# Levels of Organochlorine Pesticides in Brazilian Human Milk

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*Introduction*

Breastfeeding has been intensively encouraged, especially in developing countries, due to its beneficial properties, i.e., increase infant immune factors and resistance to chronic diseases such as asthma, diabetes or allergies. In addition, human exposure to environmental pollution has led the scientific community to study the pathways of these contaminants and the possible risks they pose to human health. Exposure to organochlorine pesticides (OCs), in special, has been the subject of great interest during recent years given their potential toxicity, resistance to degradation and bioaccumulation through the food chain. The major source of OC has been agriculture and public health campaigns to vector control. General population exposure occur mainly through the diet and human milk can be an indicator of exposure since OCs are lipophilic xenobiotics that accumulate in adipose tissue and breastfeeding is the main pathway of elimination through the fatty fraction of milk. In this study pooled samples of mothers living in the capitals of two different states of Brazil were evaluated in order to assess the trends of human exposure to persistent pollutants.

## *Methods and Materials*

*Milk sampling*

Two pooled samples were analyzed. The first one was collected in the city of São Paulo and the other in the city of Belo Horizonte. Both were part of a total of 10 pooled samples collected in Brazil for the WHO-coordinated exposure study on "Levels of PCDDs, PCDFs and PCBs in Human Milk"[i, ii]. All samples were obtained in 2001 and donors were selected according to the protocol established by WHO[iii]. All mothers were informed about the objectives of the study and gave their consent. At each area, 10 samples of 100 ml each were collected from breastfeeding mothers. Milk was expressed manually directly in a jar and kept frozen at –20°C. Before, each jar was cleaned with organic solvents and three tablets of potassium dichromate were added to avoid degradation during shipping. After finishing the collection of the 10 sub-samples, they were sent frozen to the central laboratory in Rio de Janeiro, where they were defrosted at room temperature and pooled to give a total volume of 1000 ml. One part of 500 ml was sent to the reference laboratory in Freiburg, Germany and the other aliquot was kept frozen for safety purposes (e.g. damage during transportation).

*Analytical procedure*

The applied analytical method follows the principles of Official Methods in Germany[iv]: For determination of organochlorine pesticides, human milk samples were centrifuged and from the cream the compounds of interest extracted together with fat by use of sodium sulfate and light petroleum. After evaporation of the solvent the fat content was determined by weighing out the mass of the remainder. Up to 0,5 g of the fat extract was redissolved in cyclohexane/ ethyl acetate and the internal standards were added. For separation of fat, gel permeation chromatography was performed on Bio-Beads S-X3 with cyclohexane/ethyl acetate as eluting solvent. After concentration of the eluate almost to dryness and redissolution in iso-octane chromatography on a small column of partially deactivated silica gel was used as final clean up step using toluene as eluent.

Routine determination was performed with GC/ECD using generally two different GCs (Fisons Mega 2) with two columns of different polarity (fused silica no. 1: 30 m PS-088 [97.5 % Dimethyl-2.5 % diphenyl siloxane copolymer], 0.32 mm i.d., 0.32 µm film thickness, fused silica no. 2: 30 m OV-1701-OH, 0.32 mm i.d., 0.25 µm film thickness, both columns custom-made). Results were confirmed routinely by GC-LRMS (GC: HP 6890 / MS: HP 5973; 30 m HP5-MS, 0.25 mm i.d., 0.25 µm film thickness + 2.5 m pre-column; detection mode: MSD – EI.

For validation purposes, the laboratory has successfully participated in 56 proficiency tests since 1994.

## *Results and Discussion*

The age of donors in each pool sample was about 22.5 years old in average. The fat amount of fresh weight was 4,2% for São Paulo and 4,9% for Belo Horizonte. Concentrations, based on lipid weight, of organochlorine pesticides in human milk are given in table 1. In both samples, the highest amounts were found for p,p'-DDE, which was 0.596 µg/g fat in São Paulo and 0.155 µg/g fat in Belo Horizonte, followed by *beta*-HCH, which was 0.027 µg/g fat and 0.022 µg/g fat respectively. The concentrations of the other analyzed compounds were near or, in most cases, below detection limit (0.001 µg/g fat).

***Table 1*:** Organochlorine pesticide concentrations in human milk from São Paulo and Belo Horizonte collected in 2001 (µg/g fat)

| Compound | São Paulo | Belo Horizonte |
|---|---|---|
| **HCB** | **0.006** | **0.003** |
| **o,p'-DDT, o,p'-DDE, o,p'-DDD** | **< 0.001** | **< 0.001** |
| **pp'-DDE** | **0.596** | **0.155** |
| **pp'-DDT** | **0.010** | **0.009** |
| **pp'-DDD** | **< 0.001** | **< 0.001** |
| **dieldrin** | **0.001** | **0.001** |
| **endrin, endrin ketone** | **< 0.001** | **< 0.001** |
| ***cis*-heptachlorepoxide** | **0.001** | **0.001** |
| ***alpha*-chlordane, *gamma*-chlordane** | **< 0.001** | **< 0.001** |
| **oxy-chlordane** | **0.003** | **0.001** |
| **trans-nonachlor** | **0.002** | **0.002** |
| **Σ Parlar (toxaphene)** | **< 0.001** | **< 0.001** |
| ***alpha*-HCH** | **< 0.001** | **< 0.001** |
| ***beta*-HCH** | **0.027** | **0.022** |
| ***gamma*-HCH** | **< 0.001** | **< 0.001** |
| **Σ Endosulfane** | **< 0.001** | **< 0.001** |

The high p,p'-DDE/p,p'-DDT ratios, 59.6 for São Paulo and 17.2 for Belo Horizonte, indicate that exposure happened through environmental contamination by former application of DDT since DDE/DDT ratio will increase when the use of DDT ceases[v]. The exposure to its persistent metabolite DDE still occurs mainly via foodstuffs and also through metabolic conversion of p,p'-DDT. The same assumption can also be made for the HCH group, as only the more stable *beta* isomer was present in both samples. In 1985, the use of DDT and HCH has been restricted by the Brazilian Government to public health campaigns for vector control. Until 1998, both compounds were used against the vector of malaria, which is concentrated in the Amazon region (98% of cases), northern part of Brazil[vi]. Both sampled capitals are located in the central part of Brazil.

The levels of the current study were compared to the levels found in a pool sample (40 donors, 33 *primiparae* and 7 in the second lactation) collected in Rio de Janeiro in the year 1992 (table 2)[vii]. Theold data are between 2 and 23 times higher. Results from another study (table 2) conducted in the year 2000[viii], where 50 individual samples collected from a milk bank in Rio de Janeiro were analyzed, showed also higher concentrations in average, especially for dieldrin, *gamma*-chlordane and the HCH group. Unfortunately, no information about the numbers of donor pregnancies was available. In 1999, high HCH concentrations were reported in human milk from mothers living in a area called Cidade dos Meninos, situated in the Municipality of Duque de Caxias/Rio de Janeiro State, where residues of lindane production were left behind about 40 years ago[ix]. The average amounts were 0.101 µg/g fat for *alpha*-HCH, 2.487 µg/g fat for *beta*-HCH and 0.124 µg/g fat in *gama*-HCH, obtained in four individual samples from *primiparae* donors.

***Table 2*:** Organochlorine pesticide concentrations in human milk from Rio de Janeiro collected in 1992 and 2000 ( μg/g fat).

| Compound | Rio de Janeiro 1992 | Rio de Janeiro 2000* |
|---|---|---|
| HCB | **0.012** | **0.026** |
| **pp'-DDE** | **1.520** | **0.580** |
| **pp'-DDT** | **0.180** | **0.024** |
| **pp'-DDD** | **0.006** | **0.014** |
| **Σ DDT** | **1.706** | **0.618** |
| **dieldrin** | **0.023** | **0.048** |
| **endrin** | **n.a.** | **0.013** |
| **cis-heptachlorepoxide** | **0.008** | **n.a.** |
| ***gamma*-chlordane** | **n.a.** | **0.078** |
| **trans-nonachlor** | **n.a.** | **0.016** |
| ***alpha*-HCH** | **0.001** | **n.a.** |
| ***beta*-HCH** | **0.270** | **0.253** |
| ***gamma*-HCH** | **0.005** | **0.073** |
| **Σ Endosulfane** | **n.a.** | **0.176** |

* Average concentrations (n = 50)
n.a. = not analyzed

Levels of HCB, DDT and HCH have been reported in human milk from different countries and some data are given in table 3. Data from Germany are also from samples from the third round of WHO-coordinated exposure studies [x] and reflect the situation in central Europe. Comparison of the levels from the current study in Brazil with those in other countries showed that HCB is at the lower end of concentrations, whereas sum DDT and beta_HCH are at the upper end.

***Table 3*:** Average concentrations of organochlorine pesticides from different countries (μg/g fat).

| Compound | **Iran**[xi] 1991 (n = 40) | Mexico[xii] 1997 (n = 60) | Indonesia[xiii] 2000 (n = 70) | Turkey[xiv] 2003 (n = 37) | Germany 2001/02 (n = 39) |
|---|---|---|---|---|---|
| HCB | **0.061** | **0.025** | **0.03** | **0,020** | **0,042** |
| **pp'-DDE** | **1.701** | **3.997** | **0.28** | **1,522** | **0,148** |
| **pp'-DDT** | **0.302** | **0.651** | **0.06** | **0,065** | **0,005** |
| **pp'-DDD** | **n.a.** | **0.002** | **n.a.** | **n.a.** | **< 0,005** |
| **Σ DDT** | **2.199** | **4.696** | **-** | **1,595** | **0,152** |
| ***alpha*-HCH** | **0.022** | **0.001** | **n.a.** | **< 0,001** | **< 0,005** |
| ***beta*-HCH** | **0.399** | **0.061** | **0.09** | **0,149** | **0,021** |
| ***gamma*-HCH** | **0.182** | **0.002** | **0.01** | **0,003** | **< 0,005** |

n.a. = not analyzed

**Conclusion**

OCs concentrations in the environment have been decreasing gradually worldwide. Legally banning of OCs can lead to low concentrations in the environment and consequently to low human exposure which can be observed by considering human milk as good indicator of general population exposure. This process is supported by the Stockholm Convention which is a global treaty signed now by 55 parties to take action against certain POPs, among them PCBs, PCDDs and PCDFs (xv). After ratification by France as the 50th Party, the Convention entered into force in May 2004. The effectiveness should be evaluated four years after the date of entry into force and periodically thereafter at intervals. Therefore, a Global POPs Monitoring Programme was developed. United Nations Environment Programme (UNEP) organized a workshop to provide a scientific basis for this programme. One of the conclusions was to select the following matrices: air; bivalves; wildlife species (fish, bird's eggs, marine mammals) and human milk (xvi). Therefore, future analyses of these POPs in breast milk also from Brazil are encouraged to show tendencies over time.

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