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Pyrethroid use-malaria control and individual applications by households for other pests and home garden use

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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 dichlorodiphenyltrichloroethene (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 as well as in human breast milk, 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 agriculture 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.

1. Introduction

In 2004, an estimated 350–500 million people contracted malaria and 0.85 million died (91% in Africa, 85% of them children under 5 years) (WHO, 2007, WHO and UNICEF, 2005). The World Health Organization Pesticide Evaluation Scheme (WHOPES) (WHO, 2008) supports the use of recommended insecticides for malaria control based on the evaluation of human and environmental safety conditions (WHO, 2006). In tropical Africa, these insecticides were used for treatment of mosquito nets (ITNs) (Kapp, 2004) and indoor residual spraying (IRS) on walls and roofs to kill the mosquitoes that land and rest there (Montgomery et al., 2010). In the last years increases of international funding for malaria control allowed protecting larger numbers of people in sub-Saharan Africa, from 13 million in 2005 to 75 million in 2009 by IRS as well as 66% of the 765 million at risk by use of 254 million ITNs between 2008 and 2010 (World Malaria Report. WHO, 2010). The implementation of dichlorodiphenyltrichloroethene (DDT) and pyrethroids for IRS constitute one of the major interventions for reduction and interruption of malaria transmission by vector control in all epidemiological settings (World Malaria Report. WHO, 2010).

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 (Hargeaves 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

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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 the end of 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 derivates 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 biphenthrin) 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 $\mu g\, L^{-1}$) (Laskowski, 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 analyzed 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 and April and a dry and cold season during the rest of the year.

2.2. Samples

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.

Thatch samples ($n = 14$) covering surfaces of about 10 cm^2 were collected in-door during 2006–2007 and introduced in sterile polyester bags (Kapak Corporation, Minneapolis, USA) which were closed with a heat sealer and stored at −20 °C.

Thatch was elaborated from *Typha* plants (particulary *Typha latifolia*).

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 breast milk (0.1 g dry weight) 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 (2 g/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 μL ethyl acetate for GC–NCI-MS–MS analysis (Feo et al., 2010a, 2010b).

The breast milk extracts were cleaned up by elution through C18 cartridges (2 g/15 mL) coupled to basic alumina (5 g/25 mL) and conditioned with 25 mL of acetonitrile. Then the sample was eluted with 30 mL of acetonitrile. The acetonitrile extract was evaporated under a nitrogen stream and the residue was dissolved in 100 μL 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 (15 m × 0.25 mm i.d., 0.1 μm 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 μL. 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 and Martell, 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.