Levels of selected persistent organic pollutants in blood from delivering women in seven selected areas of São Paulo State, Brazil

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ABSTRACT

Persistent organic pollutants (POPs) present in the living environment are thought to have detrimental health effects on the population, with pregnant women and the developing foetus being at highest risk. We report on the levels of selected POPs in maternal blood of 155 delivering women residing in seven regions within the São Paulo State, Brazil.

The following selected POPs were measured in the maternal whole blood: 12 polychlorinated biphenyls (PCBs) congeners (IUPAC No. 99,101,118,138, 153,156,163,170,180,183,187,194); dichlordiphenyltrichloroethane *p,p'*-DDT, diphenyldichloroethylene *p,p'*-DDE and other pesticides such as hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), hexachlorobenzene (HCB), chlordanes (t-CD and c-CD, oxy-chlordane), nanochlors (t-NC and c-NC).

Statistical comparisons between regions were performed only on compounds having levels above LOD in 70% of the samples. PCB118 congener was found to be highest in the industrial site (mean 4.97 ng/g lipids); PCB138 congener concentration was highest in the Urban 3 site (mean 4.27 ng/g lipids) and congener PCB153 was highest in the industrial and Urban 3 sites with mean concentration of 7.2 ng/g lipids and 5.89 ng/g lipids respectively. Large differences in levels of *p,p'*-DDE between regions were observed with the Urban 3 and industrial sites having the highest concentrations of 645 ng/g lipids and 417 ng/g lipids, respectively; β-HCH was found to be highest in the Rural 1 site; the γ-HCH in Rural 1 and industrial; the HCB in the Rural 1 and industrial sites and oxy-chlordane and t-NC in the Rural 2 sites. An association between levels of some contaminants and maternal age and parity was also found.

Key-words:

Pesticides exposure, delivering women, human blood, São Paulo State, Brazil

1. Introduction

Worldwide, concern is growing about the presence of persistent organic pollutants (POPs) in the living environment and their possible negative impacts both on the environment and on human health. These man-made chemicals are highly resistant to biodegradation and have high affinities for bioaccumulation and biomagnifications, both in the environment and in living organisms, including humans. A number of human studies have shown POP exposure effects on neurological development; thyroid, estrogen, and immune function; and cancer promotion. (Brouwer et al., 1998; Patandin et al., 1999; Porta et al., 2008; Ribas-Fito et al.,2001; Snedeker, 2001; Tryphonas, 1998; Wolff and Toniolo, 1995)

The most vulnerable periods for toxic impact of environmental pollutants on human development are the embryonic and foetal stages.(Daston et al., 2004; Selevan et al., 2000; Weiss, 2000) Pregnant and nursing women pass these pollutants to their babies both transplacentally and via lactation, and evaluating the maternal contamination is an indirect measurement of the exposure of the foetus to external contaminants. (Suzuki et al., 2005; Wang et al., 2004) One of the most significant concerns regarding health effects is the harmful influence of PCBs and PCDDs/PCDFs on future generations, stemming from prenatal and/or postnatal exposure.(Konishi et al., 2009; Wang et al., 2004)

Furthermore, women and children are most vulnerable as they bear a disproportionate burden from environmental pollution and degradation. Hence they are exposed to particulate pollution and other environmental toxins in the air, food and water of rural and urban areas. (Cutter, 1995)

In most of the developing countries including Brazil, the rural population is most affected by exposure to insecticides, fungicides and herbicides, as agriculture is a main source of employment, particularly for women.(Moses et al.,1993; Soares and Porto, 2007; Reports of acute toxic exposure to POPs in

adults, including a few studies on the reproductive effects of pesticides, have been published in Brazil.(Baker et al.,1978; Igbedioh, 1991; Loevinsohn 1987) Additionally, human data obtained by the Unified Health System's database ("DATASUS"), from 1994 to 2004 in the State of Paraná, Brazil, showed a significant decline in male birth rates in municipalities with high levels of pesticides present in the environment.(Gibson and Koifman, 2008)

1.1. Brazilian agriculture and the use of agrotoxics

Pesticides have been widely used in agriculture in Brazil since the 1940s. In the 1950s, organophosphate insecticides were introduced in Brazil to substitute organochlorines that were found to have a long environmental persistence.(Sato et al.,2006) According to the Brazilian Agricultural and Livestock Confederation Report of 2008, agribusiness generated 28% of the Brazilian GNP (Gross National Product), and in 2003, sales of agrotoxics amounted to 375 000 tons of commercial product, which is equivalent to 182 400 tons of the active ingredient. (Confederation, 2008) Over the years, the use of agrotoxic substances has increased significantly, and currently Brazil is considered to be one of the biggest consumers worldwide, with sales increasing by 160% between 1991 and 1998.

Historically, DDT was manufactured in Brazil between 1962 and 1982, with the total production estimated to be about 73 481 tons. In 1985 DDT was banned for agricultural use, and in 1998 it was banned for public health use. In 2009 all DDT products still in circulation were removed and currently DDT is prohibited for production, trade and use.(Brazil, 2009) In contrast, since 1995 Brazil has markedly increased the use of organophosphates with the average annual use between 1998 - 2005 being 60 935 tons. (Sato et al., 2006)

It is estimated that in the agricultural sector, about 12 millions rural employees are exposed daily to toxic substances. Although personal protective equipment (PPE) is provided, in many cases employees ignore and/or fail to use

the PPE correctly, leading to poor compliance. As a result, farm workers are considered to be the most exposed population and the main target for adverse effects.(Oliveira-Silva et al., 2001)

The National Agency of Sanitary Vigilance (ANVISA) in Brazil reported in 2002 that because of Brazil's very high usage of agrotoxics, new legislation was implemented. The reverse logistic legislation compels the producers to collect the packaging of products after they have been used, thus preventing inappropriate disposal of agrotoxics that could result in secondary pollution to rivers and soils. This legislation has an important public and environmental health benefit, if it is applied strictly. (Sato et al., 2006)

In response to the lack of comprehensive data on levels of persistent toxic substances (PTS) in pregnant women from Brazil, a pilot project was designed and carried out by the Univ. Estadual Paulista (UNESP) and the University of Tromsø, Norway, under the auspices of the Arctic Monitoring and Assessment Programme (AMAP) during the period 2007-2008. The study measured levels of selected toxic metals and POPs in maternal whole blood of total number of 155 delivering women. This paper reports on the levels of PCBs congeners and pesticides (DDTs metabolites, α-HCH, β-HCH, γ-HCH, HCB, *t*-chlordane, *c*-chlordane, *oxy*-chlordane, *t*-nonachlor and *c*-nonachlor) in maternal whole blood of delivering women in seven selected regions of São Paulo State in Brazil. The seven study sites differ in their degree of environmental pollution and were selected for this very reason.

Socio-economic and self reported health and lifestyle factors as well as birth outcomes, are also reported. Based on these findings, a longitudinal main study will be designed to investigate sources of exposure and reproductive outcomes in various parts of Brazil.

2. Materials and Methods

2.1. Study sites and population

Agricultural and industrial activities were considered when choosing the sites. The study took place in seven sites of São Paulo State situated in the south-eastern region of Brazil (Figure 1). São Paulo State is the most developed state in Brazil with an area of 248 808 km^2 and a population of approximately 40 million inhabitants.

Two rural sites (Botucatu and Ribeirão Preto), one industrial site (Campinas), one coastal site (Santos) and three metropolitan urban sites (Univ. Federal of São Paulo (UNIFESP), Vila Nova Cachoeirinha and Hospital and Maternity Leonor Mendes de Barros, HMMLMB) were selected. Two rural sites are located inland of São Paulo State (Botucatu and Ribeirão Preto) and are approximately 230 km from São Paulo city. Their economy is based mainly on agriculture, derived mostly from sugarcane, and the population is exposed to sugarcane activities, mainly burning and pesticide application. Three urban sites were selected in different areas of São Paulo city (UNIFESP, HMMLMB, Vila Nova Cachoeirinha). The urban site of São Paulo city was selected because of its large population, high-density living, heavy traffic volume and excessive air pollution. The coastal site, the city of Santos situated on the Atlantic Ocean and approximately 70km from São Paulo was selected mainly because its estuary is polluted by industrial and urban sewage discharges and by harbour activities. Additionally, it is an endemic dengue area where the spraying of insecticides was reintroduced in 2000. The industrial site (Campinas) is a large city with heavy traffic and is home to a variety of industries, including petrochemicals.

Furthermore, a national and international network was established, with the national group consisting of seven centres and a national coordinator from the bio repository centre (Botucatu).

This pilot study had a total of 155 participants; All study sites have public maternal hospitals. Enrollment criteria for this project included women who were admitted to the delivery rooms at seven maternity hospitals during 2007 and 2008 and had resided in the specific study site for at least 1 year prior to the pregnancy but duration at the present address was also recorded. Women who volunteered to participate in the study signed an informed consent form and agreed to donate blood and answer a socio-economic questionnaire and allow access to their post-delivery records. Pesticide exposure was defined as a history of exposure to spraying during pregnancy. In urban areas, domestic use of insecticides was assumed to be a mode of exposure, whereas in rural area agricultural sites, spraying was thought to be the predominant source of exposure. The questionnaire was based on prior studies of women and newborns, translated and adapted for the Brazilian population.(Sandanger et al.,2007)

2.2. Sampling procedures

From each mother, non-fasting samples of venous blood were collected within one day post-partum, employing standard Vacutainer tubes containing heparin as anticoagulant and using the sterile Vacutainer disposable system. The samples were stored at -20°C and shipped in a frozen state to the University of Tromsø, in Norway, for analyses.

2.3 Sample preparation

The whole blood samples were extracted according to a liquid-liquid extraction method published by Sandanger *et al.*(Sandanger et al., 2003) In short, an internal standard mixture (containing fifteen 13C labelled PCBs and eleven of the chlorinated pesticides) was added to 2 ml of blood before mixing with 2 ml of ethanol and 2 ml of deionised water saturated with ammonium sulfate, and extracted twice with 10 ml of n-hexane in a small glass tube. The volume of the

extract was reduced to 0.5 ml using the Rapidvap (Labconco Corp., Kansas City, MO), before clean up and fractionation on a florisil column as described previously (Sandanger et al., 2007).

2.4. Instrumentation: Gas chromatography

Whole blood samples were analysed for the following compounds; PCBs (99, 101, 118, 138, 153, 156, 163, 170, 180, 183, 187, 194), and *p,p'* – DDT, *pp*'-DDE, α-HCH, β-HCH, γ-HCH, HCB, *t*-chlordane, *c*-chlordane, *oxy*-chlordane, *t*-nonachlor and *c*-nonachlor at the Norwegian Institute for Air Research (NILU) laboratory in the Polar Environmental Center, Tromsø, Norway.

The extracts were analysed using an Agilent 7890A gas chromatograph (GC) equipped with a 5975c mass spectrometer (Agilent Technologies, Böblingen, Germany). The GC was fitted with a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness; J&W, Folsom, USA). Helium (6.0 quality, Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1 ml/min. Two µL of the sample extract were injected in splitless mode using a split / splitless injector (injector and autosampler - Agilent 7683 Series, Agilent Technologies, Böblingen, Germany). The GC temperature programme for chromatographic separation was done using an initial temperature of 70°C with a hold time of 2 min, the temperature was then ramped at 15°C/min to 180°C, followed by a temperature ramp of 5°C/min to 280°C with a hold time of 5 min. The electron capture negative ionisation (ECNI) mode was used for identification and quantification of the pesticides, while the electron impact (EI) mode was used for determination and quantification of PCBs and DDTs. In both cases, the selected ion monitoring (SIM) mode was used and the different compounds were identified from their SIM masses, isotopic ratio and retention times. Peaks with differences in isotopic ratio greater than 20%, compared with the quantification standard, were rejected and not quantified. For every 10 samples, a blank was analysed to assess laboratory-derived (i.e., inadvertent) sample contamination. A standard reference

material from the AMAP ringtests was also included in each 10 sample batch. The LODs were calculated using the signal to noise calculations in real samples.

Lipids were determined gravimetrically by evaporating the extract to dryness and weighing the extract when stable weight was achieved.

2.5 Quality assurance and control

NILU participates in the AMAP Human Health Ringtest for plasma samples. The laboratory has participated in the programme from the outset and has performed well to date. Ringtest performance and the regular analysis of certified reference materials clearly indicate that the uncertainty associated with the calculated concentrations is well within +/- 20 %, which is considered the best performance, according to the AMAP ringtest protocol. The high number of labelled internal standards also ensures the quality of the analysis.

As for the determination of lipids, the enzymatic method is considered to be the more precise measurement. In this study only whole blood samples were available; hence, lipids had to be determined gravimetrically. As shown in the work by Sandanger *et al*. 2004, the gravimetric determination is expected to yield results that are 10 – 20 % lower than the enzymatic method.(Sandanger et al., 2004) This needs to be taken into consideration when lipid weight levels are being compared to other data.

2.6. Statistical analyses

Descriptive statistics, ANOVA one way was applied to compare the differences between sites followed by Tukey test for multiple comparisons (adjusted for age and parity). Due to a non-normal data distribution, analytical results for all compounds were log transformed before statistical analysis of lipid adjusted values. All levels below the LOD were set to half LOD, and were included as such in the statistical analyses.

Only compounds with levels above LOD in more than 70% of samples (PCB 118, PCB138, PCB 153, *p,p'*-DDE, β-HCH, γ-HCH, HCB, *oxy*-chlordane, *t*-nonachlor were statistically evaluated further. The criteria of significance was set to P=0.05. The STATA software package, version 10.0 was used to perform the statistical calculations. (Stata10, 2007)

2.7. Ethical considerations

The study protocol was submitted and fully approval by the Brazilian National Research Ethical Council (CONEP-Brazil; protocol number 12388). The study subjects were women admitted for delivery at institutional hospitals in each of the seven study sites. Potential participants received plain-language information about the study and those who agreed to participate signed a written consent form, which stated that participation was voluntary, confidentiality was assured and participants could withdraw from the study at any time. Subjects were also informed that if results where cause for concern, they would be referred to an appropriate care medical facility. Ninety eight percent of potential study participants approached agreed to participate.

3. Results

The study took place during 2007 and 2008, and the analytical measurements were completed at the end of 2009. In the tables that follow, study sites are referred to according to their characteristics, and presented in a particular order: Rural 1 (Botucatu); Rural 2 (Ribeirão Preto); São Paulo metropolis is divided into: Urban 1 (UNIFESP), Urban 2 (Vila Nova Cachoeirinha), Urban 3 (HMMLMB); Industrial (Campinas) and Coastal (Santos).

3.1 Socio-economic, demographic and lifestyle characteristics

Socio-economic, demographic and lifestyle characteristics for participants at each study site are summarised in Table 1. The subsets did not differ from the

overall cohort in terms of demographic variables. The majority of participants were married or lived with their partners, owned their own homes (median 3 rooms / house) and reported a median monthly income of about US$ 480.The majority of the subjects classified themselves as being from the Caucasian race. Less than half of the women were employed. The pesticides used at home varied according to the site, the most prevalent usage being in the three urban sites (São Paulo city). The domestic use of pesticides (yes / no) was reported by 84 women, representing 53% of the study participants. Commercially available Baygon (containing mainly Pyrethroids) was commonly used. Use of pesticides outdoors was most prevalent in both rural sites with 50% of participants reporting to grow own vegetables. Almost 44% of the women reported that at least one person smoked at home. The majority (90%) of the participants reported to be in a good state of health; the study subjects who reported being most satisfied with their living environment were from the industrial site.

3.2 Maternal age, weight, height, parity and neonatal outcomes by site

Table 2 reports on maternal age, weight, height, parity and neonatal outcomes by site. The mean maternal age of all delivering women was 26.3 years; the youngest delivering woman was 14 years old and the oldest was 43 years old. The parity ranged from 1 to 6 with a median of 2. Overall 36% of deliveries were by Caesarian section. The mean maternal weight on the last prenatal care visit was 74.5 kg, varying from 45 to 114 kg. The mean maternal height was 1.59m, ranging from 1.45m to 1.73 m.

Overall birth weight for newborns ranged from 995 to 4460g, and length from 29 to 58 cm. The mean gestational age at delivery was 38.2 weeks. Gender ratio differed between sites, ranging from 32 to 71% frequency for girls.

3.3 Maternal blood levels of contaminants

To allow for comparisons with non lipid adjusted published data, the concentrations and range of the different compounds measured in maternal whole blood (pg/ml), unadjusted for lipids are summarised in Table 3. The overall median, first and third quartiles, LOD value for each compound and the percentage of detected cases higher than LOD are presented. Of the 23 compounds measured, 9 (39%) showed concentrations above LOD of the instrument in more than 70% of the samples.

Table 4 reports comparisons of log transformed results between sites for compounds with concentration above LOD in more than 70% of blood samples and adjusted for age and parity. Only nine compounds met this criteria and these are PCB 118, PCB 138, PCB 153, *p,p'*-DDE, β -HCH, γ-HCH, HCB, *oxy*-chlordane, *t*-nonachlor.

The mean concentration of PCB 118 ranged from 2.55 ng/g lipids in Coastal to 4.92 ng/g lipids in industrial sites and showed statistical significance of p=0.004 between industrial and Rural 2 and Coastal sites. The differences for PCB 138 were not significant and highest concentrations were measured in Urban 3 site (4.27 ng/g lipids). PCB 153 levels ranged from 3.93 ng/g lipids (Urban 1) to 7.2 ng/g lipids in industrial site with no significant differences between sites.

p,p'-DDE was present in 100% of samples, with the highest concentration found in the Urban 3 site (645ng/g lipids), followed by the industrial site (417 ng/g lipids) and the lowest concentration was found in coastal site (126 ng/g lipids), differences being significant (p=0.02). The highest levels of *p,p'*-DDE found in women from the Urban 3 site might be attributed to the fact that they resided in this region for 11.2 (SD 9.9) years. The fact that *pp'*-DDT was detected in only 20% of samples indicates that exposure to DDT took place in the past.

ß-HCH highest concentration was measured in the Rural 1 site (37.9 ng/g lipids) followed by the Urban 3 site (29.3 ng/g lipids).The difference between the sites was highly significant ($p<0.0001$).

The highest mean concentration of γ-HCH and HCB was measured in the Rural 1 and industrial sites (1 ng/g lipids and 10 ng/g lipids respectively) but only γ-HCH differed between sites ($p=0.03$).

Oxy-chlordane was present in all of the samples, with the highest concentration found in the Rural 2 site (5.6 ng/g lipids) followed by the Rural 1 site (3.04 ng/g lipids). The level found in the Rural 2 site was significantly higher than all other sites ($p<0.0001$).

T-nonachlor was present in 99% of the samples, with the highest concentration found in the Rural 2 site (2.0 ng/g lipids) followed by the Rural 1 site (1.42 ng/g lipids). The level found in the Rural 2 site was significantly higher than all other sites, except Urban 2 site ($p<0.001$).

Overall, concentrations of PCB138, PCB153, PCB180, *pp'*-DDE, ß-HCH, *oxy*-chlordane and *t*-nonachlor were found to be significantly higher in women of 30 years of age and above. The levels of PCB118, PCB138, PCB153, α-HCH, β –HCH and c-chlordane were found to be highest in primiparous women and also statistically significant.

4. Discussion and conclusions

Limited information is available on the prenatal exposure to POPs especially in the populations residing in the southern hemisphere. To assess possible exposure of neonates to these compounds, our study quantified the levels of selected POPs in whole blood of delivering women from seven selected regions

of São Paulo State, Brazil and has shown that these contaminants are present in women residing in the São Paulo State of Brazil.

Thus, the study detected 12 PCB congeners in the maternal blood but only three namely PCB 118, PCB 138 and PCB 153 congeners were dominant (e.g. present in more than 70% samples). We may speculate that the limited number of congeners observed might be a result of the introduction and implementation of Brazilian PCB legislation in 1981which prohibits the manufacturing, marketing, use and disposal of PCBs in Brazil.(Penteado and Vaz, 2001) The study by Klantzi et al, 2009 that measured PCBs in human breast adipose tissues in 2004-2005 in Porto Alegre, the capital of the Rio de Grande do Sul state of Brazil also detected lower levels of this PCB congener if compared with results of 1999. (Erdmann et al.,1999) Levels of PCB congeners found in the current study are comparable to those measured in the general population in other non-industrialised developing countries such as Tanzania and India, but lower than those recently reported from Guinea-Bissau and from industrialised countries in the North. (Anda et al.,2008; Linderholm et al.,2010; Todaka et al., 2010; Weiss et al.,2006) Interestingly, PCB levels in Brazilian delivering women were higher than those recently reported in the plasma of delivering women in South Africa and Vietnam. (Hansen et al.,2009; Röllin et al., 2009) They were however lower than those reported by Van Oostdam et al, 2004 in comparable populations residing in Canada, the Netherland and Spain. The imbalanced distribution of this large group of 209 PCB congeners – all of which have different physiochemical properties, different ranges of persistence and biological activity; - is important information that can possibly contribute to the global debate. (Birnbaum and Staskal-Wikoff, 2010)

The environmentally persistent *p,p'*-DDE metabolite was detected in 100% of maternal blood samples in our study and *p,p'*-DDT metabolite was detected in only 20% of the samples both at relatively low concentrations which is an indication that no recent exposure to DDT took place. Another study performed

in 2003 in Rio de Janeiro, Brazil, found *p,p'*-DDE in the breast milk of 97% of participating breastfeeding mothers; with concentrations ranging from 0.16 to 8 ppb. The results from the present study in São Paulo State indicate that DDT contaminations are comparable to those in Rio de Janeiro State. (Sarcinelli et al.,2003) As expected, the concentrations of *pp'*-DDE found in delivering women from São Paulo State are lower than those found in Mexican and South African delivering women residing in areas where DDT is used to control malaria vector. In contrast, the levels of *pp'*-DDE were found to be much higher in delivering women in our study if compared with recent similar studies performed in Vietnam and elsewhere.(Hansen et al., 2009; Röllin et al., 2009; Waliszewski et al.,2000)

Due to the extensive agricultural activities in São Paulo State, our study found higher levels of pesticides in blood of delivering women mostly in rural areas. It is thought that sources of exposure to these pollutants may be both from recent and past activities. It is common practice in Brazil to use municipal solid waste as compost to enrich the soil for agricultural use; it is very possible that this practice has led to overall increase in levels of pollutants in soil, groundwater and in plants, consequently contributing to human exposure. (Lourencetti et al., 2007)

This study also confirmed that the body burden of selected POPs increases with age and decreases with parity in accordance with other studies. (Grimvall et al., 1997; O'Grady Milbrath et al.,2009; Skaare and Polder 1990) It is of concern that chronic exposure to low levels of POPs that can lead to cumulative adverse health effects in exposed populations over an extended period of time and ultimately affecting reproductive health outcomes.(Lee et al., 2006)

The recent Global Monitoring Report of 2009 indicates that there is a gap in knowledge on levels and trends of POPs in ecosystems as well as in humans in most developing countries and in countries in transition. In addition different climatic conditions in countries situated in the southern hemisphere promote an

increase in the use of pesticides for pests and vector control. Use of outdated industrial processes combined with uncontrolled disposal of hazardous chemicals and wastes is also of concern. (UNEP, 2009)

In conclusion, our study quantified pre natal exposure to multiple organic contaminants and contributed to the knowledge of possible exposures to POPs in São Paulo State, Brazil. Although sample size was limited in this pilot stage of the investigation; it produced enough evidence for future studies in Brazil. The strength of our investigation lies in the usage of standardised methods of sample and data collection and comparable analytical methodology.

To the best of our knowledge, this pilot study is the first Brazilian investigation that concurrently measured a wide spectrum of POPs in whole blood of delivering women, in selected areas of São Paulo State, Brazil. The findings may be useful as baseline data for future investigations, particularly regarding temporal trends of exposure to POPs in São Paulo State. As there is no similar data for other parts of Brazil, it is recommended that the study be extended to other regions within Brazil and southern hemisphere, thus contributing to the debate on the climate change, contaminants and human health.

Furthermore, the study involved a multidisciplinary international team of research scientists and laid foundation for building the research capacity in Brazil that will be used in future collaborative investigations.

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Figure 1 Geographical locations of study sites within Brazil (all three urban sites are located in São Paulo city)

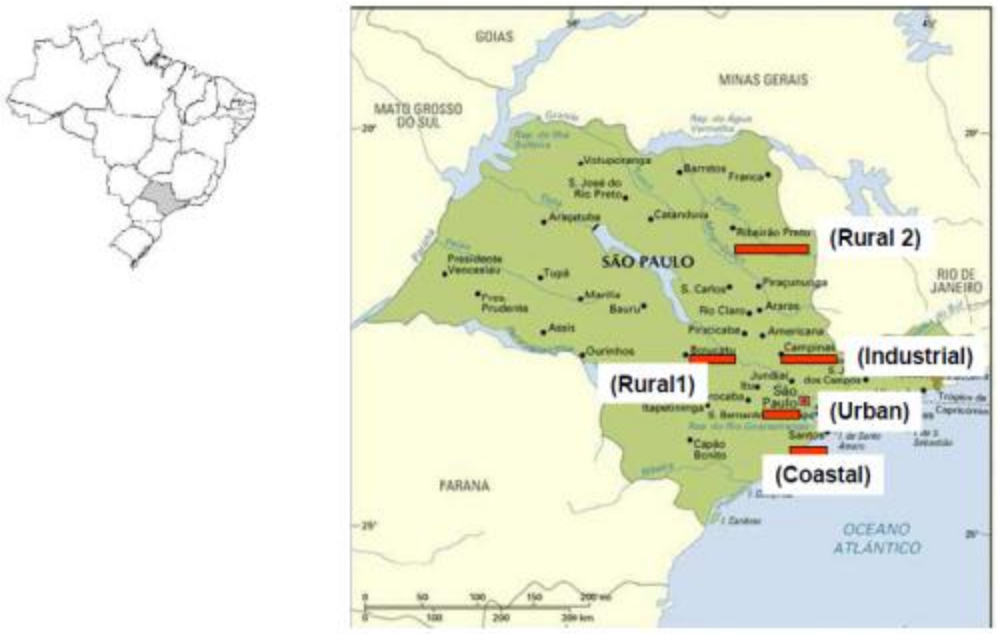

Table 1 Socio-demographic characteristics and life habits of participants by site.

Statistics	Rural 1 (n=36)	Rural 2 (n=19)	Urban 1 (n=20)	Urban 2 (n=20)	Urban 3 (n=20)	Industrial (n=20)	Coastal (n=20)	Total (n=155)
Population group (%) B=Black C= Coloured W= White	B=11 C=31 W=58	B=6 C=26 W=68	B=10 C=45 W=45	B=29 C=33 W=38	B=15 C=15 W=70	B=0 C=15 W=85	B=10 C=20 W=70	B=12 C=26 W=62
Do you consider yourself healthy(% YES)	81	100	65	90	95	100	90	89
Marital status M=married/ living together (%)	89	90	85	80	65	65	80	79
Home ownership %	63	52	60	62	75	75	65	65
Rooms	3.2	3.0	2.7	2.1	2.1	2.8	2.5	2.7
Income/ monthly-median (US$)	388	388	555	600	388	417	611	478
Employed? %	46	16	40	40	20	30	50	35
Somebody smoking In household%	56	53	60	57	40	25	30	46
Environmental pollution in neighbourhood? (%yes)	33	74	45	52	55	25	55	48
Lengths of residence (years)	6.5 (6.8)	6.3 (7.3)	3.3(4)	3.2 (4.3)	11.2 (9.9)	6.1 (7.1)	6.8 (8.7)	6.2 (6.9)

Table 2 Maternal age, weight, height, parity and birth outcomes by site

Statistics	Rural 1 (n=36)	Rural 2 (n=19)	Urban 1 (n=20)	Urban 2 (n=20)	Urban 3 (n=20	Industrial (n=20)	Coastal (n=20)	All n=155
Maternal age								
mean (SD)	29.1	24.6	29.4	22.9	25.4	24	26.1	26
	6.4	6.8	7.6	4.5	6.3	6.5	6.2	5.4
Maternal weight (kg)								
Mean								
SD	77.3	75.4	72.6	72.8	71.8	77.5	73.9	74.5
	13.6	19.5	16.1	13.6	15.6	18.1	14.2	15.8
Maternal height (m)								
Mean	1.60	1.60	1.62	1.60	1.63	1.58	1.62	1.60
SD	0.07	0.08	0.06	0.07	0.07	0.11	0.08	0.08
Parity median (range)	2 (1- 9)	2 (1- 8)	3 (2- 4)	2 (1- 3)	2 (1- 6)	2 (1- 3)	2 (1- 4)	2(1-5)
Birth weight (g)								
mean	3021	2735	3138	3259	3200	2928	3069	3050
SD	469	740	683	397	339	567	505	529
Birth length (cm)								
Mean	47	47.4	47.5	48.0	48.8	47.4	47.5	47.7
SD	5.0	3.5	3.0	2.8	2.1	4.8	2.6	3.4
Head circumference (cm)								
Mean	35.0	33.9	34.2	35.1	34.3	34.9	34.5	34.6
SD	3.6	2.3	1.6	1.4	1.3	2.8	1.6	2.4
Gestational age (w)								
mean	38.3	37.0	38.0	39.2	38.6	37.8	38.2	38.2
SD	2.1	3.9	1.6	1.4	1.5	2.2	2.1	2.1
Gender: girls (%)	36.1	31.6	55	71	31.5	65	60	50
Caesarean section (%)	39	32	50	25	10	50	45	36

Table 3: Overall levels of measured POPs in maternal whole blood at delivery (pg/ml) for all samples

Compounds	Overall Median (all samples)	Overall 1st quartile (all samples)	Overall 3rd quartile (all samples)	Overall Median (>LOD)	Overall 1st quartile (>LOD)	Overall 3rd quartile (>LOD)	LOD	% detected >LOD*
PCB 99	4.46	2.32	8.25	14.96	14.27	17.48	11.91	10.9
PCB 101	6.01	3.90	9.51	14.33	12.81	16.71	11.90	19.1
PCB118	14.93	10.13	19.31	16.36	13.47	21.00	9.5	79.6
PCB138	12.83	9.55	20.35	15.77	12.11	22.88	9.67	76.2
PCB163	3.38	1.94	5.15	12.10	9.54	14.31	9.06	11.5
PCB153	17.74	112.08	28.33	20.41	15.19	33.10	11.16	83.7
PCB156	3.12	1.87	4.63				41.07	0
PCB170	6.03	2.89	9.30	15.96	14.75	20.81	12.18	16.3
PCB 180	14.19	8.93	20.14	19.58	15.68	24.60	11.03	62.6
PCB187	4.10	2.04	7.83	13.77	12.09	18.23	10.81	12.9
PCB183	1.23	0.84	2.59	5.76	5.76	5.76	4.96	2.70
PCB194	1.86	1.22	2.24				5.00	0
p,p'-DDE	280.14	113.12	860.24	280.14	113.12	860.24	11.30	100
p,p-DDT	17.64	10.99	35.65	85.19	65.96	228.48	41.36	20.3
α-HCH	1.0	0.63	1.62	3.40	3.15	7.40	3.0	11.6
β-HCH	28.64	12.32	77.42	48.70	24.23	108.09	11.0	76.9
γ-HCH	2.8	2.15	4.11	3.20	2.55	4.49	2.0	84.4
HCB	35.5	31.4	42.52	35.58	31.39	42.69	2.0	100
t-chlordane	0.4	0.3	0.5	0.55	0.50	0.65	0.5	41.9
c-chlordane	0.2	0.15	0.3	0.60	0.60	0.70	0.5	7.0
oxy-chlordane	5.4	3.99	11.2	5.70	4.00	11.35	2.1	100
t-nonachlor	2.7	1.59	5.65	2.75	1.65	5.65	0.5	99.3
c-nonachlor	0.5	0.3	0.99	0.75	0.55	1.25	0.4	57.8

*LOD= limits of detection

Table 4. Comparison of POPs concentrations in different study sites in maternal blood (ng/g lipids) adjusted for age and parity by ANOVA one-way, followed by Tukey test for multiple comparisons

Compound	Rural 1 (n=36)	Rural 2 (n=19)	Urban 1 (n=20)	Urban 2 (n=20)	Urban 3 (n=20)	Industrial (n=20)	Coastal (n=20)
PCB 118	3.79±1.77	2.62±1.95*	3.37±1.39	3.80±2.58	3.69±1.70	4.92±2.74*#	2.55±1.59#
PCB 138	3.97±2.63	4.01±3.64	2.80±1.03	2.98±3.42	4.27±3.17	3.19±2.04	3.82±1.98
PCB 153	5.23±3.31	4.61±3.70	3.93±2.89	4.48±4.40	5.89±4.81	7.20±5.85	4.65±2.51
p,p'-DDE	198±281 #&	355±590 #@	164±358 *	145±326 *#	645±1347 *	417±1198	126±215 *&@
βHCH	37.92±63.23*	9.97±9.81	3.24±4.03	9.26±14.41	29.29±61.78	28.05±66.33	5.60±5.63
γHCH	1.04±1.40	0.74±0.34	0.48±0.37*	0.74±0.69	0.65±0.41	1.09±0.66*	0.73±0.40
HCB	10.14±4.45	9.13±3.75	8.09±1.79	8.36±2.29	9.88±4.29	10.98±4.41	8.57±2.75
oxy-chlordane	3.04±2.97	5.60±4.94*	1.18±0.66	1.08±0.56	2.72±3.61	1.68±1.22	1.18±0.44
t- nonachlor	1.42±1.83	2.00±1.97*	0.49±0.34*	0.68±0.86*	1.28±1.86	0.68±0.54*	0.69±0.46*

*,#,&,@ - significant differences by Tukey test at 5% significance