EXPOSURE TO PERSISTENT ORGANOCHLORINE PESTICIDES IN THE POPULATION OF THE METROPOLITAN AREA OF SÃO PAULO - BRAZIL **

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INTRODUCTION

The growth in agricultural production, industrialization and urbanization in Brazil has led to human exposure to multiple chemicals. Therefore, it is important to identify populations under risk of exposure to contaminants such as persistent organochlorine pesticides. These compounds cause severe toxic effects for human health and risks to public health. Persistent organochlorines (aldrin, dodecachlor, dieldrin, DDT, endrin, heptachlor, HCH) were included in the POPs list, approved in May 2001 by the Stockholm Convention on Persistent Organic Pollutants (POPs, 2001). São Paulo Metropolitan Region presents various contaminated sites (CETESB 2012) and it is one of the world highest urban concentrations. Human biomonitoring is an used tool in environmental medicine to assess the level of internal exposure of the general population, groups and individuals to environmental pollutants. Blood donors constitute a group of healthy individual and researches have shown that adequately represent the population (CERNA et al, 2007).

OBJECTIVES

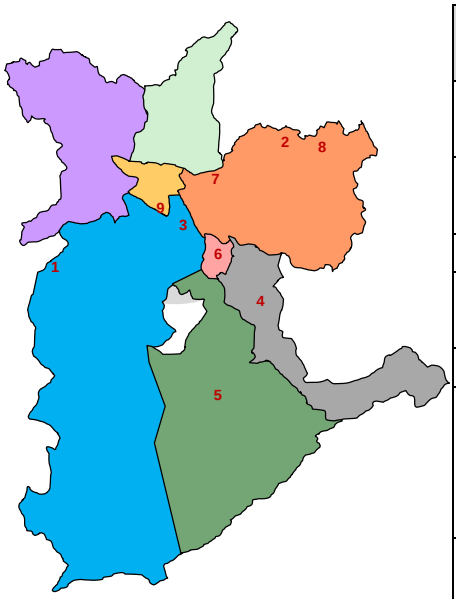
The study had as objective to evaluate exposure by persistent organochlorinated residues in the adult population from the Metropolitan Region of São Paulo (RMSP) and provide subsidies for realization of the First Brazilian Survey of Populations Exposed to Chemical Substances.

METHODOLOGY

Study Population

Biological monitoring was conducted in blood serum (N = 547) of adults of both sexes, 18 to 65 years old and living in RMSP, collected in nine blood donation stations of the Beneficent Association of Blood Donors – Colsan (Figure 1).

Figure 1: Distribution of blood collection stations of the Beneficent Association of Blood Donors – Colsan of São Paulo Metropolitan Region



	Collection Station	Location	Number of samples
1	Hospital Campo Limpo	Campo Limpo	36
2	Hospital Municipal Alípio C Neto	Ermelino Matarazzo	38
3	Hospital Ipiranga	Ipiranga	54
4	Hospital Estadual Mário Covas	Santo André	140**
5	Hospital Anchieta	São Bernardo	95
6	Núcleo Regional de Hemoterapia Dr. Aguinaldo Quaresma	São Caetano	29
7	Hospital Carmino Caricchio	Tatuapé	57
8	Hospital M. Tide Setúbal	São Miguel Paulista	28
9	Hospital Servidor Público Municipal	Vergueiro	70
			547

* Proportional number of the usual demand for each unit;
**Include 2 stations.

Sample collection

Blood samples (about 10 ml) were collected in type "vacutainer" bottle without anticoagulant addition and sent immediately to laboratory, under refrigeration. After centrifugation, serum samples were transferred to glass tube with screw cap and PTFE stopper and kept in freezer until analysis.

Ethical aspects

All procedures complied with the ethical guidelines.

Questionnaires

Questionnaires were used to obtain socio-demographic and economic characteristics (sex, race, education, birth place, age, work, residence time) and variables (past occupations involving pesticides, animal farming for consumption, greenery garden, living near industrial waste or agricultural area and water source used) for support interpretation of the analytical results.

Statistical Analysis

Were investigated correlation of variables with relation to exposure and risk factors included in the applied questionnaires, described in the Chart 1.

Chart 1 - Statistical Analysis

Statistical software SPSS version 15.0. 5	Medians, means, standard deviations, variations, and percentiles
Kolmogorov–Smirnov test	Normality of data distribution
Mann-Whitney test	Comparison for 2 variables
Kruskal-Wallis test	Comparison for 3 or more variables.

Statistical error type I = 5%; Values < LOQ = ½ LOQ

Analytical method

The method was "Hexane extractable chlorinated insecticides in human blood", Dale et al. (1966) with modifications as described in Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples (Dale, 1980). External calibration curves at least 7 levels of concentrations for each pesticide were used to quantify pesticide residues, within the linear range of detector (Table 1): of 0,01 - 0,4µg/dL for HCB, α-HCH , γ-HCH, δ-HCH; of 0,04 - 1,0µg/dL for β-HCH; of 0,02 - 0,8µg/dL for heptachlor, heptachlor epoxide (cis e trans), pp'DDE, dieldrin; of 0,03 - 1,6 µg/dL for op'DDE, op'DDD, pp'DDD, op'DDT, pp'DDT and of 0,04 - 1,7µg/dL for dodecachlor.

The identification and quantification were by GC/µECD for organochlorine pesticides and metabolites, totalling 8205 assays (Table 1).

CHART 2– Qualitative and quantitative analysis

	Column	**Instrument**	**Chromatographic conditions**
Identification and Quantification	VF-5MS (5% phenyl 95% dimethylsiloxane) fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness)	GC Agilent 6890	Injector temperature: 250°C, Detector: µECD, temperature: 310°C, Oven Temperature programmed for quantification: 60ºC (3min.), 20ºC/min to 200ºC, 3ºC/min, 280ºC, 290ºC (20min); Flow of carrier gas N_2, 1mL/min, Mode and injection volume: splitless, 2µL.
Confirmation	VF-35MS (35% phenyl 65% dimethylsiloxane) fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness	GC Thermo Scientific Trace GC Ultra	Injector temperature: 250°C, Detector: ECD, temperature: 310°C, Oven Temperature programmed for quantification: 60ºC (3min.), 10ºC/min, 220ºC, 3ºC/min., 280ºC; Flow of carrier gas N_2, 1mL/min, Mode and injection volume: splitless, 2µL.

RESULTS AND DISCUSSIONS

The blood donors sampled showed similar profile to the adult population of RMSP in terms of ethnic groups, birthplace and socioeconomic variables; however had a slight predominance of male population, opposed to that found in RMSP reality where the female population is slightly larger and better educated. The mean residence time in last address in São Paulo (MRSP) was 16 years. All medians had lower levels of organochlorine pesticides than LQs (0.02 to 0.16 µg/dL) (Table 1); β-HCH, and pp'DDE were found with higher frequencies (24% and 70%) and maximum levels of 0,45 µg/dL and 1,17 µg/dL, respectively (Table 1). DDT (isomers op'and pp'), after body absorption is distributed into tissues and highest concentrations are found in adipose tissues due to high lipid solubility. After absorption, DDT is slowly metabolized to op' and pp'DDE, DDA and DDD isomers. DDE is stored in adipose tissue and excreted via the bile, urine and milk (KLAASSEM 2007). The analysis revealed that individuals with history of exposure to pesticides (working with pesticides) had p,p'DDE levels twice higher than those with no past exposure reported. No other organochlorines were found. Studies have shown pp'DDE levels in blood serum of exposed and unexposed population (Cruz et al 2003; CHARLIER & Plomteux 2002). However, higher levels of pp'DDE regarding DDT isomers indicate earliest exposure (ZHOU et al 2006). These data are consistent with the values of pp'DDE being significantly higher among those people who had reported occupation with pesticides in the past and results with DDT isomers were less frequent. In Brazil there are no established benchmarks for organochlorines pesticides in human serum (Brazil 1994).

Table 1. Concentrations of persistent organochlorine pesticides in blood serum (µg/dL)

Organochlorine pesticides	**Mean (x)**	**SD**	**Median (Md)**	**Geometric Mean (MG)**	**Maximum value**	**P95**	**LOQ**	**Adult population not exposed References**	
								Turci et al, 2010 (Novafeltria, Pavia, Italia)	Cruz and Lino 2003 (Coimbra, Portugal)
HCB	0.01	0.001	0.01	0.01	0.03	0.01	0.02	0.03 (Md)	
α-HCH	0.01	0.001	0.01	0.01	0.03	0.01	0.02	ND (LD=0.02)	0.76 (mean)
β-HCH	**0.03**	**0.038**	**0.02**	**0.02**	**0.45**	**0.07**	**0.04**	**0.05 (Md) 0.12 (máx)**	**0.16 (mean) 0.3 (max)**
γ-HCH	**0.01**	**0.005**	**0.01**	**0.01**	**0.12**	**0.01**	**0.02**	**0.01 (Md) 0.01 (max)**	**0.07 (mean) 0.48(max)**
δ-HCH	**0.01**	**0.014**	**0.01**	**0.01**	**0.32**	**0.01**	**0.02**	**-**	**-**
Heptachlor	0.02	0.000	0.02	0.02	0.02	0.02	0.04	-	-
Heptachlor epoxide	0.04	0.000	0.04	0.04	0.04	0.04	0.08	ND (LD=0.005)	-
Dieldrin	0.02	0.000	0.02	0.02	0.02	0.02	0.04	-	-
o,p' DDE	0.04	0.000	0.04	0.04	0.04	0.04	0.08	0.04 (max)	-
p,p' DDE	**0.05**	**0.085**	**0.02**	**0.03**	**1.17**	**0.12**	**0.08**	**0.03 (Md) 0.20 (max)**	**0.16 (mean)**
o,p' DDT	0.04	0.000	0.04	0.04	0.04	0.04	0.08	0.01 (Md)	1.54 (Md)
p,p'DDT	**0.04**	**0.004**	**0.04**	**0.04**	**0.09**	**0.04**	**0.08**	**0.01 (Md) 0.07 (max)**	**1.88 (mean)**
o,p'DDD	0.04	0.000	0.04	0.04	0.04	0.04	0.08	-	-
p,p' DDD	0.04	0.000	0.04	0.04	0.04	0.04	0.08	ND (LD=0.005)	1.20 (mean)
Dodecachlor	0.08	0.000	0.08	0.08	0,08	0.08	0.16	-	-

SD: Standard Deviation; LOQ: limit of quantification; Md: Median; max: Maximum value; ND: Not detected; P95: percentil 95

CONCLUSIONS

The data obtained in this study represent significant contribution to the knowledge of the levels of organochlorine pesticides in adults of Metropolitan Area of São Paulo. The results provide integrated actions to health control authorities in order to reduce human exposure to chemical contaminants, to prevent or minimize the risks and viabilize the implementation of the First National Survey of Populations Exposed to Chemical Substances, under general coordination of CGVAM / SVS / MS.

References

1. Brasil, Ministério do Trabalho. Secretaria de Segurança e Saúde no Trabalho. Portaria nº 24 (NR-7), de 29/12/1994, D.O.U. de 30/12/94, Seção 1: 21278-280.
2. CETESB. Companhia de Tecnologia de Saneamento Ambiental - Relação de áreas contaminadas do Estado de São Paulo. Atualização em dez 2012. http://www.cetesb.sp.gov.br/areas-contaminadas/relacoes-de-areas-contaminadas/15-publicacoes.
3. CERNÁ M, SPĚVÁCKOVÁ V, BATÁRIOVÁ A, ET AL. Human biomonitoring system in the Czech Republic. Int J Hyg Environ Health. 2007; 210(3-4):495-9
4. CHARLIER JC, PLOMTEUX GJ. Determination of organochlorine pesticide residues in the blood of healthy individuals. Clin Chem Lab Med. 2002; 40 (4): 361-364.
5. CRUZ S, LINO C, SILVEIRA MI. Evaluation of organochlorine pesticide residues in human serum from na urban and two rural populations in Portugal. The Science of the Total Environment 2003; 317:23-35.
6. DALE, W.E., CURLEY, A. and CUETO, C., Hexano extractable chlorinated insecticides in human blood. Life Sciences, 5:47, 1966. In Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples, Environmental and Protection Agency, 5A, (3), (a), rev. 1980.
7. KLAASSEM CD EDITOR. Casarett and Doull's Toxicology The Basic Science of Poisons, New York, 17th Ed., Mac Graw Hill Medical, 2007.
8. POPs - Persistent Organic Pollutants, 2001: Text of the Convention of POPs, Stockholm, Sweden, 22 May 2001. http://chmpps.int/The Convention/Overview/TextotheConvention/tabid./2232/Default.aspx.
9. TURCI R. ET AL. A simple and fast method for the determination of selected organohalogenated compounds in serum samples from the general population.Toxicology Letters, 192 (2010) 66-71.
10. ZHOU R, ZHU L, YANG K, CHEN Y. Distribution of organochlorine pesticides in surface water and sediments from Qiantang River, East China. Journal of Hazardous Materials A. 2006; 137: 68-75.

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