LD50) in rats is 250 mg/kg for oral intake, 250-500 mg/kg for dermal administration of DDT:oil solution, and 3000 mg/kg for dermal administration in powder (33). The oral LD50 values vary widely according to the species metabolism: mice, 152.3-1466 mg/kg/day; rabbits, 300 mg/kg/day; guinea pigs, 400 mg/kg/day (10).

1.1.4. Human Toxicity

DDTs and other OCs can be the first contamination source for the developing organism, as they are absorbed by pregnant women, distributed in the bloodstream of mothers, pass through the placenta to the fetus and accumulate in the lipid-rich tissues (34, 35). Once chemicals reach the fetus they may lead to severe repercussions for newborns (36) and might predispose to late adverse adult effects (37, 38). Pregnant women are exposed to DDT and their metabolites by ingestion of contaminated food (39, 40) and from environment inputs in sites where DDT is used in malaria control programs (41). Studies in laboratory animals show that breast milk exposure is quantitatively more important than exposure *in utero* (42). DDT is poorly absorbed by skin.

Immunological modulation

DDT and analogues are immunotoxic in animals and human. Studies in animals have shown that acute-, intermediate-, and chronic-exposure can have damaging effects on humoral and cell-mediated immune responses. The reported effects include decrease in antibody titers, increases in γ -globulin and serum immunoglobulin (Ig) and decreased tuberculin skin reaction (43-45).

Early childhood represents a critical period of immune development, as the infant's immune system is less developed and thus potentially more susceptible to environmental exposure (46). Environmental exposure during prenatal and early postnatal development has been linked to childhood diseases in which the immune response plays an important role.

Tumor necrosis factor (TNF) is an important proinflammatory cytokine that participates in the induction of immune responses to infectious agents and possesses direct antiviral activity (47). Prenatal exposure to DDE has been related with lower *in vitro* secretion of TNF by cord blood mononuclear cells (47).

DDT and analogues have potential for modulating the human immune response via reducing natural killer (NK) cells (48), lymphoproliferative activity and IgG levels (49), and increasing IgA (50) or altering white blood cell counts (51). DDT might also damage DNA (52-54) leading to apoptosis of peripheral blood mononuclear cells (55, 56). Some of these immunological alterations may have clinical consequences. Previous studies suggest that raised prenatal and early postnatal exposure to DDT and/or DDE increase the risk of childhood diseases such as asthma (57, 58), otitis media (5), upper and lower respiratory tract infections (59), aplastic anemia (60), but others have not found these associations (61). DDT is also suspected to reduce the response to vaccinations in infants, however a study in Brazilian children exposed to DDT found no association with diphtheria immunization response (62).

DDTs have also been associated with susceptibility to infections in adults, e.g. increased frequency of upper respiratory infections (63). Adults occupationally exposed to DDT had suppressed Th1 cytokines such as IL-2 and IFN- γ and induced Th2 cytokine such as IL-4. It has been hypothesized that this immunological abnormalities caused higher frequency of infections reported by the patients (64).

Neurodevelopment effects

There is increasing evidence that environmental exposures to pesticides may affect the progressive growth and neurobehavioral development of the infant in critical periods (65), even at relatively low doses. Infants are more susceptible to the effects of pollutants because their neurological system is still developing. Their immature cells and internal organs are sensitive to contaminants and are more likely to be affected by exposure (66).

Exposure to DDT in sensitive periods of prenatal and neonatal nervous system development of rats causes significant behavioral and neurochemical changes into adulthood (67, 68). Other studies have suggested that exposure to DDT during the brain growth spurt affects the density of muscarinic cholinergic receptors of the cerebral cortex and can cause behavior abnormalities in adult life (69).

Studies suggest that DDT and DDE may be associated with neurodevelopment deficits. A dose-dependent relationship between DDE levels in breast milk and hyporeflexia in infants has been reported (70). Conversely other studies found no association of cord blood DDE levels with hyporeflexia (71). Lack of association between perinatal DDE exposure and performance on infant intelligence has been reported (72). However, other studies found negative associations between serum DDE and cognitive, psychomotor and social development at age 13 months (73) and psychomotor development at ages 3, 6 and 12 month (74). Prenatal exposure to DDT and DDE has been related with cognitive function at ages 6, 12 and 24 months (75) and DDT with decrease in verbal, memory, quantitative and perceptual performance skills among preschoolers (76).

Cancer

In 1991 the IARC classified DDT as possible carcinogenic to humans (group 2B). Some studies have found a positive association between DDTs and breast cancer (77-82) but others do not support this association (37, 83-89). Elevated mortality from pancreatic cancer has been weakly associated with long exposure to DDT in the workplace (90, 91) but other studies have found no association (92). Exposure to 4,4'-DDT and 4,4'-DDE has been associated with mutation of the k-ras oncogene in pancreatic cancer patients [Porta et al. 1999]. The risk of developing liver cancer was associated with serum DDT but not DDE (93), and elevated mortality from liver cancer has been associated with high adipose DDE concentrations (94). Some studies have suggested that DDT exposure may be associated with lymphomas (95) but others have not found evidence of this association (96). Studies have investigated the association between DDT and genital cancers such endometrial cancer (97) and prostate and testicular cancer (98) but results have been inconclusive or do not support an association. For example, McGlynn et al reported association of DDE and risk of both seminomatous and nonseminomatous testicular germ cell tumors (99).

Many of these studies have limitations such as the lack of control for exposure to other chemicals, small sample size, and short follow-up time for long-latency cancer. These and other issues, for example the role of cofounders, make it difficult to draw definite

conclusions about exposure to DDT/DDE/DDD and cancer. Thus, taking all factors into consideration, the ATSDR concluded that the existing information does not support the hypothesis that exposure to DDT/DDE/DDD increases the risk of cancer in humans (100).

Endocrine disruption and reproductive health

An endocrine disruptor (ED) is “*an exogenous agent which interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development or behavior*” (101). DDT and metabolites may act as ED. They are estrogenic, interfere with hormone receptors and mimic estrogen activity. DDT has a relative binding affinity to estrogen receptors and DDE has weak anti-androgenic properties, by inhibiting binding to androgen receptors (102-105).

DDTs stimulate aromatase activity in human cell cultures (106, 107) which is involved in the synthesis of estrogens, e.g. estrogen reduction and progesterone increase in females, and androgen reduction in males. The DDT potential to alter androgen production has also been demonstrated *in vivo* in animals by decreasing testosterone concentrations in testis (108), altering the expression of androgen-dependent genes and decreasing weights of reproductive organs (109, 110). In humans, studies report higher serum estradiol levels among adult men highly exposed to DDE and DDT (111, 112), others did not provide any direct evidence for relationships between DDE levels and androgenic hormones (113-115). DDT and DDE have also been related with reduction in semen quality in young men (116, 117), with decreased fertility and early puberty (118).

DDT is estrogenic in female mice (119). DDT has been found to bind to the estrogen receptor alpha and to be a weak estrogen agonist, affecting the menstrual cycle in women (120-122) and enhancing miscarriage (123). However other studies have not found associations with menstrual-cycle (124) or miscarriage (125).

Disruption of the endocrine system is one of the mechanism by which DDT negatively affect the neurodevelopment of fetuses since thyroid hormones (TH) regulate the process of brain development during the prenatal and neonatal phase (126). The fetus is totally

dependent on maternal TH during the first trimester of pregnancy, and a slight difference in the concentration of TH during gestation can lead to major changes in children intelligence. DDT compounds have a high degree of structural similarity to the thyroid hormones, T4 and T3, and therefore may interfere with the binding to the receptors or transport proteins of the TH. Studies in animals have found a relationship between exposure to DDTs and reduction of T4, T3 levels and increases in thyroid stimulating hormone (TSH) (127-129). The findings on the interplay between DDTs and TH and TSH in epidemiological studies are inconsistent, some have found inverse relation (111, 130-132), while others have found no association (113, 133-137).

1.1.5. Exposure assessment

DDT, DDE, and DDD can be measured in biological samples such as serum, adipose tissue, blood, fat, urine, feces, amniotic fluid, semen, breast milk, or placenta (138-142). The major urinary metabolite identified in humans is DDA (143). The biological half-lives for elimination of these compounds are ranked as follows: DDE > DDT > DDD. Importantly, the **DDT/DDE ratio** is used as a rough estimate of the period of application of DDT. Higher DDT/DDE ratios indicate recent exposure while low ratios reflect old exposure (144, 145).

1.2. Pyrethroids

Pyrethrum is a naturally-occurring chemical mixture found in extracts from some chrysanthemum flowers (*Chrysanthemum cinerariaefolium* and *C. coccineum*). The insecticidal properties of pyrethrum were discovered around 1800 in Asia and were used to kill ticks and insects such as fleas and mosquitoes. The pyrethrum extract contains six active chemical compounds with insecticidal activity, these compounds are called pyrethrins. These compounds are slightly soluble in water and organic solvents like alcohol, kerosene, nitromethane, petroleum ether, carbon tetrachloride, and ethylene dichloride. Pyrethrins are used as pesticides in household insecticides and products to

control insects on pets or livestock. They may alter the nerve function, which causes paralysis in target insect pests, eventually resulting in death. They break down quickly in the environment, especially when exposed to sunlight (146).

Pyrethroids are a class of insecticides that are synthetic versions of pyrethrum. They are more toxic and persistent in the environment than pyrethrins (147). More than 1,000 synthetic pyrethroids have been developed, but less than a dozen are currently used. Pyrethroids are highly hydrophobic ($\log K_{ow} = 5.7-7.6$) and low water soluble (a few $\mu\text{g/L}$) (148). Despite these properties there is evidence of human pyrethroid metabolism and urine excretion of these compounds (149, 150).

The primary mechanism of pyrethroid action in insects and mammals is binding to the sodium channels of neuronal membranes, causing them to remain open for long periods of time, which disrupts the normal action (151, 152). The insect sodium channels are much more susceptible to pyrethroids than those of mammals (153, 154).

Pyrethroid compounds include: allethrin, bifenthrin, β -cyfluthrin, cyfluthrin, cypermethrin, cyphenothrin, deltamethrin, esfenvalerate, fenpropathrin, tau-fluvalinate, λ -cyhalothrin, γ -cyhalothrin, imiprothrin, cis-permethrin, permethrin, prallethrin, resmethrin, sumithrin (δ -phenothrin), tefluthrin, tetramethrin, tralomethrin, and zeta-cypermethrin (Fig. 2).

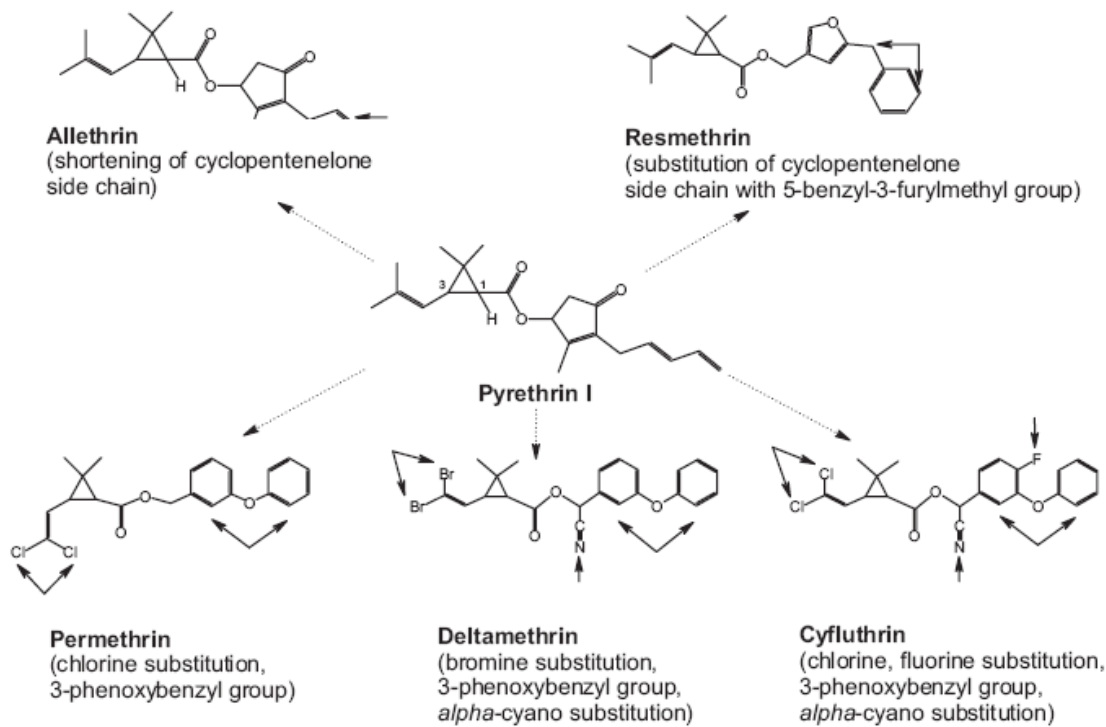


Figure 2: Chemical structures of pyrethroid insecticides (solid arrows marking modifications) (155). Some common pyrethroid insecticides, and their structural and chemical distinctions from the pyrethrins from which they have been synthesized.

Structurally, there are two different types of pyrethroids based on differences in the presence or absence of a cyano group in the α carbon of the 3-phenoxybenzyl alcohol moiety. Type I pyrethroids (allethrin and prallethrin) do not contain a cyano group, while Type II pyrethroids (deltamethrin, cypermethrin and fenvalerate) contain the α -cyano group. Both pyrethroid types show insecticidal action and low toxicity to mammals (156). In laboratory rodents, the symptoms of poisoning include whole body tremors for type I and salivation and sinuous writhing for type II. Type II pyrethroids have greater toxicity, and acute oral LD50 values are generally lower in Type II than Type I pyrethroids (149).

Pyrethrins and pyrethroids pose relatively little hazard to mammals (including humans) by common routes of exposure (inhalation, oral, dermal) at levels likely to be encountered in the environment or resulting from the normal use of pyrethrin- or pyrethroid-containing substances (149). The evidence of the toxicity of pyrethroids is mainly based on acute exposures in highly exposed populations. Studies have demonstrated that pyrethroids

produce reversible impairment of motor function and ‘knockdown’ in flying insect species that may be followed by death, depending upon the exposure level (157). In water invertebrates they induced chronic toxicity and cytotoxicity (158). The effects of intrauterine exposure to pyrethroids on the immune response in the offspring have been shown in animal models (159, 160).

In addition to urine, appreciable pyrethroid levels have also been detected in breast milk (161, 162). Studies have suggested that exposure to agricultural pesticides during the first trimester of pregnancy may increase the risk of gestational diabetes mellitus and hypertensive disorders of pregnancy (163, 164). Some studies have observed that chronic exposure alters plasma biochemical profile and decrease lipid peroxidation (165, 166). Pyrethroids have been related with higher prevalence of abnormal glucose regulation in humans (167). Potential hormonal effects have also been attributed to these compounds (168-170).

Little is known about the effect of these compounds in children and the role of intrauterine exposure in subsequent development. In vitro human models have shown that pyrethroids inhibit IFN- γ and IL-4 (171). Even though effects to humans are still unclear, some of them (cypermethrin, permethrin and biphenthrin) has been classified as possible human carcinogens(172).

1.3. DDT use for Public Health

1.3.1. Malaria

Malaria is a mosquito-borne infectious disease caused by *Plasmodium* protozoa. It is widespread in tropical and subtropical regions. The disease symptoms in humans result from the multiplication of malaria parasites within red blood cells, causing fever and headache, among other symptoms and, in a small percentage of cases, progressing to severe disease and death.

Malaria is a preventable and treatable disease. It is a public health problem in more than 100 countries inhabited by about 40% of the world's population. In 2009 there were an estimated 225 million cases of malaria and 781000 deaths worldwide (173). Children are especially vulnerable to malaria. In Africa, where 80% of malaria cases are treated at home, the disease kills one child in twenty before the age of five. Pregnant women are also at high risk of disease and death, with adverse impacts for their developing babies-including low birth weight, growth retardation, still births and death. In African countries, up to 60% of hospital admissions may be due to malaria (174).

Malaria is one of the major public health problems in Mozambique. Malaria is endemic throughout the country but the intensity of transmission varies from year to year and from region to region, depending on rainfall, altitude and temperature. Malaria is the most common cause of admission to hospital wards, accounting for approximately 40% of all outpatient attendance, 60% of paediatric admissions and 29% of all hospital deaths (case fatality rate vary between 1.8% and 9.9% depending on the level of the health facility), and contributes to the high maternal mortality observed (1500 per 100.000 births (175). Malaria results in significant economical losses in Mozambique, greatly limiting productivity, particularly among rural populations, and being a leading cause of school absenteeism.

In the late 1990s an intense debate erupted when negotiations for DDT ban were initiated against an increasing malaria burden. In 1999 the United Nations Environment Program

(UNEP) began negotiating a treaty to reduce and/or eliminate the use of 12 initial POPs (176). This led many groups such as Greenpeace, World Wildlife Fund and environmental organizations to advocate for a total DDT ban. The Malaria Foundation International (MFI) and the Malaria Project (MP) initiated a campaign against a DDT ban without replacement, arguing that DDT remains the most effective and least expensive method for preventing the transmission of malaria in many regions of the world. The MFI and MP campaign ended successfully and in 2000 the UNEP called for the complete elimination of eleven POPs and the continued use of DDT limited to disease vector control (177). In 2001 the Stockholm Convention on POPs classified DDT as restricted and allowed its continued use for disease control when there are no available safe, effective, and affordable alternatives, as recommended by and under the WHO. WHO 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 lives associated with unstable malaria transmission and epidemics is greatest. At present, DDT is still used in public health programs of several countries endemic for malaria.

1.3.2. Indoor Residual Spraying (IRS)

IRS is “*the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces*”; the effect is to reduce the life span of vector mosquitoes thus that they can no longer transmit malaria parasites from one person to another, and reduce the density of the vector mosquitoes (178). IRS has saved millions of lives around the world over the past 60 years (179).

IRS was shown to be efficient in reducing or interrupting malaria transmission in different epidemiological settings since the 1940s and 1950s, which led to introduction of IRS as a primary intervention for malaria control and eradication. The malaria incidence was reduced by about 90% in major areas of tropical Asia and Southern America (through a combination with other measures). Between 1950s-1970s, within the African malaria eradication pilot projects in which many countries participated (except sub-Saharan

countries), IRS reduced significantly the Anopheline population and malaria (178). In 1955 the WHO launched the Global Malaria Eradication Campaign based mainly on periodic use of DDT for IRS against the malaria vector mosquito in malarious areas.

The application of IRS consistently over time in large areas led to a shift in the geographical distribution of malaria coupled with a decline in the level of transmission and reduced to negligible levels the vector populations in Botswana, Namibia, South Africa, Swaziland and Zimbabwe (8, 180, 181). The WHO Pesticide Evaluation Scheme (WHOPES) evaluated and recommended 11/12 pesticides (Table 2) according to their safety, susceptibility, efficacy and cost-effectiveness, which can be used for malaria vector control: OC+ compounds, pyrethroids, organo-phosphates and carbamates. The insecticide selection in a given area is done on the basis of resistance, residual efficacy, costs, safety and type of surface to be sprayed.

DDT is a contact toxin, irritant and repellent for mosquitoes. It has been shown to be effective in controlling malaria, prevent entry into and promote exit from sprayed dwellings and, limiting the survival of the mosquito. It either kills mosquitoes resting on the walls, or repels them from entering into the dwelling (182).

During the mosquito gonotrophic cycle, the female *Anopheles* spends most of its time digesting blood meals on resting places because they are too heavy to fly far. Insecticide is sprayed on the walls and other surfaces inside dwellings where mosquitoes rest before or after a blood meal, thus IRS targets these mosquitoes by killing them before they have a chance to transmit malaria to others in the community. The greatest impact of IRS is not protection of individual residents, but community-level protection, which it accomplishes by cutting short the malaria transmission cycle. For the IRS programs to be completely effective at the community level, it is recommended spraying at least 80% of houses in a targeted area (178). This coverage is usually accomplished through IRS campaigns conducted once or twice a year according to the duration of the insecticide's effectiveness and the length of the malaria transmission season (179).

Table 2: Insecticides recommended for IRS against malaria (183)

Product	Class/Group	Dosage (g/m²)	Hazard classification of active ingredient	Duration of effective
DDT (WP)	Organochlorine	1_2	Class II	>6 months
Malathion (WP)	Organophosphate	2	Class III	2-3 months
Fenitrothion (WP)	Organophosphate	2	Class II	3-6 months
Pirimiphos-methyl (WP, EC)	Organophosphate	1_2	Class II	2-3 months
Bendiocarb (WP)	Carbamate	0.1-0.4	Class II	2-6 months
Propoxur (WP)	Carbamate	1-2	Class II	3-6 months
Alpha-cypermethrin (WP, SC)	Pyrethroid	0.02-0.03	Class II	4-6 months
Cyfluthrin (WP)	Pyrethroid	0.02-0.05	Class II	3-6 months
Deltamethrin (WP, WG)	Pyrethroid	0.01-0.025	Class II	2-3 months
Bifenthrin (WP)	Pyrethroid	0.025-0.05	Class II	3-6 months
Etofenprox (WP)	Pyrethroid	0.1-0.3	U	3-6 months
Lambda-cyhalothrin (WP, CS)	Pyrethroid	0.02-0.03	Class II	3-6 months

WP= water-dispensable powder; EC= emulsifiable concentrate; SC= suspension concentrate; WG= water dispersible granule; class II, moderately hazardous; class III, slightly hazardous; U, unlikely to pose an acute hazard in normal use.

IRS is effective if the target vectors are susceptible to the insecticide in use. The development of resistance to insecticides constitutes a major threat to the chemical control of malaria vectors, as it compromises the efficacy of insecticides. Resistance to DDT, pyrethroids and carbamates has been found in major malaria vectors. Resistance to carbamates has a mechanism that also induces cross resistance to organophosphates. DDT is the only insecticide within OCs which is still recommended for IRS, since resistance development is no longer influenced by other uses such as agriculture (178).

The Stockholm Convention encouraged reducing reliance on DDT and promoting research and development on safe alternative pesticides and strategies in Africa where malaria killed 709,000 people in 2009 (WHO 2010). Many of these efforts have been concentrated on the use of pyrethroids as an alternative to vector combat, which has been successful in some countries. However the malaria vector has become resistant to pyrethroid (184-186). Accordingly, more than 24 countries, most of them from Sub-Saharan Africa, requested exemptions on the ban of DDT for malaria vector control. These exceptions were ground on the evidence that DDT was the most effective insecticide due to its persistence (it can be sprayed just once a year in the homes), relatively low cost compared to others insecticides (cost per house for 6 months: DDT 1.6\$, deltamethrin 1.6\$, malathion 8.2\$, λ-

cyhalothrin 8.6\$, bendiocarb 13.8\$, fenitrothion 14.8\$ and propoxur 18.8\$) (187, 188) and efficiency. Reintroduction of DDT for IRS in malaria endemic countries showed a rapid decline in the number of malaria cases (179, 189).

Despite its contribution to the success of malaria eradication and control efforts, there are concerns about the potential harmful effects of DDT on the environment and on human health. High non-occupational exposure to DDT was shown in people who live in sprayed dwellings in the tropics and in the arctic populations who consume marine mammals. Biomonitoring data on general population non-occupationally exposed to DDTs showed that the levels in population of the tropics (IRS-treated houses) is about 60 times higher than the population living in the arctic (Fig. 3) (190). The use of DDT in IRS is rising in the tropics, and due to the global distillation effect this use could involve an ongoing input of DDT in the arctic.

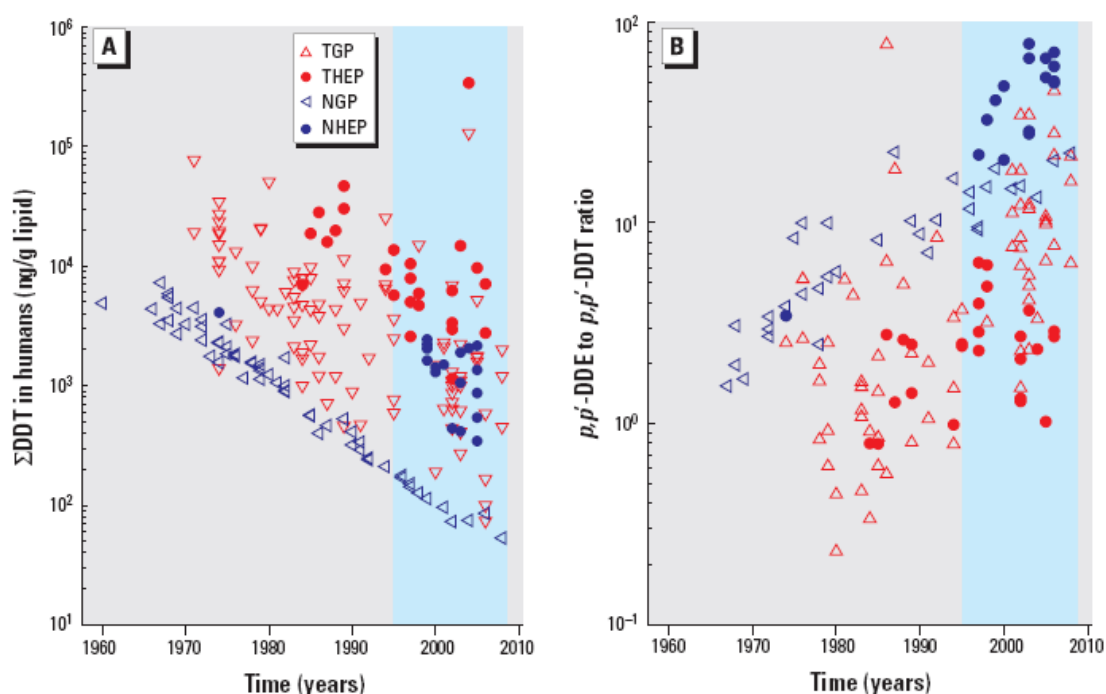


Figure 2: Temporal trend in human biomonitoring data. (A) ΣDDT. (B) 4,4'-DDE/4,4'-DDT. TGP (Tropics general population; THEP (Tropics highly exposed population- IRS-treated houses); NGP (North general population); NHEP (North highly exposed population- marine mammals' consumers)

1.3.3. Mozambique: malaria control and IRS

The strategies adopted by the Mozambican Ministry of Health (MISAU) for malaria control and prevention mainly in the most vulnerable groups (children < 5 years of age and pregnant women) included early diagnosis and appropriate treatment, integrated vector management (IRS, pyrethroid ITNs and environmental management), artemisinin-based combination therapy (ACTs), intermittent preventive treatment of pregnant women (IPTp), and health promotion at community level (191). Presently the burden of malaria in Mozambique is decreasing (179, 192). Several neighbouring countries, including South Africa, Zimbabwe and Zambia have large-scale IRS programs using DDT (173).

DDT has been widely used in Mozambique between 1946 and 1988 in agriculture and health programs. IRS with DDT and HCB for malaria control started in 1946 in the southern part of the country and stopped in 1956 resulting in reducing cases in children less than 5 years. In 1960 IRS with DDT was initiated again as part of malaria control programmes until 1971, when it was restricted to main towns due to the civil war (8).

After the cessation of the civil war in 1992, the range of insecticides used for agriculture increased in rural and peri-urban areas (193) but DDT use was forbidden. In 1993 there was a change in policy and the National Malaria Control Program (NMCP) decided to replace DDT by pyrethroids (Deltamethrin and λ -cyhalothrin) in IRS programs. In 1994 a limited control action was initiated in which λ -cyhalothrin, Deltamethrin, Cyfluthrin, all pyrethroids and baythroid insecticides were evaluated. Of these, λ -cyhalothrin was selected as the insecticide of choice (194).

In addition to the NMCP 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, and established to develop the Lubombo region into a globally competitive zone for trade and tourism, commenced operations in southern Mozambique in 1999 (195). In 1999 initial baseline resistance to pyrethroid began to be detected in malaria vector mosquitoes in the southern part of the country (185, 193, 196-198), which led to the implementation of changes from insecticide pyrethroids to

carbamate bendiocarb (two spray rounds annually) in November 2000 based on vector susceptibility, as part of the LSDI(199).

At the end of 2005 the Mozambican government withdrew its ban on DDT for IRS and it was re-introduced in the Maputo province. DDT has now become the main insecticide used for malaria vector control as IRS with no resistance detected in vectors (193, 198, 200, 201). The houses and structures are sprayed once a year and the application rate is 3 g per m². Nowadays bendiocarb and pyrethroids (λ -cyhalothrin and Deltamethrin) are used with DDT for IRS. However, anopheles became slightly carbamate-resistant and highly pyrethroid-resistant (198, 201). The primary insecticide used is DDT; bendiocarb and pyrethroids are applied on structures not suitable for DDT (painted walls) (192). At present DDT and carbamates are used exclusively for IRS, synthetic pyrethroids are used for impregnation of ITNs and organophosphate larvicides are used in environmental management.

Currently the use of DDT in agriculture is still banned, being restricted to malaria vector control in accordance with the WHO recommendations and guidelines. To prevent illegal uses, DDT is exclusively distributed through the MISAU, applied by their workers (Fig. 4). Teams were organized by the Provincial Health Department, Ministry of Environment and the Ministry of Agriculture which visit local markets and make unscheduled stock inspections of all operational base stores (191, 202). However, misuses as a consequence of poor management in rural areas cannot be excluded (202, 203). Until 2008 the MISAU have imported and distributed about 144 tons of DDT (191).

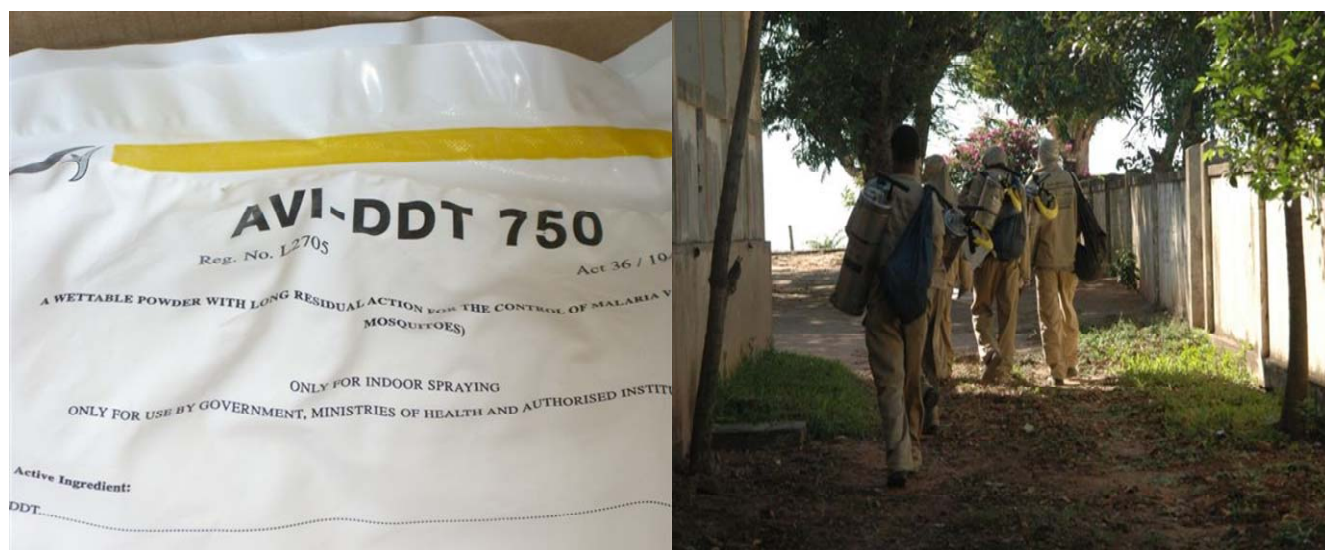


Figure 4: A DDT wettable powder pack imported by MISAU and spray workers in Manhica

1.4. Pyrethroids in malaria control programs

The synthetic pyrethroid used in IRS is deltamethrin (pure compound). It has 6 days mean half-life and it is estimated that 6 months after exposure the levels return to levels observed before exposure (204). The use of pyrethroid insecticides in malaria vector control has increased dramatically in the past decade through the scale up of ITN distribution programs and IRS campaigns (205). High coverage with ITN or IRS interventions can result in a dramatic reduction in malaria associated morbidity and mortality. These insecticides are the only class approved for impregnation of mosquito nets due to their relative safety for humans contact, their low mammalian toxicity and high knock down effect, irritancy and efficacy at low dosages for mosquitoes (206, 207).

Six pyrethroid insecticides have been evaluated by the WHOPES and recommended for treatment of nets: α -cypermethrin, cyfluthrin, deltamethrin, λ -cyhalothrin (α -cyano pyrethroids) and etofenprox and permethrin (non-cyano pyrethroids; Table 3) (208, 209). Pyrethroid resistance of malaria vectors has already developed in several malarious countries (184-186, 196-198). However, ITNs continue to have a powerful impact on

vector populations (210). There is not a suitable alternative insecticide for impregnation of mosquito nets (208).

Table 3: Chronic and oral/dermal toxicity of insecticides commonly used for treatment of mosquito nets. ADI (acceptable daily intake) and LD₅₀ (amount to kill 50% of tested population of rats), milligrams per Kg of body weight for rats (206)

Insecticide	ADI (mg/kg bw)	LD ₅₀ (oral)(mg/kg)	LD ₅₀ (dermal))(mg/kg)
alpha-cypermethrin SC	0-0.02	4932	2,000
Cyfluthrin EW	0-0.02	2100	>5,000
Deltamethrin 1% SC	0-0.01	>10 000	>10,000
Etofenprox EW	0-0.03	>5,000	5,000
Lambdacyhalothrin CS	0-0.01	>5000	4,000
Permethrin EC	0-0.05	5000-6000	4000-10 000

SC= suspension concentrate; EW= emulsion oil water; EC= emulsifiable concentrate.

1.5. Malaria prevalence in Mozambique

In Mozambique malaria transmission was never interrupted. There was a dramatic reduction in malaria prevalence following the introduction of IRS with DDT, mainly in the southern regions of the country. In 1946 malaria admissions dropped from 16% to about 8% in 1947 and to 3% and 1% in 1953 and 1954, respectively (181).

Recently, reductions in malaria infections have also been reported in the Maputo province

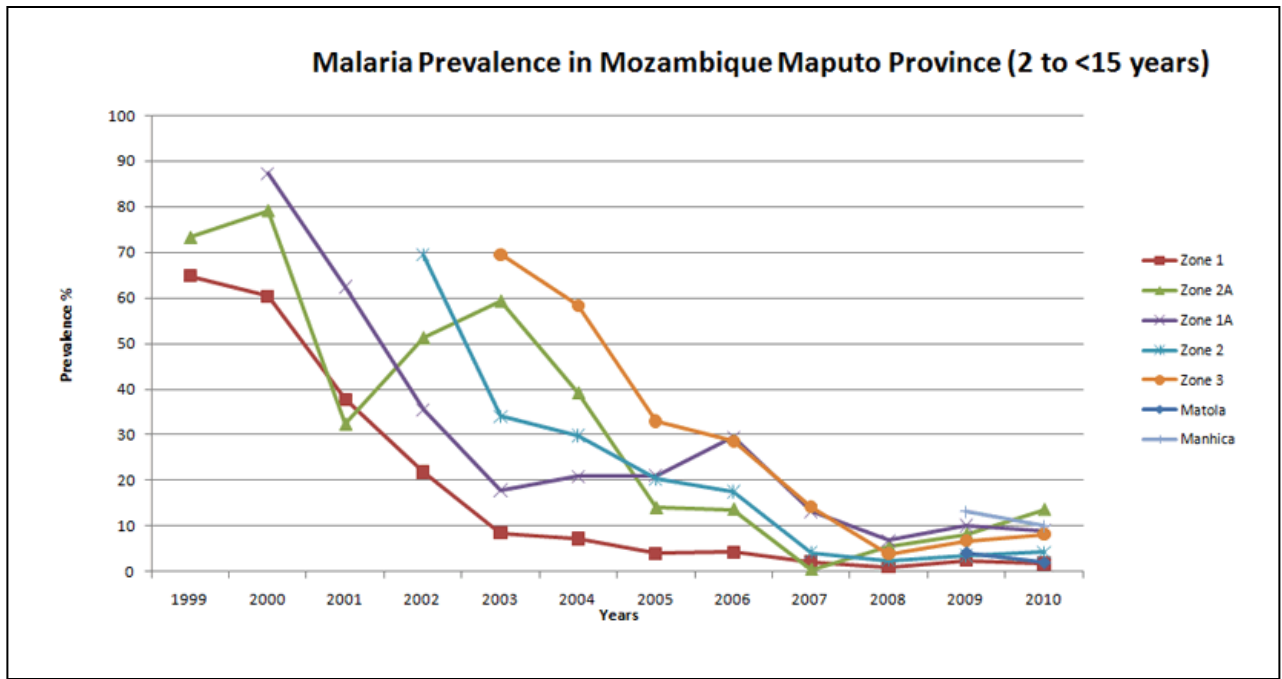


Figure 5: Malaria prevalence pre and post spray (1999 – 2010). Zone 1 (Catuane, Salamanga, Bela vista, Changanane, Namaacha, Zitundo, Ponta de ouro), Zone 2A (Boane), Zone 1A ((beleluane, Malhampsene, Mussumbuluco, Djuba, Matola), Zone 2 (Marracuene, Bobole, Machubo, Moamba, Ressano Garcia, Corumane, Mavuquane), Zone 3 (Mapulanguena, Motaze, Panjane, Chobela, Magude)

after years of successful control of vectors by IRS as part of the LSDI (Fig. 5) (211). Since 1999, annual parasite prevalence surveys have been conducted by LSDI in 3 zones of southern Mozambique: zone 1 which extends from the Kwazulu-Natal border to Maputo, zones 2 and 3 which extend north of the province along the Kruger national park border. In Zone 1 the average infection rate at baseline was 62 %, and reduced to 1.9% in 2007. In Zone 2, overall prevalence of infection at baseline was 70% and dropping to 5.1% in 2007. In Zone 3 the prevalence before spraying was 69.6%, after successive spray rounds the prevalence has fallen to 15.2% in 2007 (211).

1.6. Pesticide use in Manhiça

Manhiça district is a rural area located 80 km north of Maputo (Fig. 6 and 7). The CISM (Manhiça Health Research Center) has established a demographic surveillance system (DSS) since 1996. This system encompasses a census by numbering the houses and given identification to the inhabitants of each house. The district has an estimated population of 140,000 inhabitants of which 84,000 are under DSS.

The Incomati River crosses this area with great plains that are very favorable for agriculture. Most residents are farmers who grow sugar cane, bananas and rice, and some work in the two sugar processing factories. The houses are made of reeds with thatched or tin.

Manhiça has a sub-tropical climate with two distinct seasons: one warm and rainy between November-April and another cool and dry during the rest of the year. Full descriptions of the geographic and socio-demographic characteristics of the study area are reported elsewhere (212).

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Figure 6: Mozambique and Manhica district location

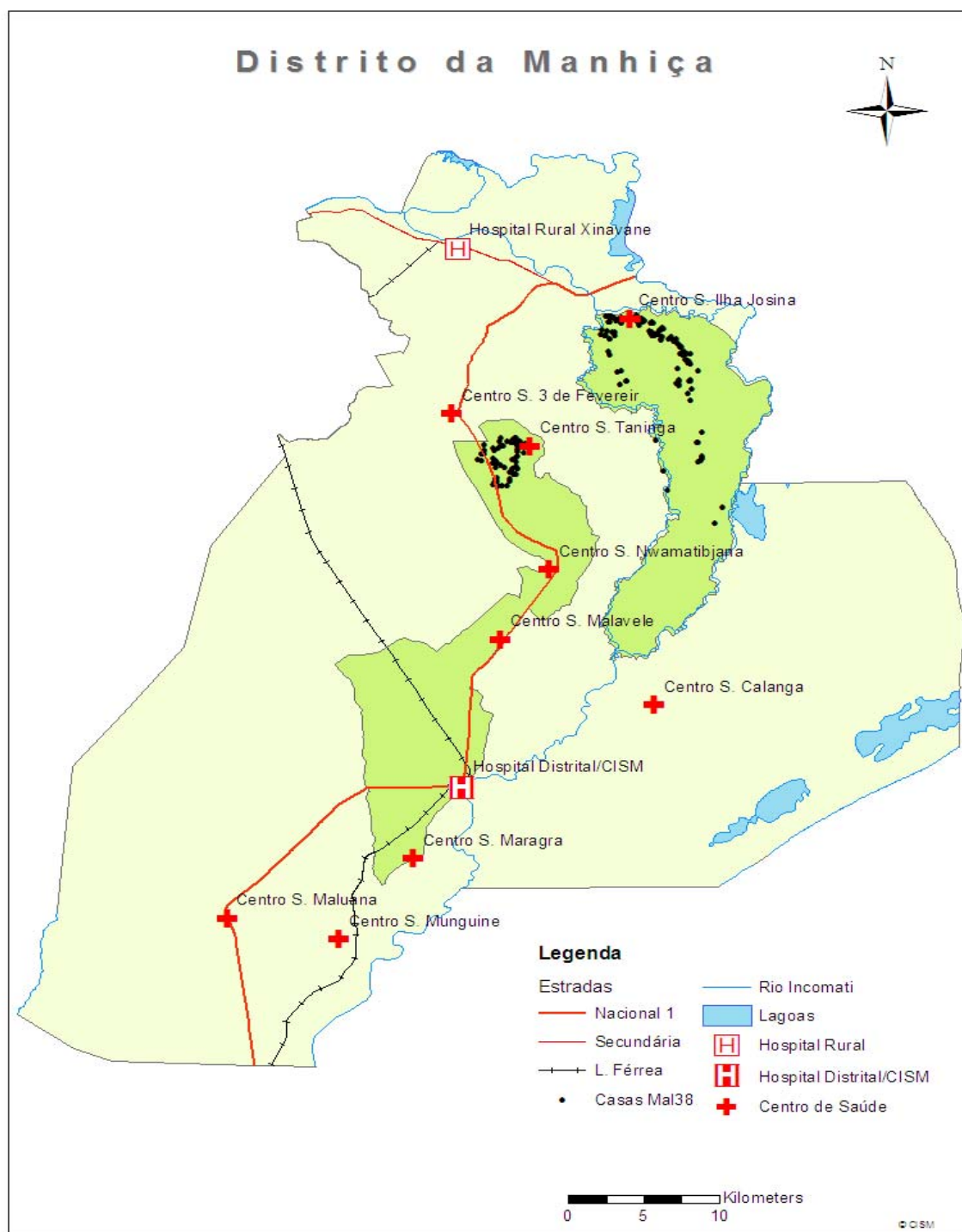


Figure 7: Map of Manhica district, (213)

In the Manhiça district, about 4% of the population is less than a year old. Neonatal mortality is 41.5 per 1000 and, in the first year of life, 88.9 per 1000. Malaria, respiratory and intestinal infections are the leading causes of hospitalization among children under 5 years. It is estimated that 100% of Manhiça children are breastfed at birth. However, at around 2 months of life they begin to take other liquids such as water and tea.

Malaria transmission is perennial with marked seasonality; IRS is done once a year to prevent the transmission of malaria through its vector. Until 2005 pyrethroids were used for IRS. The pyrethroids are widely used in agriculture and many families use mosquito nets impregnated with pyrethroids.

In the Manhiça district, DDT was reintroduced for IRS in September 2006 after a period of disuse. This compound is now used together with bendiocarb. Between September 2006 and 2010 there were five complete rounds of spraying.

The effective implementation of IRS also depends on the community acceptance and cooperation, by allowing access and removing some household contents prior to spraying. Reduced acceptability has been an impediment to effective IRS implementation in various parts of the world. The IRS in Manhiça is well accepted, mainly due the involvement of local government leaders. The acceptability isn't due to perceived efficacy of the spraying but is relates to political and socio-cultural issues such as citizenship and identity. (214).

Chapter 2. OBJECTIVES

General

To assess DDT exposure in women, newborns and human environments of the Manhica district (southern of Mozambique) prior and at the early stages of IRS with DDT for malaria vector control

To assess exposure to pyrethroids in women and human environments of the Manhica district prior and during reintroduction of IRS with DDT for malaria vector control.

Specific

1. To determine DDT levels and analogous compounds on thatch samples from dwellings during the reintroduction of IRS in late 2006.
2. To determine DDT levels on thatch from dwellings after its indoor spraying in 2007.
3. To determine the background DDT levels in breast milk before its reintroduction as IRS in 2006.
4. To determine the DDT levels in breast milk during IRS reintroduction in late 2006.
5. To establish the levels of DDT and analogous compounds in newborns before IRS reintroduction between 2003 and 2006.
6. To assess pyrethroid exposure in women through breast milk analysis.
7. To assess pyrethroid levels in thatch material of dwellings.

PART II. METHODOLOGY AND RESULTS

Chapter 3. METHODOLOGY

3.1. Samples available for the study

This thesis is part of the project EPSIMA (*Exposicao a Pesticidas y Saude Infantil em Manhiça*) which began in late 2006 as a collaborative study between the *Centro de Investigação em Saude de Manhiça* (CISM), the *Centre de Recerca en Salut Internacional de Barcelona* (CRESIB), the Institute of Environmental Assessment and Water Research (IDÆA-CSIC) and the Centre for Research in Environmental Epidemiology (CREAL).

3.1.1 Biological samples

Biological samples were obtained from studies conducted at the CISM in the Manhiça district between 2003-2009. As consequence of those studies there were archived samples stored at -80°C , which were subsequently used for this thesis. After sample selection women who participated in previous studies were identified. The research team explained the objectives of the study and asked permission to use part of their cord blood or breast milk sample for the EPSIMA study. The samples used in the present PhD thesis are from those who gave their written informed consent. Samples were identified and shipped in dry ice by *World Courier* from CISM to CRESIB and then IDÆA-CSIC in Barcelona for determination of pesticide levels.

3.1.1.1. Breast milk

The samples for this PhD research were obtained from two studies:

“The Epidemiology and Burden of Measles, *Salmonella* Typhi and *Shigella* Infections in a Rural Area of Southern Mozambique” (2002)

These samples were initially collected as part of the MEASLE study. One of the proposed studies inside the project was to determine the prevalence of measles, *S. typhi* and *Shigella* antibodies in breast milk of 50 lactating mothers who attended the maternal child clinic for EPI in 2002. The inclusion criteria were living in the study area, lactating, and agreeing to provide the informed consent. Mothers were asked to collect 5 mL of breast milk and to complete a brief questionnaire in which information of their name, birth date and residence neighbourhoods were collected (215).

“Descriptive study of the microbiological and biochemical composition of breast milk in a rural area of southern Mozambique-MICROBIOTA” (2006)

Those samples were initially collected as part of a study of breast milk microbiota. For the study, 121 women were enrolled in 2006. The inclusion criteria were living in the study area, giving birth in the last 12 months, breastfeeding, and providing informed consent. An exclusion criterion was having had fever and/or mastitis. The selection of subjects was randomized and stratified by area and age of the child. Demographic data collected from the mothers were: name, birth date, neighborhood of residence, disease, medical treatment, child birth, type of delivery, Middle Upper Arm Circumference of the mother, and number of children alive. After that, 5 mL of breast milk were collected by self-expression and a baby stool sample.

3.1.1.2. Umbilical cord

The samples for this PhD research were obtained from three studies:

“Study of the physiopathological mechanisms involved in placental malaria infection and its impact on fetal outcome-EPIC” (2003).

The objective of the study was to investigate the physiopathological mechanisms involved in placental malaria infection from a multidisciplinary histologic, cytometric, immunologic, parasitologic and molecular perspective and to evaluate the impact of placental malaria on fetal outcomes.

Pregnant women giving birth at the maternity ward in the CISM from 2003 to 2005 were included in 3 groups: active placental malaria infection (n=50), past placental malaria infection (n=50) and no placental infection (n=50). Peripheral, placental and cord blood were collected from each women.

The main inclusion criteria were: delivery at the maternity ward in the CISM, having a healthy newborn and giving informed consent. The exclusion criteria were: being part of other studies, abnormal delivery and having more than one newborn. The data collected were: obstetric history, age of the mothers, birth weight and gestational age. From this study, 50 women without placental infection were selected for this PhD thesis

“Intermittent Preventive Treatment during Pregnancy and use of ITNs -TIMNET-” (2003)

This study was aimed to evaluate whether administration of sulfadoxine-pyrimethamine as IPTp had any additional benefit over sleeping under ITNs. 302 pregnant women attending the Maternity Clinic between 2003 and 2006 were asked to give informed consent. Information on age, parity, gestational age and clinical history were collected onto standardized questionnaires. Cord blood samples were collected at delivery. Details of the study have been described elsewhere (216, 217). From this study, 50 women were randomly selected for this PhD thesis.

“Age of exposure and immunity to malaria in infants-AGEMAL” (2005)

The objective of the study was to determine the role of age and exposure to *Plasmodium falciparum* in infants on the rate of acquisition of naturally acquired immunity. 387 HIV-negative pregnant women resident in the Manhiça study area were recruited during the third trimester of pregnancy at the antenatal clinic from 2005 to 2007. Informed consent was sought to enrol their newborn children in the study upon birth. The inclusion criteria for newborns were birth weight ≥ 2 kg, lack of apparent health problems and having a healthy mother. Exclusion criteria included same gender twins, congenital malformations or birth asphyxia. Cord blood samples and demographic information (number of children, age) were collected at delivery. For the children the data collected were: birth weight, gender, use of ITNs, IRS and exclusive breastfeeding. A socio-economic questionnaire was also asked to the mothers. All cord samples available from this project were selected for this PhD thesis.

3.1.2. Environmental samples (thatch)

The thatch samples were collected, placed in sterile and resistant polyester bags (Kapak Corporation, Minneapolis, USA) provided by IDÆA, sealed using a heat sealer and stored at -20°C until the shipment. The samples were shipped at room temperature by *World Courier* from CISM to CRESIB and then to IDÆA for the pesticide analyses.

2006

When the EPSIMA project began in 2006 the sample collection for the MICROBIOTA project was underway. Under the supervision of the principal investigator of the MICROBIOTA project, the CISM fieldworker asked to women permission to also collect the thatch in the roof or wall of their dwellings to measure the pesticides concentrations and assess their environmental exposure. Information related to their activities, use of ITN and IRS was related.

2007

To measure the pesticide levels on the thatch after IRS, a second sampling was performed in 2007. The sampling and questionnaire were done by the same fieldworker who collected the straw in 2006 and the supervision and review of the questionnaires was made by the predoctoral student.

2008

A third sub-sampling was done in 2008 in different parts of the same dwellings. The objective of this sampling was to assess the degree of DDT variability in the same house. Four houses were chosen for study after the concentrations found in 2007; the two lowest and highest were selected. The thatch samples were taken from different parts of each house, packed and transported to IDÆA by the predoctoral student for the subsequent analysis of DDTs.

Chapter 4. RESULTS

4.1. Assessment of prenatal exposure to DDT from cord blood serum analysis in Manhiça (Mozambique) prior to its reintroduction for malaria vector control.

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Assessment of prenatal exposure to DDT from cord blood serum analysis in Manhiça (Mozambique) prior to its reintroduction for malaria vector control

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Abstract The concentrations of DDT compounds in cord blood of 214 children born between 2003 and 2006 in Manhica (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 despite these generally low concentrations the inputs of these compounds arriving to children *in utero* originated from recent uses of the insecticide. Children from mothers with secondary school level exhibited lower concentrations of these pesticides than mothers with lower degree of education. However, the strongest factor affecting DDT concentration was parity. Children from multiparae women showed much lower concentrations than primiparae women. 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.

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, the Stockholm Convention encouraged reducing reliance on DDT and promoting research and development on safer alternative pesticides and strategies. Many of these efforts were concentrated on the use of pyrethroids as an alternative to combat the vector, which was successful in some countries. However, the malaria mosquito became 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 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).

DDT was also introduced in 1946 in Mozambique where it was used widespreadly in agriculture and health programs until 1988. IRS programs with DDT started in the southern part (1946) and in 1950 all target areas were covered. 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, 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 this year initial baseline resistance to pyrethroids began to be detected in malaria vector mosquitoes (Casimiro et al. 2006a, b; Sharp et al. 2006) which led to change pyrethroids by carbamates (bendiocarb) in November 2000 (LSDI, 2006). In the Manhiça district, DDT was reintroduced for IRS in 2006 and was used together with bendiocarb. Between September 2006 and 2008 there were three complete rounds of spraying.

DDT monitoring in humans in the early ages 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 (Gladden 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. 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 of IRS for public health policies. However, no studies had previously been carried out to monitor the DDT levels in this country.

The present study focuses on establishing the levels of DDT and its metabolites in human cord blood in a rural area located south of the country. Samples were collected between 2003 and 2006, they provide representative examples of DDT accumulation prior to reintroduction of DDT for IRS. This baseline information will be useful for assessment of the intake of this compound in newborns from these communities as a consequence of the subsequent implementation of DDT for fighting against malaria.

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 babies' born from 2003 to 2006 at the Manhica Health Centre in the context of several birth cohorts 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 mothers signed an approved consent before they were enrolled in the study. Information on maternal age, parity, educational level, babies' sex, was obtained from the study data base. 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°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). 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). Lipid content could not be calculated in the samples due to the low volumes available. Concentrations below LOQ were substituted by half of the LOD. Σ 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 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).

3. Results and Discussion

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 cases.

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, mean 4,4'-DDE concentration in the whole study cohort was 0.8 ng/ml (standard deviation 0.9 ng/ml). Mean 4,4'-DDT concentration was 0.4 ng/ml (standard deviation 0.6 ng/ml). 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). Concentrations 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 et 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 in the Manhiça cohort 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 known to the authors of the present study since the official IRS program for implementation of DDT use in the region started by the end of 2006.

DDT concentrations and gender

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 ($p = 0.01$). These results are in agreement with a previous study on newborns from Veracruz ($n = 60$) but the higher concentrations of 4,4'-DDE in female children was not significant (Waliszewski et al. 2001). Conversely, in a previous study of a cohort from Menorca, gender comparison of cord blood levels showed higher 4,4'-DDE mean concentrations in male (1.85 ng/ml) than female (1.6 ng/ml) and higher mean 4,4'-DDT concentrations in female (0.2 ng/ml) than male (0.15 ng/ml) (Grimalt et al. 2010). In none of these cases the mean differences were statistically significant. The present results are in agreement with the Menorca study for 4,4'-DDT but not for 4,4'-DDE. That study involved 410 newborns (49% male). The population number in the present study is about half (Table 1) but the results for 4,4'-DDE were statistically significant. Further studies are needed for assessment of gender trends in the accumulation DDTs and OCs in newborns.

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 ($p = 0.015$) for 4,4'-DDT. No difference was observed between the groups of mothers having primary school and those illiterate. These results differ from observations in other cohorts in which maternal education level was also considered. 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 and to higher fish consumption in the group of more elevated OC and healthier women (Vizcaino et al. 2010). The reverse trend in Mozambique may reflect that mothers who have received higher education may have a higher degree of understanding of the risks of DDT and better skills to keep exposure to this insecticide low. This risk perception has perhaps been incorporated to the live style of their families. However, the number of individuals in this group was small ($n = 18$) involving that any interpretation of the observed difference must be done with caution.

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 ($p = 0.073$; 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). This difference between multiparous and primiparous mothers has also been 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). In all these cases lower concentrations were found in multiparous than primiparous. 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 concentrations in cord blood of newborns.

No relationship was observed between maternal age and cord blood concentrations of DDT species. Older women have higher body burden of these compounds than younger women (Sala et al. 2001; Vizcaino et al. 2010; Rhainds et al. 1999) and, in some cases, brought to live children with higher OC cord blood levels (Sala et al. 2001; Vizcaino et al. 2010; Rhainds et al. 1999; Carrizo et al. 2007). In Manhiça, there is a significant age difference between the primiparae and multiparae maternal groups, 18.7 and 26.0 years, respectively. According to these previous studies, this difference should be reflected in higher concentrations of DDT compounds and metabolites in the multiparae group. Conversely, 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 contrast between Manhiça and these previous cases is likely related to differences in the age distribution and parity of the cohort mothers. 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 younger, 22.8 years (age range 15.5-43.4 years), and the proportion of multiparae women (68%) was much higher than primiparae women (32%). Age was having less weight than parity in this last cohort whereas in Valencia the relative weight of these two factors 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. Nevertheless, this small increase between these two years was not significant considering the low number of participants in 2003 ($n = 5$). Comparison of 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. All these data reflect higher exposure to DDT in earlier dates than in the more recent period, just before implementation of IRS.

Examination of the temporal trends between 2004 and 2006 separating between primiparae and multiparae mothers showed 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 but the temporal evolution was towards decreasing concentrations in both groups. However, the study of the 4,4'-DDT/4,4'-DDE ratio showed a significant difference between primiparae and multiparae mothers in 2004 and 2005 being considerably higher in the latter (although the differences are 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 essentially affects older women. This group, despite their higher 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 which may reflect the increased detoxification trend of the latter group.

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. Children from mothers with higher degree of education (secondary school) had lower concentrations of these pesticides than illiterate mothers or those having primary studies. In this cohort, 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 Manhiça parity overcomes the age effect.

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 multiparae than primiparae women, and a subsequent detoxification as consequence of the maternal activities (deliver and breastfeeding). In this respect, the similar average 4,4'-DDT/4,4'-DDE ratios of primiparae and multiparae women in 2006 suggest that the qualitative effects of this previous exposure are no longer involving a significant difference between the two groups in this year.

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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
Min-Max	0.5 - 2.2	0.1 - 2.03	0.7 - 3.4	0.2 - 1.5
p95	2.2	2.03	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
Min –Max	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
Min –Max	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
Min –Max	0.04 - 4.02	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
Min –Max	0.04 - 6.3	0.02 - 3.4	0.08 - 8.5	0.04 - 2
p95	2.6	1.8	4.5	1.4

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

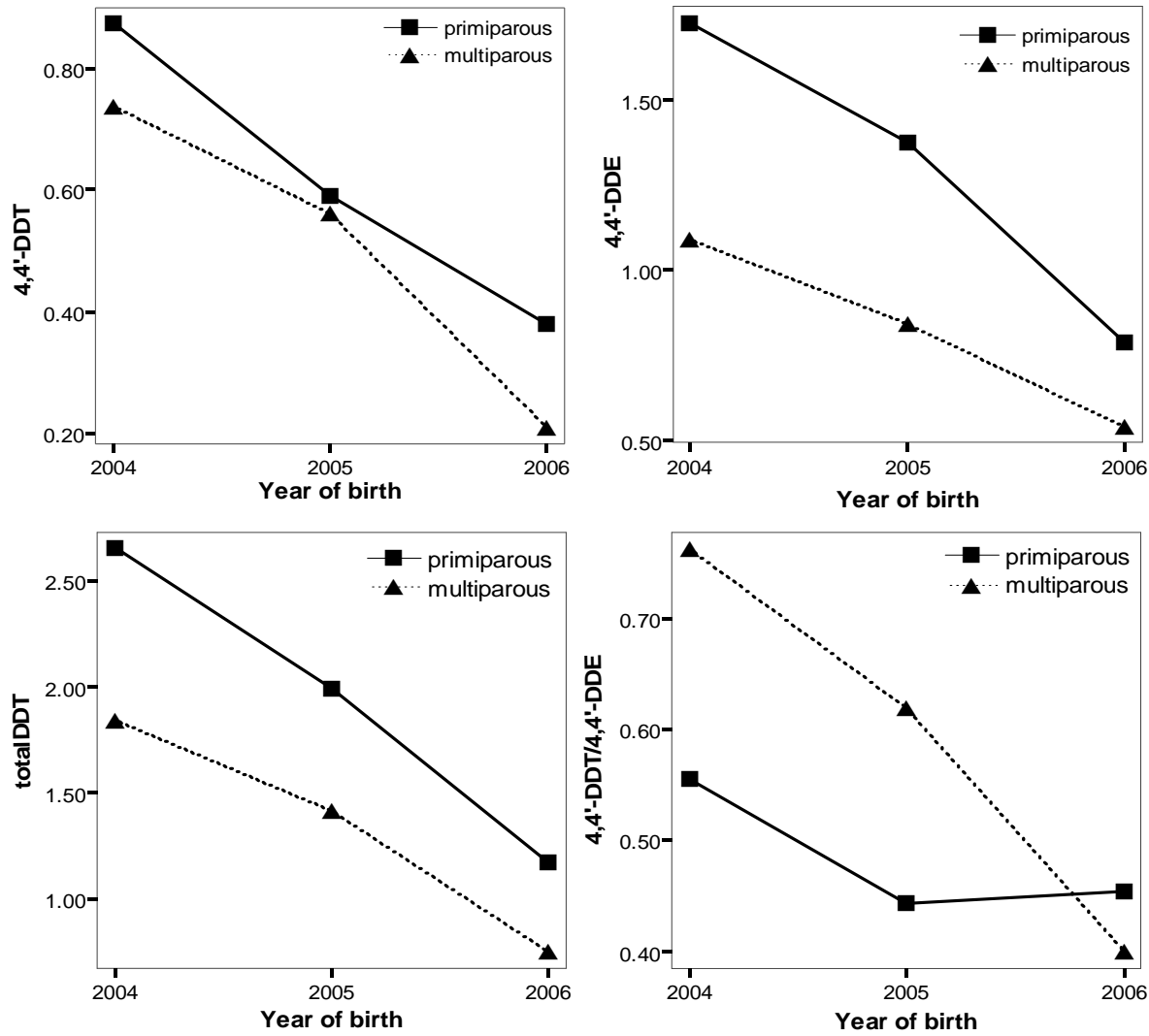
Table 4: Mean organochlorine compounds in cord blood grouped by newborn gender, parity and maternal education level.

	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
	<i>p value</i>	0.010	0.19	0.024
	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
	<i>p value</i>	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
	<i>p value</i>	0.353	0.015	0.159

*t student test. **Multiple comparisons using the F test

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).



4.2. Human exposure to pyrethroids due to agricultural and domestic applications for malaria prevention (Manhiça, Mozambique).

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Human exposure to pyrethroids due to agricultural and domestic applications for malaria prevention (Manhiça, Mozambique)

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Abstract

Presence of pyrethroid insecticides in human breast milk and in thatch wall material of dwellings from Southern Africa subtropical area (Manhiça, Mozambique) was investigated to assess potential pyrethroid route of human exposure. Human breast milk samples were collected during 2002 when pyrethroids were widely used as insecticides for mosquito bed nets in Mozambique for malaria control. The median concentration value of total pyrethroids ranged between 87 and 1200 ng/g lw, with λ -cyhalothrin being the most predominant pyrethroid in human breast milk contributing for 35% of the total amount. Moreover, and for the first time, an isomer-specific enrichment was found in human breast milk, showing a selective isomeric accumulation or metabolism in the human body. Based on the calculated pyrethroid concentrations in human breast milk, the daily ingestion rate of pyrethroid was estimated. The nursing infant dietary intake ranged from 0.67 to 9.0 μg (kg of body weight)⁻¹ day⁻¹. In addition, thatch materials collected after the reintegration of dichlorodiphenyl-trichloroethene (DDT) as insecticide residual spraying (IRS) in Mozambique, showed the presence of pyrethroids with concentration values ranging between 6.9 and 700 ng/g dw. In thatch material pyrethroid contamination was mainly attributed to the agriculture usage of this insecticide knowing that agriculture represent the 80% of the economy in Mozambique. However, a possible usage of this insecticide as IRS in Mozambique cannot be excluded despite their low efficiency for malaria control. The continued use of these compounds (both for agricultural and malaria prevention) and the ingestion rates calculated from the breast milk concentrations indicate that these insecticides cannot be overlooked for the assessment of the lactation risks of breastfeeding infants from the Manhiça region.

Introduction

In 2009, an estimated 250 million people contracted malaria and 0.85 million died (91% in Africa, 85% of them children under 5 years) ([WHO 2007](#)) The World Health Organization Pesticide Evaluation Scheme (WHOPES) ([WHO 2008](#)) supports the use of recommended insecticides for malaria control based on the evaluation of their human and environmental safety conditions ([WHO 2006](#)). In tropical Africa, domestic use of these insecticides involves treatment of bed nets ([Kapp, 2004](#)) and indoor residual spraying (IRS) on walls and roofs to kill the mosquitoes that land and rest there ([Montgomery et al., 2010](#)). These walls are constituted by thatch and branches, usually stems of *Typha* plants (particular *Typha latifolia*). One of the insecticides used for IRS is dichlorodiphenyltrichloroethene (DDT) but 11 others are also recommended including pyrethroids. The latest were introduced as alternative pesticides to reduce the use of DDT by international initiatives such as the Stockholm Convention and the Roll Back Malaria campaign.

In Mozambique DDT was introduced in 1946 for agriculture and health programs. The IRS program with DDT broke down in the late 1970s due to the civil war. After this event (1993), the National Malaria Control Program (MNMCP) decided to restart IRS with pyrethroids in suburban areas of most provincial capitals. However, *Anopheles funestus*, one of the main mosquito vectors became resistant to this group of insecticides ([Hargreaves et al., 2000](#); [Sereda and Meinhardt, 2005](#)). Thus, in 2000 carbamates (bendiocarb) were used in the rural areas of Maputo province within a coordinated effort for protection of the population of the Lubombo region (Mozambique, Swaziland and South Africa; [Mabaso et al., 2004](#)) while the use of pyrethroids was continued for mosquito nets. By end 2005, DDT was reintroduced for

IRS following the WHO recommendations for areas of potential human life loss as consequence of unstable malaria transmission and epidemics (WHO, 2006),

Pyrethroids are synthesized derivatives of pyrethrins, which are natural insecticides produced by certain species of chrysanthemum (*Chrysanthemum cinerariaefolium*). Even though effects to humans are still unclear, the US Environmental Protection Agency (EPA) has classified some of them (cypermethrin, permethrin and bifenthrin) as possible human carcinogens (Cox, 1996). Pyrethroids are persistent compounds with high hydrophobicity ($\log K_{ow} = 5.7-7.6$) and low water solubility (a few $\mu\text{g L}^{-1}$) (Laskowski, DA., 2002). Despite these properties there is evidence of human pyrethroid metabolism and urine excretion of these compounds (ATSDR, 2003).

The accumulation of some pyrethroids in human milk has been considered in a limited number of studies (Bouwman et al., 2006; Sereda et al., 2009; Zehringer and Herrmann, 2001) showing appreciable pyrethroid levels in breast milk together with DDT. In some individuals, pyrethroid levels were higher than DDT levels suggesting domestic and home garden use of the former, while the presence of DDT was attributed to activities for control of malaria vectors. Except for DDT, safety of insecticide residues in breast milk has not been considered during the WHOPES evaluation and very little is known on the effect of these chemicals to infants. This issue is important because milk is the best sole nutrient source for infants, particularly in Africa.

In the present study assessment of pyrethroid exposure in a rural area located in the south of Mozambique (Manhiça district) is undertaken. The study encompasses a comprehensive examination of the compounds belonging to the pyrethroid group, e.g. bifenthrin, λ -cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, fenvalerate, fenpropathrin, deltamethrin, tetramethrin, phenothrin and resmethrin, including the isomeric composition of some of these compounds. Human milk was

analysed as body burden estimate. Moreover, pyrethroid content in walls (thatch material) of dwellings was also determined for assessment of potential human exposure. To the best of our knowledge this is the first time in which this combined human-environmental approach is addressed.

2. Material and Method

2.1. Study area

Manhiça district is a rural area located in the Northern of Maputo province in Mozambique. The climate is subtropical characterized by a warm and rainy season between November-April and a dry and cold season during the rest of the year. Agriculture is one of the main economic activities, mostly dominated by rice, bananas and sugar cane.

2.2. Sample

Mature breast milk samples were collected in 2002 (n = 22) in the context of studies conducted at the Centro de Investigação em Saúde da Manhiça (CISM). The research protocol was approved by the ethic committees of Mozambique and Hospital Clinic in Barcelona. All women signed an informed consent before they were enrolled in the study. Samples were stored in sterile polyester containers at -80°C at CISM and at -20°C in IDÆA-CSIC until analysis, which was performed in this institute.

Dwelling thatch samples (n = 14) were collected during 2006-2007 in sterile polyester bags (Kapak Corporation, Minneapolis, USA) which were closed using a heat sealer and stored at -20°C.

2.3. Standards and reagents

All certified pyrethroid standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). They encompassed a standard mixture of seven pyrethroids, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin, phenothrin and tetramethrin and single analytical standards of bifenthrin, λ -cyhalothrin, esfenvalerate, fenpropathrin and resmethrin. d_6 -trans-permethrin and d_6 -trans-cypermethrin were used as surrogate standard. Hexane, dichloromethane and acetonitrile were obtained from Sigma Aldrich (St. Louis, MO, USA). The solvents used in this study were all pesticide grade.

The standard solutions were prepared in ethyl acetate. In order to check the linearity of the method two calibration curves were prepared at five different concentrations ranging between 0.08 and 2.5 ng mL⁻¹ (first curve) and between 5 and 45 ng mL⁻¹ (second curve). These calibration lines contained d_6 -trans-permethrin and d_6 -trans-cypermethrin at 45 ng mL⁻¹ and 22 ng mL⁻¹, respectively.

2.4. Sample preparation

Thatch material (0.3 g) and lyophilized breast milk (0.1 g) were placed in 40 mL glass-centrifuge tubes. They were fortified with d_6 -trans-permethrin (4.5 ng) and d_6 -trans-cypermethrin (2.5 ng) as surrogate standards. The samples were stirred and extracted by sonication with 20 ml of hexane:dichloromethane (2:1) in a Raypa, UCI-200 bath for 15 min. Then, the samples were centrifuged at 3500 rpm for 5 min. The organic phase remained at the top of the conical tube and was entirely transferred to a vial and evaporated under a nitrogen stream. This extraction step was repeated two additional times and all the solvent residues were collected together.

Thatch material extracts were cleaned up by elution through Florisil cartridges (2g/15 ml). Each cartridge was conditioned with 15 mL of ethyl acetate:dichloromethane (2:1). The sample was loaded onto the cartridges and the pyrethroids were eluted with 25 mL of ethyl acetate. The eluate was evaporated under a nitrogen stream and re-dissolved with 100 μ L ethyl acetate for GC-NCI-MS-MS analysis (Feo et al., 2010).

The breast milk extracts were cleaned up by elution through C18 cartridges (2g/15ml) coupled to basic alumina (5g/ 25ml) and conditioned with 25 ml of acetonitrile. Then the sample was redissolved in 30 ml of acetonitrile and then passed through the cartridge for pyrethroid elution. The acetonitrile extract was evaporated under a nitrogen stream and the residue was dissolved in 100 μ l of ethyl acetate for GC-NCI-MS-MS analysis.

2.5. GC-NCI-MS-MS operating conditions

GC-MS-MS analysis was performed in NCI mode on Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quad. A DB-5MS capillary column (15m x 0.25mm i.d., 0.1 μ m film thickness) containing 5% phenyl methyl siloxane was used with helium as carrier gas at constant flow of 1 ml min⁻¹. The temperature program was from 100°C (held for 1 min) to 230°C at 15°C min⁻¹, then from 230 to 310°C (held for 2 min) at 10°C min⁻¹, using the splitless injection mode during 0.8 min. Inject volume was 3 μ l. The inlet temperature was set at 275°C and ion source temperature at 250°C. Ammonia was used as reagent gas at 2.04×10^{-4} torr. More details on MS-MS condition and selected transitions were reported elsewhere (Feo et al., 2011).

2.6. Lipid content

Total milk lipid content was determined by crematocrit method ([Mayans et al., 1994](#)). However, due to the low breast milk volume available, lipid content was not calculated in all the collected samples, thus a median value was used for the calculation of pyrethroid concentrations.

2.7. Quality control

Recovery tests were carried out by addition of each pyrethroid to a thatch sample at concentrations of 16 ng/g dry weight (dw) and to a breast milk sample at concentrations of 100 ng/g lipid weight (lw) (Table 1). These samples were previously analyzed in order to determine pyrethroid presence before spiking. Five replicates were prepared for evaluation of the reproducibility of the method. Recovery values were higher than 77% in thatch and ranged between 48 and 91% in breast milk with relative standard deviation values lower than 3-20% (n=5; Table 1). Method detection limits (MLODs) defined as the minimum amount of analyte which produces a peak with a signal-to-noise ratio equal to 3 were determined for each single pyrethroid isomers by estimating the relative isomer abundance of the relative peak areas. They ranged between 0.10 to 75 pg/g dw and 3.1 to 1100 pg/g lw for thatch and breast milk, respectively (Table 1). Limits of quantification, defined as the minimum amount of analyte that produces a peak with a signal-to noise ratio equal to 10, ranged between 0.33 to 230 pg/g and between 8.3 to 3600 pg/g lw for thatch and breast milk, respectively.

2.8. Estimation of Nursing Infant Dietary Intake

In order to evaluate the magnitude of exposure to pyrethroids by infants, the daily intake (EDI) was estimated as $EDI_i = C_i F Mb$ where EDI_i is the estimated intake [micrograms per kilograms of body weight (bw) per day], C_i is the median

concentration of pyrethroid in milk samples (micrograms per grams of lipid weight), F is the lipid content in milk samples (grams of lipid per 100 g of milk) and Mb is the daily consumption of milk (grams per kilograms of body weight per day). The infant's average milk consumption (Mb), 175 g of milk (kg bw)⁻¹ day⁻¹, was calculated from US EPA recommendations (US EPA, 2002) by assuming an average daily intake breast milk rate of 702 mL of milk per day (723 g of milk per day) and a 1-month-old infant body weight of 4.14 kg. The mean value of lipid content in analyzed samples was used for F estimation, with a value of 4.4 g of lipid per 100 g of milk

3. Results and Discussion

3.1. Pyrethroid levels in human breast milk

Basic statistics of pyrethroid levels found in breast milk from Manhiça mothers are reported in Table 2. λ -Cyhalothrin and permethrin were detected in all samples followed by esfenvalerate/fenvalerate (found in 21 samples), cypermethrin (found in 20 samples) and tetramethrin and bifenthrin (found in 19 samples) while cyfluthrin was only detected in 9 samples. Phenothrin, resmethrin and deltamethrin were not found in any milk sample. The concentration ranges were 1.1-36, 16-440, 10-230, 11-220, 3.3-160, 9.7-200 and 6.7-230 pg/g lw for bifenthrin, λ -cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate and tetramethrin, respectively. The median values estimated from casewise data were 110 ng/g lw for λ -cyalothrin, 70 ng/g lw for tetramethrin, 60 ng/g lw for cyfluthrin, 55 ng/g lw for permethrin and 42 ng/g lw for esfenvalerate/fenvalerate. Total pyrethroid concentration ranged between 87 and 1200 ng/g lw. Figure 1 shows single pyrethroid contribution (%) to the total concentration. In this case, a pairwise statistical approach was used: the most predominant pyrethroid

was λ -cyhalothrin (35%) followed by permethrin (21%) cypermethrin, esfenvalerate/fenvalerate and tetramethrin (14%).

The literature on pyrethroid levels in human breast milk samples is very scarce. Our results can be compared to those found in human breast milk in Basle (Switzerland) during 1998/99 ([Zehringer and Herrmann, 2001](#)). In that study, 13 pyrethroids were analyzed with median concentration values ranging between 15 and 31 ng/g lw. In our study median concentration values of the 10 detected pyrethroid ranged between 87 and 1200 ng/g lw showing higher levels than those found in Basle. This was probably due to the different use of these insecticides. In Basle, pyrethroids were used only for agricultural and, in minor part, urban (e.g. pet sprays) applications ([Zehringer and Herrmann, 2001](#)).

More recently, Sereda et al., 2009 found high permethrin levels up to 1.2 μ g/g lw (mean value) which occurred together with cypermethrin and cyfluthrin at lower concentration in human breast samples collected from northern KwaZulu Natal, South Africa ([Sereda et al., 2009](#)). The authors associated the pyrethroid contamination to home garden and indoor use. In the same region, during 2006 Bouwman et al. found permethrin, cyfluthrin, cypermethrin and deltamethrin at concentrations of 14.5, 42, 4.2 and 8.4 μ g/l, respectively (Σ pyrethroid concentration of 31.5 μ g/l) which the authors associated to agriculture ([Bouwman et al., 2006](#)). These levels are higher than those found in our study. According to the estimated fat content of 4% ([Bouwman et al., 2006](#)) total pyrethroids ranged between 110 and 1050 with mean concentrations of 790 ng/g lw.

The pyrethroid concentrations found in Manhiça mothers are similar to those found in South Africa (Bouwman et al., 2006; Sereda et al., 2009). Thus, a significant concentration of pyrethroids in breast milk was found in 2002 despite the substitution

of these insecticides by carbamates for IRS. One source of these pyrethroids could be the bed nets that were still treated with this insecticide. In Manhiça, the main source of pyrethroid contamination can be attributed to their agricultural application. However, their continued use for IRS cannot be excluded despite the official change to another insecticide.

3.2. Pyrethroid levels in thatch materials.

Basic statistics of pyrethroid levels found in thatch material are reported in Table 2. Cypermethrin was detected in 13 samples followed by λ -cyhalothrin and tetramethrin (detected in 12 samples). Cyfluthrin and esfenvalerate/fenvalerate were found in 11 samples. Permethrin was found in 9 samples while deltamethrin, the pyrethroid used during the IRS program together with λ -cyhalothrin, were found in 8 samples. Bifenthrin, phenothrin and resmethrin were also found in a few samples. The concentration ranges were 0.45-7.7, 0.45-510, 0.45-695, 0.75-150, 0.50-210, 1.2-18, 2.9-30, 0.18-2.3, 0.52-3.1 and 0.05-0.76 ng/g dw for bifenthrin, λ -cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate, deltamethrin, tetramethrin, phenothrin and resmethrin, respectively. The median values estimated from pairwise data were 7.4 ng/g dw for deltamethrin, 4.5 ng/g dw for cyfluthrin, 3.5 ng/g dw for λ -cyhalothrin, 3.2 ng/g dw for cypermethrin, 2.8 ng/g dw for permethrin and esfenvalerate/fenvalerate. Total pyrethroid concentration ranged between 7.0 and 700 ng/g dw. In Figure 1 the most predominant pyrethroid was cypermethrin (contribution of 37% of the total amount) followed by cyfluthrin (25%) and λ -cyhalothrin (19%).

3.3. Exposure and bioaccumulation of pyrethroids

In Mozambique the agriculture represent the 80% of the economy, thus it makes sense to attribute pyrethroid contamination found in the thatch of dwellings to agricultural usage. However, pyrethroid half lives are estimated to range between 11.5 and 425 days in aerobic and anaerobic soils and between 1.83 and 619 days in water ([Oros et al., 2005](#) and [Laskowski et al., 2002](#)). This information and the common four year period for replacement of thatchs from the roofs of the dwellings indicate the presence of pyrethroid in the thatch of the dwellings during 2006-2007 must reflect a continued application despite the general regulations involving the use of other compounds as preferred insecticides against malaria vectors. Moreover, pyrethroid levels found in thatch materials collected during 2006-2007 were of the same order of magnitude to those found for DDTs in Manhica during the same years (total DDT median concentration values of 56 and 180 ng/g during 2006 and 2007, respectively) ([Manaca et al, submitted](#)). Thus, the amounts of pyrethroids used in the 2006-2007 were not small.

On the other hand, results on pyrethroid levels found in human breast milk demonstrate that mothers exposed to insecticide contamination accumulate pyrethroids that could be transfer to infants via breast milk. Some studies reported that pyrethroids are metabolized by humans: the chrysanthemic acid ester is usually cleaved via esterase or mixed function oxidase activity and the resulting alcohol moieties are converted to their corresponding acids. It is reported that these metabolites are partly conjugated to glucoronide and both the conjugates and free acids are excreted in urine ([ATSDR, 2003](#)). However, and based on our results, bioaccumulation of pyrethroid in women is evident and it seems to differ depending on the pyrethroid. Figure 1 shows the percentage contribution of each detected pyrethroid in thatch material and human breast

milk. The distribution patterns are different which may indicate changes in pyrethroid use through time or the combination of different pyrethroid sources, e.g. domestic and agricultural applications, in breast milk.

Pyrethroid molecules typically contain 2-3 asymmetric carbon atoms (chiral centers), making them a family of pesticides with high chirality. Figure 2 shows the relative contributions of the two isomers of permethrin and esfenvalerate/fenvalerate found in a commercial technical mixture (standard), as well as in thatch material and human breast milk. For permethrin, the abundance in the commercial technical mixture is 84% and 16%, for isomer I and II respectively. In thatch material, an abundance of 69% and 31% was found, showing a roughly similar distribution. However, for human breast milk samples, the percentage contribution of both isomers is very similar, with 52% and 48%, respectively. The observed enrichment in isomer II may reflect higher bioaccumulation potential of this compound or, conversely, a higher degree of human metabolization of isomer I. To the best of our knowledge this selective enrichment in isomer composition is described here for the first time. Analysis of milk samples in forthcoming studies are needed for a better understanding of the processes leading to this preferential accumulation.

In the case of esfenvalerate/fenvalerate, the abundance in the commercial technical mixture is of 60% and 40%, for isomer I and II respectively. These percentages were similar to those found in thatch material and also in human breast milk samples, with 60% and 40% and 56% and 44% for isomer I and II in thatch material and human breast milk, respectively. In this case, no differential isomeric behavior was observed.

3.4. Nursing Infant Dietary Intake Estimation

The concentrations of each pyrethroid found in breastmilk (C_i) can be used for the calculation of the infant's daily intake rates as described in section 2.8 of the present study. Maximum EDI values were 0.12, 0.28, 1.5, 1.7, 1.75, 1.8 and 3.4 $\mu\text{g}/\text{kg bw}$ and per day for cypermethrin, bifenthrin, esfenvalerate/fenvalerate, cyfluthrin, permethrin, tetramethrin and cyhalothrin, respectively (Table 3). These values can be compared to recommended EDI values reported by FAO and WHO 2005 for bifenthrin ($4 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$), cyfluthrin ($20 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$), cypermethrin ($20 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$), deltamethrin ($10 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$), λ -cyhalothrin ($5 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$) and permethrin ($50 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$) (FAO and WHO 2005 and FAO 2005). In general, the rates estimated from the mothers in Manhiça are lower than these recommended levels but the levels of λ -cyhalothrin in some mothers, the pyrethroid used during IRS program in Mozambique, were close to the EDI WHO-recommended value ($5 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$). Thus, pyrethroids cannot be ignored when considering the lactation risks of breastfeeding infants in this region.

4. Conclusions

Pyrethroids were found in human breast milk despite the discontinuation in the use of these compounds for IRS. Their occurrence may reflect an influence from the insecticide impregnated bed nets, agricultural sources or use for IRS in some cases despite their known low efficiency for malaria control. The presence of these compounds in breast milk confirms their bioaccumulation potential in humans. Some pyrethroid compounds are accumulated with isomeric discrimination. The concentrations of some of these insecticides found in some mothers, namely λ -

cyhalothrin used for IRS in Mozambique, involves EDI values close to the upper limits recommended by FAO. The presence of pyrethroids in wall thatch from dwellings evidences that these insecticides are still used for IRS. The observed occurrence of pyrethroids in dwellings despite the preferential use of other insecticides for IRS and the concentrations of some of these compounds found in human breastmilk evidence that these compounds need to be considered in the evaluation of infant risks associated to lactation in areas where insecticides are used for elimination of malaria vectors.

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Table 1. Analytical quality parameters of pyrethroid methodologies applied to thatch material and breast milk samples.

	Thatch material					Breast Milk				
	Blank (ng/g dw)	Recovery (%)	RSD (%)	MLODs ^a (pg/g dw)	MLOQs ^a (pg/g dw)	Blank (ng/g lw)	Recovery (%)	RSD (%)	MLODs ^a (pg/g lw)	MLOQs ^a (pg/g lw)
Bifenthrin	1.2	86	5	1.7	5.1	0.82	70	20	32	97
λ-Cyhalothrin	2.2	78	5	1.1	3.3	9.05	82	20	3.6	11
Permethrin	3.8	82	8	11; 9.5	33; 28.5	24	80	4	1100; 1100	3200; 3200
Cyfluthrin	1.1	85	20	2.7; 2.5; 0.10	8.1; 7.6; 0.30	nd	60	20	160; 160; 3.1	480; 480; 9.2
Cypermethrin	1.6	90	19	2.0; 4.5; 3.7	6.0; 13.5; 11	3.55	72	6	140; 140; 140	430; 430; 430
Es/fenvalerate	2.8	98	20	1.0; 0.90	3.0; 2.7	nd	70	7	63; 63	190; 190
Deltamethrin	3.0	104	20	13	39	nd	48	8	280	8.3
Tetramethrin	0.75	77	3	0.41; 0.75	1.2; 2.25	17	86	20	45	140
Phenothrin	0.84	60	20	67; 69	200; 210	nd	50	20	1100; 1100	3200; 3600
Resmethrin	Nd	87	20	69; 75	210; 230	nd	91	16	800; 780	2400; 2300
Total	17					54				

^a MLODs and MLOQs were estimated for each isomer of a specific pyrethroid.

Table 2. Basic statistics for single pyrethroid and Σ pyrethroid concentrations in thatch materials (ng/g dw) and human breast milk (ng/g lw) of Mozambique.

	Bifenthrin	λ -Cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Es/Fenvalerate	Deltamethrin	Tetramethrin	Phenothrin	Resmethrin	Σ PYR
<i>Thatch</i>											
Mean	2.9	59	80	17	24	5.4	11	0.68	1.4	0.43	162
Median	2.6	3.5	2.8	4.5	3.2	2.8	7.4	0.54	1.1	0.47	55
Max	7.7	510	695	150	210	18	30	2.3	3.1	0.76	700
Min	0.45	0.45	0.45	0.75	0.50	1.2	2.9	0.18	0.52	0.05	7.0
SD	2.6	150	230	45	60	5.2	9.1	0.59	1.0	0.36	220
<i>Milk</i>											
Mean	6.5	140	79	80	54	55	nd	80	nd	nd	425
Median	4.0	110	55	60	34	42	nd	70	nd	nd	370
Max	36	440	230	220	160	200	nd	230	nd	nd	1200
Min	1.1	16	10	11	3.3	9.7	nd	6.7	nd	nd	87
SD	8.0	120	62	65	50	44	nd	64	nd	nd	265

Table 3. Basic statistics of pyrethroid nursing dietary intake evaluation (expressed as $\mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$) in Mozambique.

	Bifenthrin	λ -Cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Es/Fenvalerate	Tetramethrin	ΣPYR
Mean	0.05	1.05	0.61	0.61	0.42	0.42	0.62	3.3
Median	0.03	0.85	0.42	0.46	0.03	0.33	0.54	2.8
Max	0.28	3.4	1.75	1.7	0.12	1.5	1.8	9.0
Min	0.01	0.13	0.08	0.09	0.00	0.07	0.05	0.67

Figure 1. Percentage contribution of each pyrethroid to total contamination estimated in thatch material and human breast milk collected from Manhiça (Mozambique).

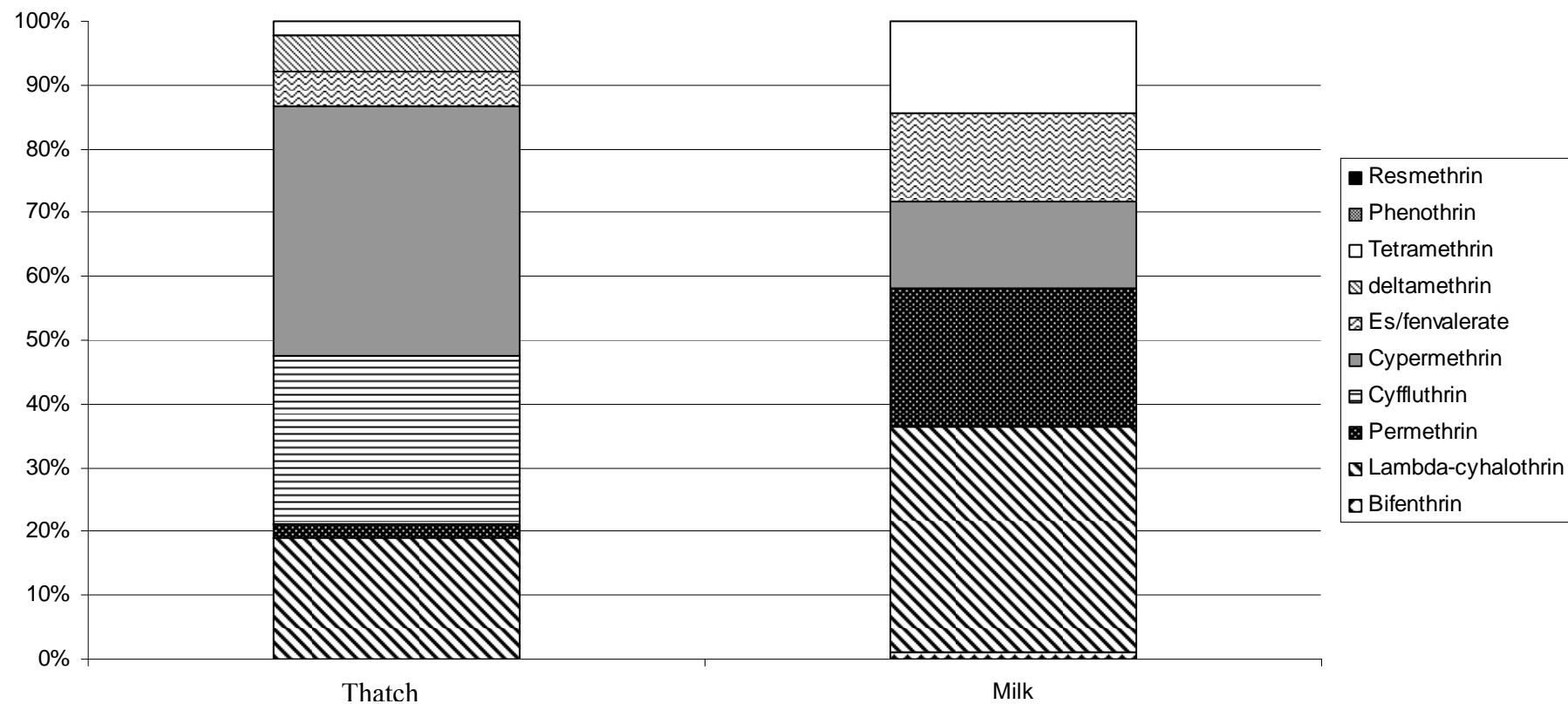
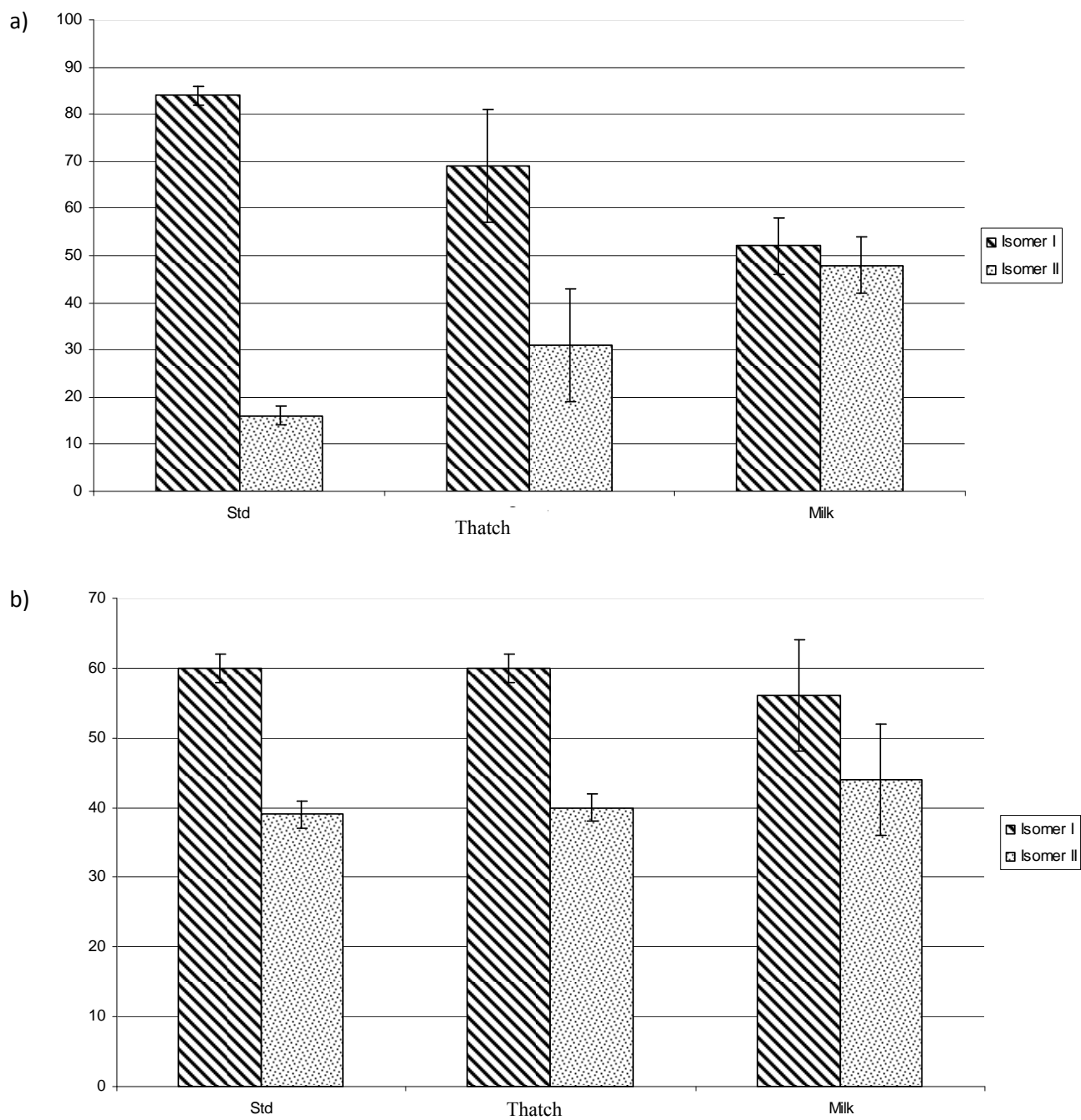


Figure 2. Abundance (%) of the two isomers of permethrin and esfenvalerate/fenvalerate in thatch material and human breast milk collected from Manhiça (Mozambique).



4.3. Assessment of exposure to DDT and analogous compounds after indoor residual spraying through the analysis of thatch material from rural African dwellings.

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Assessment of exposure to DDT and metabolites after indoor residual spraying through the analysis of thatch material from rural African dwellings

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Abstract

We report on the analysis of 4,4'-DDT and its metabolites in thatch and branch samples constituting the wall materials of dwellings from south African subtropical areas. This approach was used to assess the exposure to DDT in the residents of the dwellings after indoor residual spraying (IRS) following recommended sanitation practices against malaria vectors. Examination of the distributions of DDT compounds (2,4'-DDT, 4,4'-DDT and its metabolites) in 43 dwellings from the area of Manhica (Mozambique) has shown median concentrations of 19, 130 and 23 ng/g for 2,4'-DDT, 4,4'-DDT and 4,4'-DDE, respectively, in 2007 when IRS implementation was extensive. The concentrations of these compounds at the onset of the IRS campaign (n = 48) were 5.5, 47 and 2.2 ng/g, respectively. The differences were statistically significant and showed an increase in the concentration of this insecticide and its metabolites. Calculation of 4,4'-DDT levels in the indoor air resulting from the observed concentrations in the wall materials led to the characteristic values of environments polluted with this insecticide.

Key words: DDT, thatch, Typha, indoor residual spraying, air concentrations, malaria

1. Introduction

Indoor residual spraying (IRS) is one of the primary interventions for vector control in the efforts for reducing malaria transmission. Accordingly, long-acting chemical insecticides are sprayed on the walls and roofs of all structures in a determined area to kill the mosquitoes that land and rest there (Montgomery et al 2010). Dichlorodiphenyltrichloroethane (DDT) is one of the most effective insecticides due to its persistence and relatively low cost, it either kills mosquitoes resting on the walls, or repels them from dwelling. IRS with DDT is still recommended by WHO (2006; 2007) and at present it is an important source and direct input of the insecticide residues in African domestic environments (van Dyk et al 2010, Sereda et al 2009). Because of its toxicity, DDT use is at present restricted to public health purposes (UNEP 2008).

Residents are exposed to DDT through their daily activities in and around the house (Van Dyk et al 2010). Various studies have shown high DDT levels in the environment and populations of areas where DDT has been applied in IRS programs for malaria control (Van Dyk et al 2010, Röllin et al 2009, Barnhoorn et al 2009, Yañez et al 2001). They included measurements of the concentrations of this compound in indoor air, floor dust, outdoor soil, birds, sediment, food and water (Van Dyk et al 2010, Barnhoorn et al 2009, Singh et al 1992). However, the quantification of the insecticide residue remaining in the thatch and branches (usually stems of *Typha* plants, particularly *Typha latifolia*) of the wall materials of many dwellings (many of them also covered by daub) remain to be quantified in rural areas of malaria endemic African countries. In Mozambique, these materials are sprayed directly during IRS and constitute the DDT standing stock after application. Partial volatilization of the insecticide accumulated in the walls likely determines the DDT indoor air concentrations and therefore contribute to the exposure of inhabitants

At the end of 2005 house spraying with DDT in Mozambique was reintroduced in public health programs. DDT has now become the main insecticide for malaria vector control because no resistance to this insecticide has been detected in Mozambican mosquitoes (Casimiro et al., 2006; 2007). In the Manhiça district, in particular, DDT was reintroduced for IRS in 2006.

The present study describes the analysis of DDT levels in wall materials with the purpose of assessing the exposure of dwelling residents to the insecticide after implementation of IRS as a public health tool for malaria vector control. The study was carried out in dwellings from the Manhiça district, a rural area of southern Mozambique. This subtropical area provides a representative example of the current exposure levels associated with IRS in people living in thatch walled huts.

2. Materials and methods

2.1. Study area

The Manhiça district is located in the north of the Maputo province, 80 km from the capital city (Maputo), and has 159,000 inhabitants. There are two distinct seasons; one warm and rainy between November and April and another dry and cooler for the rest of the year. Most of the inhabitants are farmers who grow sugar cane, banana and rice, and some work in two nearby big sugar cane factories. The typical houses in the Manhiça area are cylindrical, with diameters and height of about 6 and 2 m, respectively. They are mostly made of reeds with roofs of corrugated zinc or thatch. The thatches from the roofs of the houses are usually replaced every four years. These dwellings are similar to others studied in Vhembe District (Limpopo Province, South Africa; van Dyk et al, 2010) that are also built in a compact circular arrangement (diameter 3-5 m). Their roofs are also made of thatch and the walls consist of a mixture of mud and cement.

The DDT used for IRS in Mozambique is a 75% wettable powder (CAS register number: 59-29-3). One sachet of 670 g of DDT is mixed into a portable sprayer with 10 L of water and shaken before spraying. This is done by the IRS spray teams from the Mozambique Ministry of Health (MISAU).

Typical dwellings in Manhica involve a total wall and roof surface area of about 38 and 28 m², respectively. In the current IRS practice, about 3 g/m² of DDT are sprayed, which involves about 200 g per regular dwelling.

2.2. Sample collection

The dwelling thatch samples included in the study were collected in May 2006 (n = 48) and February-March 2007 (n = 43) and were taken from the same dwellings. In September 2006 the MISAU expanded the IRS with DDT into the Manhica district through the National Malaria Control Program.

The samples were collected, placed in sterile and resistant polyester bags (Kapak corporation, Minneapolis, USA), sealed using a heat sealer and stored at -20°C until extraction.

Ethics approval for the study was obtained from the National Mozambican Ethics Review Committee. Informed consent was obtained from all owners of the dwellings prior to thatch collection.

2.3. Chemical analysis

Standards of tetrabromobenzene (TBB), polychlorinated biphenyls (PCB) 142, PCB 200, PCB 209 and DDTs 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 sulphuric acid, dichloromethane (DCM), methanol, cyclohexane, and *n*-hexane were also from Merck.

2.4. Extraction procedures

Samples were thawed at room temperature before extraction. Then, they were minced by stainless steel scissors and homogenized with liquid nitrogen in mortars. About 0.5-2.5 g of the homogenized material was put in 25 mL tubes and extracted with DCM: hexane (1:4). TBB, PCB 209 and PCB-200 were added as surrogate standards and the mixture was

vortexed for 1 min at 2000 rpm for homogenization. Extraction was performed after addition of 20 mL of solvent mixture and sonication for 20 min and subsequent centrifugation for 5 min at 2000 rpm. This last step was repeated three times. The combined extract of 60 mL of solvent was reduced to 1 mL by vacuum rotary evaporation operated at 30°C. The solution was transferred into a 10 mL centrifuge tube using several rinses of *n*-hexane (approximately 3 mL), and 2 mL of concentrated sulfuric acid were added. After reaction, the mixture was stirred for 30 s, centrifuged for 10 min and the remaining sulphuric acid solution was discarded. Subsequent clean-up with concentrated sulphuric acid was done. The supernatant *n*-hexane phase was transferred to a conical tube and reduced to nearly dryness under a gentle nitrogen flow. The extract was transferred to gas chromatography (GC) vials with four 25 µL rinses of isooctane. Before injection it was re-evaporated under nitrogen and 100 µL of a PCB142 standard were added as injection standard (Grimalt et al., 2010).

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 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°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). The 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. $LOD = \text{mean of all blanks} + 3 \text{ times the standard deviations}$. $LOQ = \text{mean} + 10 \text{ times the standard deviation}$. When the compound was absent from the blanks, LOD and LOQ were determined from instrumental limits of detection using injection of dilutions of standards.

Concentrations below LOQ were substituted by half of the LOD. Univariate statistics were calculated as customary (Rothman et al., 2008). This method performed satisfactorily in repeated international intercalibration exercises within the Arctic Monitoring and Assessment Programme (AMAP, 2004). Total DDTs (ΣDDT) were calculated by the sum of the concentrations of 4,4'-DDT, 2,4'-DDT, 4,4'-DDE, 2,4'-DDE, 4,4'-DDD and 2,4'-DDD. The DDT/DDE ratio was calculated by the division of 4,4'-DDE by 4,4'-DDT. In order to obtain a normal distribution, data were log transformed for application of t-tests to compare mean concentrations of the same dwellings sampled in 2006 and 2007. All statistical analyses were performed using the Statistical Package for the Social Science (SPSS for Windows version 15), and the figure was produced with GraphPad Prism5. The statistical significance was set at $p < 0.05$ (two sided).

3. Results

The variation of the concentrations in the wall materials after IRS could be assessed by replicate sampling of wall materials from different sites in the same dwelling and independent analysis of each collected material ($n = 3$ or 4 ; Table 1), this subsampling was done in 2008. The observed standard deviation of 4,4'-DDT concentrations from the same dwelling ranged between 17-65% of the mean values. Similar percent values were observed for the standard deviations of 4,4'-DDE and 4,4'-DDD except when the concentrations of some metabolites were found below the limit of detection which

involved higher standard deviation values. Within these ranges, the degree of variation between samples from the same dwelling did not seem to be influenced by the absolute concentrations. Table 2 shows the levels of DDTs (ng/g) in thatch samples from this study in Mozambique compared to DDT levels found in similar (but not equivalent) materials in studies conducted in other countries.

Thatch collected in 48 and 43 dwellings in 2006 and 2007, respectively, were analyzed. In 2007 we could not repeat sampling in five dwellings sampled in 2006 and two other dwellings were not sprayed in 2007. In order to perform a close t-test mean comparison, we excluded from the calculation of the mean values of 2006 the dwellings that were not sprayed or sampled in 2007. Therefore, the results in Table 3 are just based on 41 thatch pairs. 4,4'-DDT, 2,4'-DDT, 4,4'-DDE and 4,4'-DDD were quantifiable in 94%, 92%, 98% and 98% of the samples of the first group, respectively. In the 2007 group, 4,4'-DDT, 2,4'-DDT, 4,4'-DDE and 4,4'-DDD were found at quantitative levels in 98%, 95%, 93% and 93% of the samples, respectively. Total DDT concentrations ranged between 0.6 ng/g and 6100 ng/g in the materials collected in 2006. The predominant isomer was 4,4'-DDT (median = 49 ng/g) followed by 4,4'-DDD (median = 5.2 ng/g) and 4,4'-DDE (median = 2.2 ng/g). The 2,4'- isomers for DDE, DDD and DDT (0.2, 1.0 and 7.5 ng/g, respectively) were in lower concentration than the 4,4'- isomers except in two samples from the 2006 series.

The materials sampled in 2007 were from sprayed dwellings, the concentrations of total DDT ranged between 12 ng/g and 29000 ng/g (Table 3). The predominant isomer was 4,4'-DDT (median=150 ng/g) followed by 4,4'-DDD (median=23 ng/g) and 4,4'-DDE (median= 21 ng/g). The concentration of the 2,4'- isomers for DDE, DDD and DDT (0.3, 5.4 and 21 ng/g, respectively) were lower than those among the 4,4'- isomers. Both in 2006 and 2007 the concentrations exhibited large standard deviations as a consequence of the high values in some dwellings. The 2007 dataset exhibited higher maximum concentrations than that of 2006. These high values were consistent with the spraying in September 2006 of the studied dwelling. They showed a strong dispersion in DDT concentrations among the different dwellings after IRS.

The concentrations of all DDT isomers and their metabolites were higher in thatch samples collected in year 2007 than in 2006. Comparison of the means of the log-transformed data of these two sample populations show that their differences were statistically significant in all cases ($p < 0.05$) (Fig. 1). The comparison is useful to support that average concentrations were significantly higher after IRS despite referring to two independent episodes. As mentioned above, the two means referred exactly to the same dwellings before and after IRS.

4. Discussion

In this study we found that the concentrations of 4,4'-DDT were higher (10 times or more) than those of the other isomers in all samples, except in two from the 2006 series. This finding is consistent with recent use of the commercial insecticide. Conversion of 4,4'-DDT into 4,4'-DDE starts after application but the half-life of the former could be years. Accordingly, the historical environmental DDT record is dominated by 4,4'-DDE because this is the most chemically stable metabolite of the insecticide. In Manhiça dominance of 4,4'-DDT was observed even in samples with low total DDT levels, indicating that the whole area has been under the influence of emissions from this insecticide, maybe as a consequence of misuses and poor management (MISAU 2006).

Total DDT levels in thatch in 2007 were significantly higher than in 2006. The strong difference may reflect the more widespread application of IRS in 2007 compared to 2006. The 4,4'-DDT levels measured in 2007 were considerably higher than those found in other types of applications such as thatch treated with DDT in Mexico (Waliszewski et al 2004). However, they were lower than those found in Tanzania in dried hay samples collected in the vicinity of an old storage site, which represented a point source of contamination (Marco et al., 2005). It has been reported that DDT levels increase in commodities stored in rural houses treated with DDT for malaria control (Battu et al 1989, Singh et al 1991).

The total 4,4'-DDT medians in the Manhiça thatch samples were similar to those found in Indian wheat straw samples stored in dwellings treated with IRS (Battu et al 1989).

The results shown in Table 2 indicate that the standard deviation of the distribution of 4,4'-DDT as consequence of IRS is in the order of 17-67% of the mean concentration values. The DDT metabolites exhibited about the same figures.

Part of the DDT adsorbed in the thatch evaporates into the dwellings. The concentrations in the wall materials can be used to estimate these levels in the indoor air using the octanol-air constant, $K_{OA} = C_O/C_A = 10^{6.9}$ (De Bruijn et al., 1989), according to the expression: $C_{air,eq} = C_{thatch}/K_{OA}$, which provides an estimate of the equilibrium in-door air concentrations ($C_{air,eq}$). Having in mind kinetic aspects and a partial renewal of the atmosphere in the dwellings, more realistic estimates could be operationally defined as 50% of $C_{air,eq}$. Thus, according to these calculations the median 4,4'-DDT concentrations of 2006 and 2007 (Table 3) corresponded to air concentrations of 3.5 and 8 ng/m³, respectively, and the maximum concentrations observed in 2006 and 2007 corresponded to 300 and 1250 ng/m³, respectively. Studies of in-door 4,4'-DDT spraying with 2 g/m² under controlled conditions in rooms from dwellings with plaster covered walls and furnished as per normal household practices have shown gas phase air concentrations in the range of 400-8600 ng/m³, depending on the time of sampling after spraying (1 h – 240 days; Singh et al., 1992). In contrast, remote areas exhibit open air concentrations of 4,4'-DDT in the order of 0.01-0.001 ng/m³ or even less (van Drooge et al., 2002; 2004). A study of DDT contamination due to IRS for malarian control in two districts of South Africa showed indoor air concentrations in the ranges of 750-6000 ng/m³ (mean 2200 ng/m³) and 1.5-28 ng/m³ (mean 7.2 ng/m³) in treated and untreated dwellings, respectively. The calculated values in Mozambique are consistent with these previously described air concentrations (van Dyk et al., 2010). They suggest that DDT exposure through inhalation is a probable mechanism for exposure of their inhabitants. Other exposure routes could be related to dust or particles falling from the treated walls or roofs of the dwellings, e.g. DDT has been found in floor dust (van Dyk et al, 2010). These particles could eventually be inhaled through respiration or ingested if they become mixed with food or water.

5. Conclusions

Measurement of the concentrations of DDT and its metabolites adsorbed onto the thatch and branches of the wall materials provide useful information to estimate the exposure levels of the residents from the dwellings in which this insecticide has been sprayed. Analysis of these wall materials in dwellings from Manhica have shown higher concentrations of 4,4'-DDT than its metabolites, including 4,4'-DDE, in all cases except two from the 2006 series. Independently of absolute concentrations, the dominance of 4,4'-DDT evidences that the origin of the compound is likely related with the recent use of this insecticide.

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Table 1: Concentrations (ng/g dry mass) of DDT and metabolites in repeated thatch sampling (n = 3 or 4) from the same dwelling in 2008.

Dwelling	Sample	4,4'-DDE	4,4'-DDD	2,4-DDT	4,4'-DDT
A	1	180	34	260	2100
	2	56	54	190	950
	3	90	28	680	1800
	4	72	29	87	330
	Mean	99	36	310	1300
	SD	54	12	260	810
	%SD/Mean	55	33	85	62
B	1	98	22	47	160
	2	82	43	69	290
	3	46	13	25	99
	4	33	11	14	63
	Mean	65	22	39	150
	SD	30	15	24	100
	%SD/Mean	47	65	63	65
C	1	11	10	86	210
	2	<LOD	7.4	23	85
	3	19	11	150	350
	Mean	10	9.6	86	210
	SD	9.5	2.0	63	130
	%SD/Mean	94	20	73	62
D	1	8.4	6.7	40	220
	2	<LOD	6.2	30	170
	3	15	4.8	32	140
	4	<LOD	8.6	38	220
	Mean	5.9	6.6	35	190
	SD	7.1	1.6	5.1	38
	%SD/Mean	120	23	14	20

LOD = 0.01 ng/g

Table 2: Comparison of DDTs concentrations (mean,ng/g) in materials sampled after IRS with DDT for malaria control in various countries (ng/g).

Country	survey year	Sample n	Type	4,4'-DDE	4,4'-DDD	4,4'-DDT	ΣDDT	Reference
Mozambique	2006	48	Straw	2.2	5.5	47	56	present study
Mozambique	2007	43	Straw	20	23	130	180	present study
India	1986	20	wheat straw	30	—	260	460	Singh et al 1988
Mexico	2003	50	Rye straw	8	—	44	—	Waliszewski et al 2004
Tanzania*	2002	6	dried hay	72	641	6900	79000	Marco et al 2005

*mean

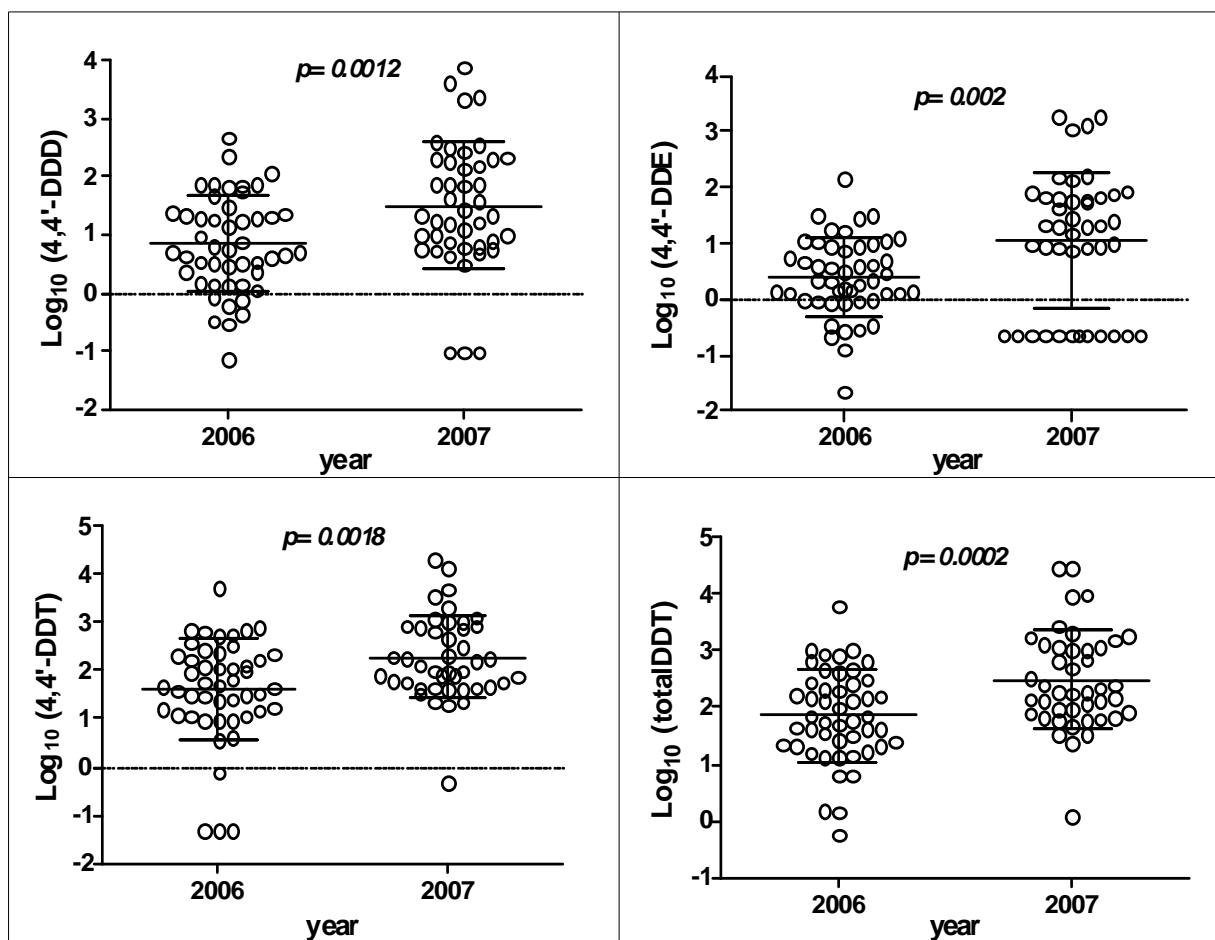
Table 3: Concentrations of DDTs (ng/g wet weight) and metabolites in thatch sampled in 2006 and 2007

(ng/g)	4,4'-DDE	4,4'-DDD	4,4'-DDT	2,4'-DDE	2,4'-DDD	2,4'-DDT	Σ DDT	DDE/DDT
2006 (n = 41)								
Mean	9.4	35	270	0.7	4.8	28	350	0.3
Median	2.2	5.2	49	0.2	1.0	7.5	70	0.0
SD	23	83	790	1.4	9.9	63	960	1.1
Min	0.0	0.1	0.05	0.03	0.1	0.1	0.6	0.01
Max	140	480	5000	8.7	55	390	6100	5.7
25% Percentile	1.0	1.9	12	0.03	0.4	2.2	21	0.04
75% Percentile	10	23	210	0.5	3.5	26	270	0.1
2007 (n = 41)								
Mean	190	470	1300	7.5	110	240	2400	0.1
Median	21	23	150	0.3	5.4	21	220	0.1
SD	470	1400	3700	22	360	650	6400	0.1
Min	0.2	0.1	0.5	0.3	0.1	0.01	1.2	0.001
Max	1900	7700	20000	95	2100	3200	29000	0.6
25% Percentile	0.2	6.3	45	0.3	0.1	11	74	0.003
75% Percentile	73	200	850	0.3	39	140	1200	0.2
Wilcoxon test	0.002	0.002	0.002	0.001	0.008	0.002	0.000	

*calculated over log-transformed data for normalization

FIGURE CAPTIONS

Figure 1. Comparison of log-transformed DDTs levels (mean and SD ng/g wet weight) in thatch samples from 2006 (n = 48) and 2007 (n = 43).



4.4. Concentration of DDTs in breast milk from African women at the early stages of domestic indoor residual spraying in Manhica, Mozambique.

Manaca, M.N., et al. Concentration of DDT compounds in breast milk from African women (Manhica, Mozambique) at the early stages of domestic indoor spraying with this insecticide. Chemosphere (2011), doi:[10.1016/j.chemosphere.2011.06.015](https://doi.org/10.1016/j.chemosphere.2011.06.015). Article in press.

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PART III. DISCUSSION AND CONCLUSIONS

Chapter 5. DISCUSSION

5.1. Pesticide concentrations in Manhica

Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are two pillars of malaria vector control in Africa. Both methods have proven to be efficient in reducing the risk of infection with malarial parasites, clinical disease and child mortality (218). This PhD thesis describes for the first time the concentrations of DDT in humans and the human environment in Mozambique, a malaria endemic country where DDT was reintroduced in IRS programs in late 2005, 12 years after the suspension.

5.1.1. DDTs

DDT continues to be the most produced and used persistent organic pollutant listed in the Stockholm Convention. At present the main DDT source is the use of this insecticide in IRS for public health programs in developing countries. Other sources are related to its use as an intermediate product in the industrial synthesis of the acaricide dicofol (219) and its incorporation in antifouling paints in China (220).

Other insecticides are available for IRS programs. However, in areas with long season periods of malaria transmission it is difficult to ensure permanent lethal residual insecticide concentrations on walls. This alternative may need multiple spray rounds which is more difficult to achieve and maintain in large areas to be covered. DDT is the only insecticide which only requires one round spray per year.

The results obtained in this study clearly show that IRS with DDT contributes to increasing the concentrations of this insecticide as demonstrated when comparing breast milk sampled in 2002 and 2006 and thatch sampled in 2006 and 2007.

4,4'-DDT was found in all breast milk and thatch samples analyzed in this study and in 95% of the umbilical cord samples. The presence of this compound in high concentrations, often above DDE concentrations is consistent with recent applications of this insecticide.

Nearly all examined mothers in this study were not exposed to DDT by IRS during their pregnancy. 24% (n=52) of the total of 214 newborns were exposed during gestation and the average exposure time was about 38 days. The low DDT concentrations found in umbilical cords in the population examined are consistent with this low exposure. Despite these low concentrations, 4,4'-DDT has been found in 95% of the umbilical cord samples.

The higher proportions of 4,4'-DDT than 4,4'-DDE observed in all matrices suggest recent inputs of this insecticide.

The concentrations in breast milk and umbilical cord range between 0.5 and 190 ng/ml and between 0.08 and 8.5 ng/ml, respectively. Comparison of these breast milk concentrations with those of other sites in Africa shows that the Manhiça levels were lower than those in breast milk in countries where IRS with DDT had been reintroduced and several applications had been done (161). The samples from Manhiça exhibited moderate 4,4'-DDT concentrations which is consistent with the initial period of IRS in this region.

The neonates studied in Manhiça had detectable but quite low DDT levels. The highest concentrations were found for 4,4'-DDE. The concentrations of DDTs in fetal blood in this population were much lower than those found in sites where IRS with DDT is taking place (221, 222).

In thatch, 4,4'-DDT was found in higher concentrations than 4,4'-DDE in all samples from 2006 except one, and in all samples from 2007. It is observed as the DDE/DDT ratio is positively related to a recent DDT application, the samples have a very high content of 4,4'-DDT. The high DDT levels in thatch analyzed in our study were similar to those found in other countries in which wood and straw material were analyzed immediately after IRS (23).

5.1.2. Pyrethroids

ITNs and IRS are the main strategies adopted as prevention measures against mosquito bite in Mozambique. Since 1999, ITNs are provided to children under five years and pregnant women free of charge (202).

Samples were analyzed for 10 pyrethroids, λ -cyhalothrin, permethrin, esfenvalerate/fenvalerate, cypermethrin, tetramethrin, bifenthrin, cyfluthrin, phenothrin, resmethrin and deltamethrin. λ -cyhalothrin was the largest component of the mixture in the milk samples with a mean value of 140 ng/g lw. This compound and permethrin were found in all samples. In contrast to the breast milk samples, permethrin was the most abundant component of the thatch mixtures with a mean of 80 ng/g dw. In these samples, cypermethrin was the compound found most often (93% of total).

Comparison of the pyrethroid breastmilk concentrations with others reported elsewhere show that women from Manhiça have generally low concentrations in relation to those from other African countries (161, 162).

In the present study p,p'-DDE, p,p'-DDT, λ -cyhalothrin, permethrin, esfenvalerate/fenvalerate, cypermethrin, tetramethrin, bifenthrin and cyfluthrin, were quantified in breast milk samples collected in year 2002. Deltamethrin, phenothrin and resmethrin were not found. In 1993 λ -cyhalothrin and deltamethrin were used for IRS but pyrethroids are not used for this purpose. Currently there are three long-lasting insecticidal nets recommended by WHO for malaria prevention that contain either permethrin, deltamethrin or α -cypermethrin (195). In 2003, one year after breast milk sampling, the pyrethroid insecticides recommended by WHO for bednet treatment were deltamethrin, λ -cyhalothrin, cyfluthrin and α -cypermethrin (223). Given the relatively low half life of these compounds, the likely source of high λ -cyhalothrin and cyfluthrin level in breast milk was not IRS but ITNs. Their use could lead to food contamination. Domestic uses of insecticide

dust or spray in indoors against insects could also be a possible source of these compounds. Locally grown food, inhalation and skin contact (through domestic insecticides dust) and work on farms have been claimed to be possible pyrethroid sources in breast milk (162).

In that, all 4,4'- and 2,4'- DDT isomers, λ -cyhalothrin, permethrin, esfenvalerate/fenvalerate, cypermethrin, tetramethrin, bifenthrin, cyfluthrin, phenothrin, resmethrin and deltamethrin were found at quantifiable levels. Possible sources of these compounds in the dwellings are IRS and agriculture for DDTs and pyrethroids, respectively.

In summary, IRS is an important DDT source in Manhiça as shown by the high 4,4'-DDT concentrations compared to 4,4'-DDE. Prior to the use of DDT for IRS some contributions of this insecticide from uncontrolled sources cannot be excluded. The indoor environments are important sources of pesticide exposure to the mothers, as they spend significant time periods inside dwellings where they could be in contact with pesticides.

5.2. Limitations of the work

The main limitation of this study concerned sample availability. Samples were collected for other studies conducted at CISM having different objectives to those of the present PhD research. Thus, cord blood and breast milk samples correspond to different periods and are not related. No correspondences could be established between sample groups. In some cases, a limited number of cases were available, e.g. cord blood samples 2003. These cases introduce incertitude when comparing with larger datasets.

The strongest difficulty in this PhD research has involved the absence of samples from periods after full implementation of IRS with DDT.

Another limitation concerned the ambiguities in the information of pulverization of the houses based on questions asked to the mothers. Systematic information collected by official agencies was not available.

Another limitation of the study is lipid adjustment. Due to the high lipid solubility of the studied compounds breast milk and cord blood data should be adjusted by lipid content (224). In the present study lipid content could only be calculated in breast milk samples from year 2006. The adjustment of the milk samples from 2002 and the serum samples was not possible due to the low volume available.

5.3. Future works

In the present study, the exposure to pesticides, particularly DDT, in early stages of application of this insecticide for IRS has been assessed. One obvious follow up should involve representative sample collection of breast milk and cord blood from infant-mother pairs in the period after full implementation of this insecticide for IRS. This would provide very good data for comparison with that examined in the present study. Overall, a precise picture of DDT intake in general population as a consequence of the use of this insecticide for IRS would emerge.

- One of the secondary questions of EPSIMA was to study the impact of prenatal exposure to insecticides in the development of infant immunity. This included (i) analysis of immunology data already collected in the AgeMal, TIMNET and EPIC studies in relation to the DDT and metabolite levels in cord plasma, and (ii) a pilot study measuring antibody responses to selected EPI vaccines in AgeMal and/or TIMNET infants.
- Another of the secondary questions of EPSIMA was to study the impact of prenatal exposure to insecticides in the neurological development of children. This included (i) validation of the methods and (ii) neurological psycho-motor assessment of older AgeMal and TIMNET children in relation to the DDT and metabolite levels in cord plasma.
- Because of the potentially interesting findings in pyrethroids in a limited set of samples, the above mentioned new samples could be analyzed for pyrethroids, as they might have more relevant or confounding effects on health. The number of pesticides analyzed should also be extended to carbamates as some of them were also used for IRS after pyrethroid use.
- Further studies should be performed on the usefulness of thatch samples for evaluation of the impact of DDT in the inhabitants of the dwellings. These studies should involve multiple samplings in the dwellings and a good coordination with IRS activities to establish correspondences between these activities and DDT concentrations.

Chapter 6. CONCLUSIONS

Composite samples from diverse wall sites provide realistic estimates of the exposure levels. Analysis of these wall materials in dwellings from Manhiça have shown higher concentrations of 4,4'-DDT than its analogous compounds, including 4,4'-DDE, in nearly all cases. Independently of absolute concentrations, this composition evidences a 4,4'-DDT origin related with the recent use of this insecticide.

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 the IRS program. The differences in median concentrations from one population of mothers whose breast milks were collected in 2006, several months after the program started, and one reference population whose breast milks were collected in 2002, gave statistically significant differences. In general, the concentrations of the 4,4'-DDE metabolite were higher than those of the 4,4'-DDT precursor in all breast milk samples collected in 2006. Those with highest total DDT concentrations had higher 4,4'-DDT concentrations, suggesting some degree of saturation of the metabolic transformation mechanisms.

The average 4,4'-DDE and 4,4'-DDT cord blood concentrations in the population of Manhiça 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.

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 multiparae than primiparae women, and a subsequent detoxification as consequence of the maternal activities (deliver and breastfeeding).

Pyrethroids were found in human breast milk despite the discontinuation in the use of these compounds for IRS. Their occurrence may reflect an influence from the ITNs, agricultural sources or use for IRS in some cases despite their known low efficiency for malaria control. The presence of these compounds in breast milk confirms their bioaccumulation potential in humans.

The concentrations of some of these insecticides found in some mothers, namely λ -cyhalothrin used for IRS in Mozambique, involves the estimated daily intake values close to the upper limits recommended by FAO.

Chapter 7. REFERENCES

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