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Evaluation of the degree of contamination of breast milk by organochlorine pesticides in the Lower Amazon region: a preliminary study

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ABSTRACT. The main source of human exposure to organochlorines is through food, especially food with a high fat content, such as breast milk, which is critical for child hood development. Therefore, it is important to analyze milk and other biological matrices for organochlorine pesticides (OCPs), and then determine the source of the OCP contamination. The objective of this study was to evaluate the degree of contamination by OCP residues and their metabolites in breast milk samples from the municipalities of Mojuí dos Campos and Belterra-Pará, Brazil. The OCPs analyzed were dichlorodiphenyltrichloroethane (DDT) and its metabolites (o,p'-DDT, p,p'-DDT, o,p'-DDE, p,p'-DDE, o,p'-DDD, and p,p'-DDD), aldrin, endrin, dieldrin, hexachlorocyclohexanes (α-HCH, β-HCH, lindane (γ-HCH), and δ-HCH), endosulfan α and β, endosulfan sulfate, heptachlor, and heptachlor epoxide. These substances were analyzed in 22 breast milk samples using gas chromatography with an electron capture detector (GC-ECD). Seven were contaminated with p,p'-DDT and/or p,p'-DDE. The mean p,p'-DDT and p,p'-DDE content in the contaminated samples was 0.0022 and 0.0152 $mg \cdot kg^{-1}$ of milk, respectively. This contamination may have been due to the inadequate application of DDT until the end of the 1990s for the control of tropical diseases, including malaria. Despite being a preliminary study, the number of contaminated samples was significant, which suggests the need to expand this study to other environmental and biological matrices.

Keywords: DDT and metabolites; GC-ECD; SPME; Persistent organic pollutants; Human milk.

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Introduction

Persistent organic pollutants (POPs) are divided into three categories: organochloride pesticides (OCPs), polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs), which are characterized by their high resistance to chemical transformations and environmental toxicity (Jiménez & Pastor, 2012).

OCPs were widely used between the 1940s and the 1980s for pest control in agriculture (Rehwagen, 2006; Tsygankov, 2019), and in combating tropical diseases such as malaria, typhus, dengue, and Leishmaniose visceral (Silva, 2015; Carvalho et al., 2018).

Organochlorine pesticides include chlorinated derivatives of hexachlorobenzene (BHC), diphenyl ethane (dichlorodiphenyltrichloroethane, DDT; dichlorodiphenyldichloroethane, DDE; dichlorodiphenyltrichloroethane, DDD; and methoxychlor) (Rêgo et al., 2019; Batool, Shah, Abu Bakar, Maah, & Abu Bakar, 2021), hexachlorocyclohexanes (HCH; four isomers of lindane: α-HCH, β-HCH, δ-HCH, and γ-HCH) (Navarro, De La Torre, Sanz, Arjol, Fernández, & Martínez, 2019), cyclodienes (aldrin, dieldrin, endrin, chlordane, nonactor, heptachlor, and heptachlor epoxide) (Qu, Albanese, Lima, Li, Doherty, Qi, & De Vivo, 2017; Ross et al., 2019), and chlorinated hydrocarbons (dodecachlor, toxaphene, and chlordecone) (Nasution & Bakti, 2018; Shanthi, Vasanthy, & Mohamed Hatha, 2022).

DDT and lindane are widely used both in agriculture and medicine, mainly in South America, due to their low cost and high efficiency (Girones, Oliva, Marcovecchio, & Arias, 2020). Following ecological studies, Sweden was the first country to ban the use of these pesticides in 1970 (Nygård, Sandercock, Reinsborg, & Einvik, 2019). In Brazil, only in 1985 was the use of DDT and other OCPs banned in the control of agricultural pests. However, the use of DDT was allowed to control vectors of tropical diseases (Guimarães, Asmus, & Meyer, 2007; Nascimento et al., 2017), and it is still routinely used for this purpose in the Amazon (D'Amato, Torres, & Malm, 2002).

These compounds are liposoluble, resist biotransformations, accumulate in adipose tissues, and transmit via the trophic chain (Song, Zhang, Zhang, & Zhang, 2019; Parra-Arroyo et al., 2021). Humans and animals can be exposed to OCPs through contaminated food, water, air, or soil (LaKind, Amina Wilkins, & Berlin, 2004; Vilca, Cuba, Nazato, & Tornisielo, 2017). OCP accumulated in adipose tissue can migrate to the produced human or animal milk (Lee, Porta, Lind, Lind, & Jacobs, 2018; Pan, Li, Li, Yang, Qin, Li, & Li, 2020). In the Amazon region, DDT and its metabolites were detected in several environmental matrices (water, soil, sediment, fish) (Mendes et al., 2019; Peng, S. et al., 2020; Umulisa, Kalisa, Skutlarek, & Reichert, 2020) and in people (blood, urine, breast milk, and hair) (Appenzeller et al., 2017; Peng, F. J. et al., 2020; Amir et al., 2021; Hardy et al., 2021).

Because organochlorine pesticides are poorly reactive and difficult to break down, they persist in the environmentand enter the food chain. Therefore, it is important to measure the levels of pesticides in biological tissues, especially adipose tissue. Mammalian milk fat, including human milk, does not escape the accumulation of these liposoluble compounds. Due to biological magnification, the adipose tissue of carnivores and omnivores contains more organochlorines residues than that of herbivores (Saldanha, Bastos, Torres, & Malm, 2010).

The present study aimed to evaluate the presence of OCPs in breast milk in Mojuí dos Campos and Belterra-Pará, Brazil. Additionally, we identified and quantified the main OCP present in these matrices to identify their most probable sources.

Material and methods

Selection of participants and sample collection

Initially, a data survey was performed at health posts and the municipal hospital of Santarém on lactating women living in the municipalities of Belterra and Mojuí dos Campos, Brazil, who had recently given birth. Subsequently, a survey was conducted considering inclusion and exclusion criteria for the selection of milk donors.

The inclusion criteria were as follows: participants who signed a free and informed consent term (FIC), aged between 18 and 49 years; the mother and the child should be in good health; the mother should have had a normal birth and be between the first and sixth month of lactation; participants should have resided in the target cities of the study for at least the last three years and should have full use of their mental faculties and be able to make decisions autonomously.

Exclusion criteria included infants with no possibility of manual milking, a history of infectious diseases during pregnancy (e.g., HIV, hepatitis B, gestational syphilis), infants with a history of postpartum disease (hypertension, gestational diabetes, sepsis, or mastitis), lactating smokers, users of drugs or working in tanneries, and agricultural or metallurgical industries. Those not all the inclusion criteria were excluded as well.

The 22 samples were collected between August and October 2017 through manual milking at the locations shown in Figure 1. Approximately 15 mL of each participant's milk was collected and stored in sterile 50 mL Falcon tubes for further analysis.

Solid phase extraction (SPE)

The extraction procedure used was based on the method described by Avancini, Silva, Rosa, Sarcinelli, and Mesquita (2013), with some modifications. Breast milk samples were warmed in a hot bath at 37°C for 20 min. An aliquot of 1 mL was transferred to Falcon® tubes and 10 mL of a solution of ethyl acetate:methanol:acetone (2:4:4) was added. The samples were then homogenized by vortex mixing (2 min), extracted in an ultrasonic bath for 20 min, and centrifuged for 15 min at 2000 RPM. Then, 10 mL of ultrapure water was added to the supernatant, and the resulting mixture was passed through a C_{18} solid-phase extraction cartridge (C_{18}-SPE), which was previously conditioned with n-hexane (2 mL), ethyl acetate (2 mL), methanol (2 mL), and ultrapure water (2 mL).

The column was washed with 2 × 1 mL of 25% aqueous acetonitrile solution, dried under vacuum for 30 min for further addition of the pesticides retained in the column and eluted with 2 ml of n-hexane. The resulting solution was then separated into two 1 mL aliquots, one for subsequent clean-up and the other for lipid content analysis. The n-hexane solution (1 mL) containing the pesticides was passed through a Florisil SPE column preconditioned with 10 mL of dichloromethane, 10 mL of ethyl acetate, and 10 mL of an acetone/15% ethyl acetate mixture. The pesticide was eluted with 10 mL of n-hexane and 5 mL of an acetone mixture in 15% ethyl acetate. The solution containing the pesticides was then evaporated to 1 mL at low temperature, and 100 µL of the internal standard 1,2-dichloronaphthalene (DCN) at 108 ng·mL^{-1}.

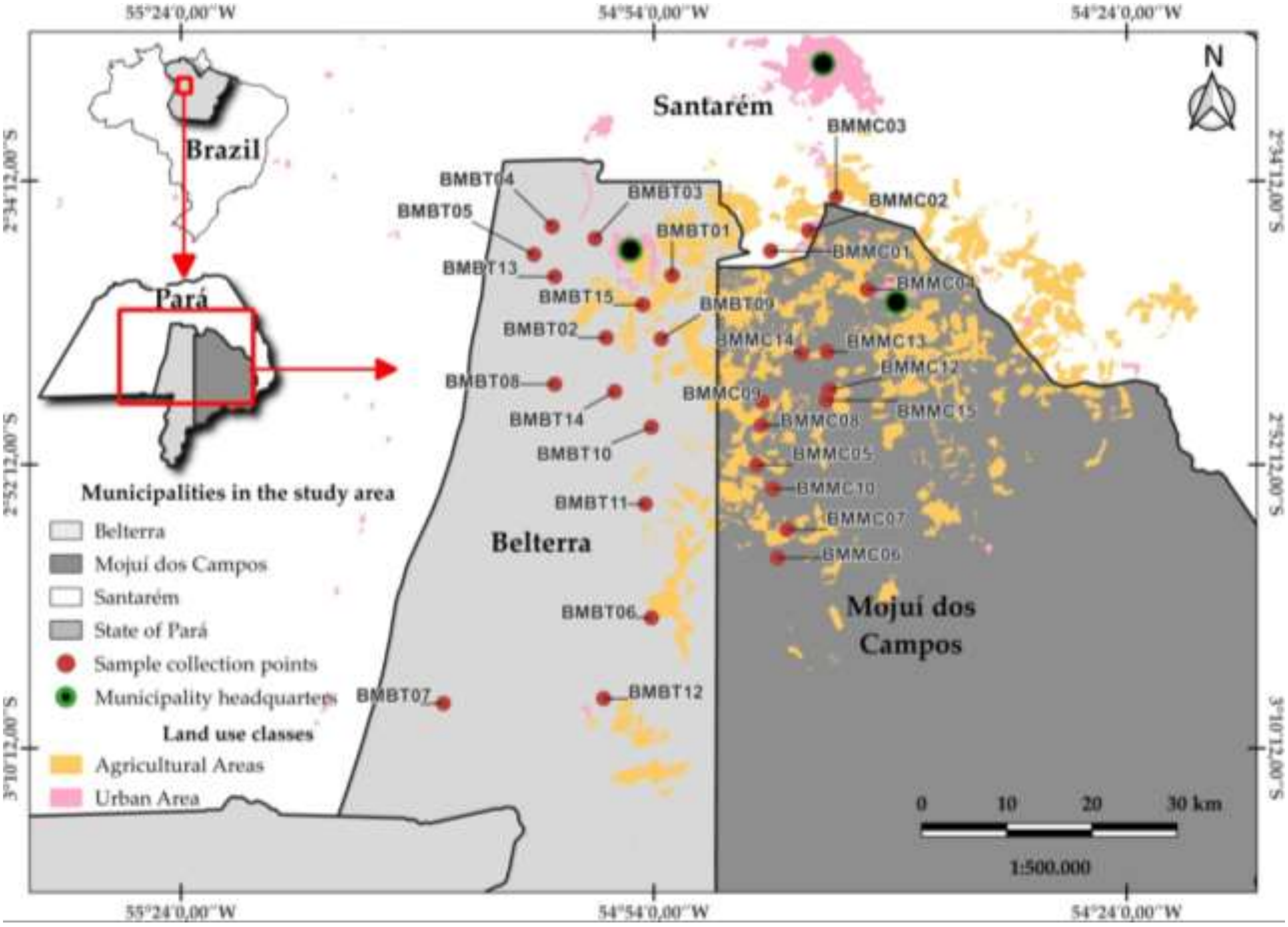

Figure 1. Spatial distribution of collection points of breastmilk samples in rural and urban areas of Mojuí dos Campos and Belterra, Pará, Brazil.

Determination of lipid content

Considering that organochlorine compounds have preferential bioaccumulation in adipose tissues, determination of the fat content in milk samples was necessary. Thus, the fat content of the samples was determined to minimize the source of variation in the results obtained, and the lipid content was determined during the final phase of sample extraction. After the pesticides retained in the column were eluted with 2 ml of n-hexane, 1 mL was used for the clean-up phase and a volume of 1 mL was used for lipid extraction, concentrated in a vacuum rotary concentrator for 10 min at 3000 RPM for drying and subsequent mass measurement of the lipid extract (Avancini et al., 2013).

Chromatographic analysis

For quantitative analysis, a CP 3800 Varian gas chromatography-electron capture detector (GC-ECD) equipped with an OV-5 silica capillary column (30 m x 0.32 mm and 0.25 µm film thickness) (OHIO VALLEY Specialty Chemical, OHIO, EUA) was used. Data were acquired and processed using Workstation 5.0 software. Ultra-pure nitrogen (99.9999%) was used as the entrainment gas at a flow of 1.2 mL·min^{-1}.

The oven temperature ramp for the column was programmed as follows:150 °C for 1 min and 150–250 °C (6 °C·min^{-1}) for 5 min. The injector was operated at 250 °C in splitless mode (1:20). The detector temperature was set to 300 °C.

For the qualitative analysis, a Thermo Scientific™ TSQ™ 8000 Gas Chromatograph/Triple Quadrupole Mass Spectrometer (GC-MS) equipped with a DB5 silica capillary column (30 m x 0.32 mm and 0.25 µm film thickness) (OHIO VALLEY Specialty Chemical, OHIO, EUA) was used. Data were acquired and processed using Workstation 5.0 software. Ultra-pure helium (99.9999%) was used as the entrainment gas at a flow of 1.0

mL·min^{-1}. The oven temperature ramp for the column was programmed as follows:80 °C for 3 min and 80 °C to 280 °C (10 °C·min^{-1}) for 3.5 min. The injector was operated at 250 °C in splitless mode (1:20). The detector temperature was 260 °C, and the ion source was 275 °C.

Quality control

Blanks were performed in parallel with the samples and did not show peaks in their chromatograms. To evaluate pesticide recovery, breast milk samples were fortified with all evaluated pesticides. The extraction method presented a recovery of 86% and 80% with a coefficient of variation of 5.4% and 4.5% for p,p'-DDE and p,p'-DDT, respectively. The limit of detection (LD) and limit of quantification (LQ) were calculated, respectively, in triplicate and as the standard deviation. The obtained values for p,p′-DDEand p,p′-DDT were 0.0060 and 0.0180 ng·mL^{-1} and 0.0070 and 0.0210 ng·mL^{-1}, respectively.

Compliance with ethical standards

This study was approved by the Ethics Committee of the State University of Pará (CEP-UEPA), with CAAE approval opinion 0616.0.146.000-08. It follows all ethical standards required.

Results and discussion

Descriptive analysis of the samples

The mean age of the milk donor motherswas 26 years, with a minimum of 18 years and maximum of 39 years. The number of individuals per residence varied from three to seven, with an average of four. More than 90% of the participants claimed to be brown. All mothers had partners.

In addition, they all had resided in the countryside for at least four years. Sixteen reported that they work or worked in the field. However, all declared that they did not work with organochlorine pesticides during this period.

All patients underwent prenatal care during the gestational period, with an average of seven visits. None of the participants reported smoking. The mean weight of the newborns was 3.348 g, with a minimum of 1.770 g and a maximum of 4.040 g. The mean age of the breastfed babies was 3.97 months.

Regarding the distribution of participants within the municipality of Mojuí dos Campos, 10 were residents in rural areas and five in urban areas. In Belterra, five participants lived in rural areas and two in urban areas. The mean proximity to agricultural crops was 498 m, with a minimum of 2 m and a maximum of 1000 m. The predominant crops were rice, soybean, and corn.

Information aboutthe husband's or partner's habits shows that most of them had lived or worked in the countryside. Currently, 13 partners workin rural areas, of which two are in direct contact with some type of pesticide (connect, belonging to the chemical class of neonicotinoids, and decis of the chemical class pyrethroids) during their work. Smoking habit was present in 12 of the husbands/companions; of these, four admitted to having smoked near the nurse during gestation.

Regarding housing, half of the participants resided in masonry houses. In 12 residences, the water consumed was untreated and obtained from artesian wells or natural sources. Finally, in 15 residences, there was no municipal garbage collection, and the garbage was discarded or improperly burned, in some cases, near the water wells.

Analysis of pesticides in breast milk

Of the 22 breast milk samples analyzed, 15 showed no contamination by any organochlorine pesticide evaluated within the limits of detection. In the other seven samples, p,p'-DDT and/or p,p'-DDE were detected, of which two were from Belterra and five from Mojuí dos Campos (Table 1). Of these samples, four were from rural area and three from urban areas. In Mojuí dos Campos, two samples collected from urban areas and three from rural areas showed pesticide contamination. In Belterra, of the two contaminated samples, one was from a neighborhood located in the city center, and the other, from the rural community.

Nd, not detected; BMBT, breast milk - Belterra; BMMC, breast milk; Mojuí dos Campos. The maximum concentration of DDT and its metabolites established by the FAO/WHO (2018) was 0.005 mg kg^{-1}of milk.

The organochlorine pesticides o,p'-DDD, o,p'-DDD, p,p'-DDD, aldrin, endrin, dieldrin, α-HCH, β-HCH, lindane (γ-HCH), δ- HCH, endosulfan α and β endosulfan sulfate, heptachlor, and heptachlor epoxide were below the limit of detection in the analyzed samples.

Table 1. The concentration of OCPs in breast milk in mg kg^{-1} of milk.

Sample ID	p,p'-DDT	p,p'-DDE
BMBT02	0.0020	0.0097
BMBT07	Nd	0.0071
BMMC06	0.0031	0.0328
BMMC07	Nd	0.0149
BMMC10	Nd	0.0060
BMMC13	0.0012	0.0298
BMMC14	0.0028	0.0062
Mean Value	0.0022	0.0152
The concentration of OCPs in breast milk in mg kg^{-1} of lipids.		
BMBT02	0.052	0.252
BMBT07	Nd	1.014
BMMC06	0.087	0.924
BMMC07	Nd	0.710
BMMC10	Nd	0.408
BMMC13	0.054	1.330
BMMC14	0.193	0.428
Mean Value	0.055	0.724

The mean p,p'-DDT content in the contaminated samples was 0.0022 mg·kg^{-1}, with a maximum value of 0.0031 mg·kg^{-1}, whereas the pp'-DDE content was higher, ranging from 0.0060 to 0.0328 mg·kg^{-1}. As organochlorine compounds bioaccumulate, especially in adipose tissue, the fat content in the analyzed milk samples was calculated.

The mean concentration of p,p’-DDT was 0.055 mg·kg^{-1} of lipids, with a maximum value of 0.193 mg·kg^{-1} and a minimum of 0.052 mg·kg^{-1}. The concentration of p,p’-DDE ranged from 0.252 to 1.330 mg·$kg^{-1}$of lipids, with a mean value of 0.724 mg·kg^{-1}.

The maximum residue limit (MRL) represents the acceptable limit of pesticides in foods to protect the health of consumers. For the organochlorines evaluated, the concentrations of pp’-DDT and pp'-DDE were above the limits established by the FAO/WHO (2018) of 0.005 mg·kg^{-1}.

The levels of maternal contamination in the samples evaluated in this study were lower than those reported in other Brazilian studies (Azeredo et al., 2008; Ferronato, Viera, Prestes, Adaime, & Zanella, 2018; Souza et al., 2020). Azeredo et al. (2008) evaluated the degree of contamination of women directly exposed to DDT in the 1990s in order to combat malaria in the Madeira River region. DDT and its metabolites were detected in all analyzed samples, with an average of 0.370 mg·kg^{-1} of lipid (Azeredo et al., 2008).

In addition, the number of contaminated samples and the contents of p,p'-DDT and p,p'-DDE were lower than those found in the city of Lucas do Rio Verde in Mato Grosso, which could be a result of the recent agricultural expansion in the western region of Pará (which began before the ban on these pesticides for agricultural purposes in Brazil) (Palma, Lourencetti, Uecker, Mello, Pignati, & Dores, 2014). The levels of p,p'-DDT and p,p’-DDE detected in the analyzed samples were also lower than those detected in Norway (Polder, Skaare, Skjerve, Løken, & Eggesbø, 2009), India (Mishra & Sharma, 2011), Colombia (Rojas-Squella, Santos, Baumann, Landaeta, Jaimes, Correa, Sarmiento, & Ramos-Bonilla, 2013) and the Canary Islands in Spain (Vall et al., 2014).

It is worth noting that samples BMMC06 and BMMC07, which presented higher levels of p,p'-DDE, were collected in a community where monoculture activities were carried out, mainly soybeans, maize, and rice, which showed high levels of p,p'-DDE.

p,p-'DDE has the most frequent occurrence in the general population due to its low rate of metabolization and the contamination of the food supply (Parra-Arroyo et al., 2021; Song et al., 2019). The prevalence of p,p'-DDE in addition to p,p'-DDT suggests past exposure to DDT, since the mean time required to metabolize p,p'-DDT to p,p'-DDE is 12 months (ATSDR, 2019; Palma et al., 2014).

Until the mid-1990s, DDT was used to control malaria vectors in the Amazon region. Other reports have shown contamination by DDT in fish, the main source of protein in the local diet (Torres, Pfeiffer, Markowitz, Pause, Malm, & Japenga, 2002; Saldanha et al., 2010; Mendes, Costa Lopes, Souza, Oliveira Lima, & Santos, 2016, 2019). In addition, Rodrigue et al. (2017) analyzed the soil in areas close to the city of Belém, finding DDT residues and their metabolites, demonstrating the abundance of these pesticides.

The presence of DDT in Amazonian soil occurs through direct application or by the superficial runoff of DDT applied to house walls, even when deposited in the air and adsorbed by clay and/or organic matter

(Rodrigues, Souza, Silva Rocha, Costa, & Mendes, 2017; Torres et al., 2009). DDT is also volatile, with a vapor pressure of 1.60 $\times 10^{-7}$ torr at 20°C, and can be transported long distances from the application site. Therefore, the risk of water contamination increases. Its n-octanol/water partition coefficient ($logK_{ow}$) is 6.91. The contamination of aquatic animals causes bioaccumulation and biomagnification, which can cause health problems in living beings (Durante, Santos-Neto, Azevedo, Crespo, & Lailson-Brito, 2016; Mazzoni et al., 2020).

Conclusion

The results showed that some samples of breast milk were contaminated by p,p'-DDT and/or p, p'-DDE, indicating contamination in communities in the Lower Amazon.

Of the seven mothers who presented contamination in the milk, five had worked in pepper, beans, cassava, maize, rice, pineapple, and coriander, lettuce, and chicory crops. However, none of them had contact with organochlorine pesticides during these activities.

Since the occupational use of organochlorine pesticides was not observed in any locality, it is possible that this contamination was due to public health campaigns. Until the end of the 1990s, DDT was applied without great care to the walls of the houses due to insufficient information on its toxicity.

Despite being a preliminary study, the number of samples contaminated by pp'-DDT and its metabolite pp'-DDE was significant, which calls for an expanded study of these areas. Additionally, other environmental and biological matrices should be evaluated to determine the source of contamination.

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