EVALUATION OF HUMAN EXPOSURE TO DDT IN SANTOS AND SÃO VICENTE ESTUARY, SÃO PAULO, BRAZIL

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Introduction

The Santos and São Vicente Estuary, sited on São Paulo's coast, is one of the areas deemed to have high levels of environmental degradation in Brazil[1]. Over 40 years ago, industries used the cities within Santos and São Vicente Estuary to dump tons of hazardous wastes that contained persistent organic pollutants (POPs) in to the environment. During this time period, researchers identified POPs within the areas. Of those pollutants, Dichlorodiphenyltrichloroethane (DDT) is an organic compound that was found in the environment (i.e. sediment, fish, mussels) and blood/breast milk of the population[1,2,3,4]. Although the use of POPs is illegal, there still remain exposure routes from which the population can contract these contaminants[5].

To analyze the extent of DDT contamination, hair samples were chosen because they are a non-invasive matrix, that is easy to collect, transport, and store. It can reveal internal, external, short, and long-term exposures to organohalogenated pollutants in any population without restrictions[6,7,8].

This study aims to measure the concentration of DDT in hair samples of exposed members from contaminated areas in Santos and São Vicente Estuary, and Bertioga.

Materials and methods

Sampling: Four contaminated estuarine areas established around the hazardous wastes were selected (**area 1**: Pilões and Água Fria; **area 2:** Cubatão Center; **area 3:** Continental São Vicente; and **area 4:** Guarujá). One area outside the estuary without a history of contamination was sampled (**area 5:** Bertioga). In 2009, 121 hair samples of the studied populations were collected. The number of samples collected per area are the following: 11 in Pilões and Água Fria; 13 in Cubatão Center; 41 in Continental São Vicente; 29 in Guarujá and 27 in Bertioga. The hair samples were cut close to the root from the occipital region and storage in a plastic bag. Figure 1 shows the five selected areas.

Chemical analysis: The preparation of the samples was based on Wielgomas *et.al.,* (2012) study[8]. About 100mg of hair was cut in small fragments and spiked with the internal standard PCBs: 103 and 198 (200ppb). Then, 4ml of HCl (4M) and 4ml of hexane:acetone (4:1vv) was added to the hair samples and there were incubated overnight at 40°C. The samples were agitated and centrifuged; then, the organic phase was transferred to another tube (three times). The clean up was done with a column of 1g of silica, 1g of acidified silica (1%), and 1g of anhydrous sodium sulfate. Samples were then eluted with 10ml of hexane and 10ml of acetone. Eluates were transferred to a vial and dried to 50µL. Lastly, the internal standard 2-4-5-6 tetrachlorometaxylene (200ppb) was added.

The instrumental analysis of the extracts was carried out by gas chromatography (Agilent GC 7890A) with a DB-5MS capillary column (60 m x 0.25 mm i.d., 0.25-µm film thickness, Agilent Technologies) coupled to a mass spectrometry (Agilent 5975C MS), using negative chemical ionization (NCI). The conditions of NCI analysis and selection of ions have been described elsewhere[9]. Injection was splitless at 265°C, and the oven program was the following: 90°C (1 min); 150°C (10°C.min^{-1}); 240°C (3°C.min^{-1}); hold for 5 minutes; and then 300°C (10°C.min^{-1}) and hold for 5 minutes. The extracts were analysed for o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT.

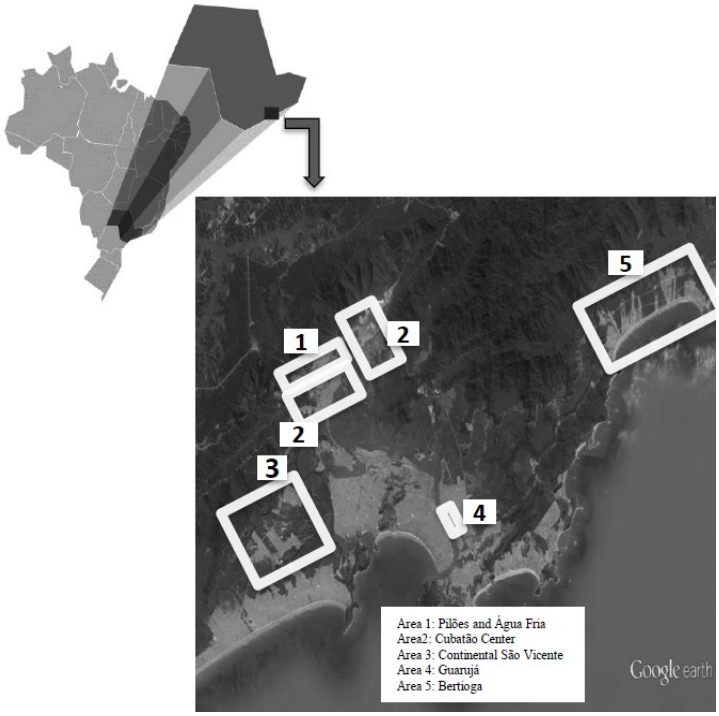

Figure 1. Five areas studied.

Quality assurance/quality control: Blanks were performed during each analytical batch to evaluate interferences. The limit of detection (LOD) for p,p'- DDT was 11.1 $ng.g^{-1}$ and for the other DDT metabolites, the LOD ranged from 3.7 to 6.8$ng.g^{-1}$. The mean recovery value of DDT and its metabolites (□DDT) from the tests of method validation was 74.4%.

Statistical test: The distribution of DDT in the five studied areas was statistically tested with a one-way ANOVA followed by a Kruskal Walles test to compare the five sampling areas. We adopted a statistical significance level of 5 %. All statistical analyses were performed on Statistical Package for the Social Sciences (SPSS) 17.0 for Windows.

Results and discussion

The internal standard recovery mean in the samples was 79% for PCB 103 and 92% for PCB 198. DDT was present in the five studied areas. Table 1 shows DDT (□DDT) concentrations per area.

Table 1. Concentration of □DDT ($ng.g^{-1}$) in the five studied areas.

	□ DDT						
	Mean	**SD***	**1ºQ+**	**Median**	**3º Q+**	**Minimum**	**Maximum**
Pilões and Água Fria	136.2	159.6	38.3	87.8	191.2	0.0	470.9
Cubatão Center	47.7	40.9	19.6	37.1	79.9	0.0	128.3
Continental São Vicente	99.8	124.7	21.2	60.5	124.9	0.0	506.6
Guarujá	78.2	104.5	0.0	41.6	144.1	0.0	372.1
Bertioga	48.5	88.8	0.0	13.3	60.1	0.0	346.7

*SD= Stantard deviation; + Quartiles.

Pilões and Água Fria was the area with the highest □DDT concentrations, subsequent by Continental São Vicente, Guarujá, Bertioga and Cubatão Center. The lowest DDT concentration was revealed in Cubatão Center. There was no significant difference between the five areas (ANOVA p=0.172). Figure 2 shows the contribution of each DDT metabolite to the □DDT.

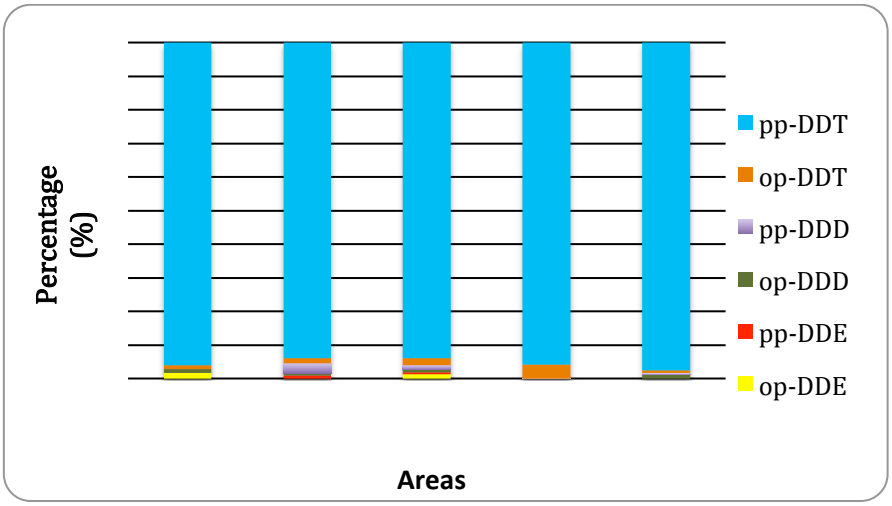

Figure 2. Contribution (%) of DDT metabolites to □DDT

The DDT metabolites concentrations ranged from non detected (0.0ng.g^{-1}) to o,p-DDT concentration in Guarujá (3.56±10.71ng.g^{-1}). The p,p-DDE was present only in Continental São Vicente (0.72±2.47ng.g^{-1}) and Cubatão Center (0.51±1.62ng.g^{-1}). The p,p'-DDT metabolite was the most present in the five areas. Its contribution in the □DDT concentration was 95.4%. Table 2 shows the p,p'-DDT concentrations in the five areas.

Table2. Concentration of p,p'-DDT (ng.g^{-1}) in the five studied areas.

	p,p'-DDT						
	Mean	**SD***	**1ºQ+**	**Median**	**3º Q+**	**Minimum**	**Maximum**
Pilões and Água Fria	130.6	156.7	38.3	79.0	174.0	0.0	463.4
Cubatão Center	44.8	40.0	16.9	37.3	62.7	0.0	128.3
Continental São Vicente	93.7	125.6	15.9	55.0	122.2	0.0	506.6
Guarujá	80.9	95.3	0.0	40.8	144.5	0.0	331.0
Bertioga	47.3	88.1	0.0	9.3	59.1	0.0	343.3

*SD= Stantard deviation; + Quartiles.

Pilões and Água Fria showed the highest p,p'-DDT concentration followed by Continental São Vicente, Guarujá, Bertioga and Cubatão Center was the area with the lowest concentration. There was no significant difference on p,p'-DDT concentrations in the five studied areas (ANOVA p= 0.260). The relation DDE/DDT was close to zero.

Previous studies in Santos and São Vicente Estuary, have identified □DDT found in sediment (301 ng.g^{-1}) since 1974. The concentration has been reducing throughout the years and in 2001 it was not detected. The same has been observed through fish and mussels from the region's rivers[1].

Furthermore, the presence of DDT was revealed in the blood (□DDT=0.13 ng.g^{-1}; n=9) and breast milk (□DDT=40.62 ng.g^{-1}; n=23) of members from Continental São Vicente[4]. Another study detected the presence of DDT in the blood of the members from Pilões (□DDT=3.71 µg.L^{-1}; n= 222). The highest concentration was revealed in females that were over the age of 40 years old[3]. Lastly, DDT was found in the blood of children from Pilões (□DDT=0.06µg.L^{-1}; n= 242)[2]. These concentrations are much lower than the □DDT concentrations observed in hair samples of this study.

However, the p,p'-DDT concentration in this study is ten times lower than the concentration revealed in the hair of members from the Amazon riverside in 2009 (p,p'-DDT=1017.9ng.g^{-1}). In this area, DDT was widely used against malaria[10]. In addition, the concentrations identified in the five studied areas are lower than the concentrations found in other countries such as Poland in 1968 (□DDT=1658.0ng.g^{-1})[8], Poland in 1989

(□DDT=143.9 ng.g^{-1})[8], and adolescents from Romania in 2002/2003 (□DDT=394 ng.g^{-1})[7]. With the exception of Cubatão Center and Bertioga, the concentrations revealed in this study are similar to those from Greece in 1996 (□DDT=101ng.g^{-1})[6], but higher than concentrations from Belgium in 2000 (□DDT =18.8 ng.g^{-1})[6], Romania in 2000 (□DDT=52.2ng.g^{-1})[6], and Poland in 2009 (□DDT=36.5 ng.g^{-1})[8] . The concentrations revealed in the contaminated areas investigated in this study suggest a relative increase of DDT availability as well that there are still exposure routes in Santos and São Vicente estuarine populations for this organic compound, hence, this matter deserves further investigation.

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References

1. Companhia de Tecnologia de Saneamento Ambiental (CETESB). (2001) http://www.cetesb.sp.gov.br/Agua/praias/publicacoes.asp.
2. Santos Filho, Silva RS, Barretto HHC, Inomatan ONK, Lemes VRR, Sakuma AM and Scorsafava MA. (1993) *Rev de Saúde Pública* 27, 59-67.
3. Santos Filho E, Silva RS, Barretto HHC, Inomatan ONK, Lemes VRR, Kussumi TA and Rocha, SOB. (2003) *Rev Saúde Pública 3*7, 515-522.
4. Silva AS, Barreto HH, Lemes VRR and Kussumi TA. (1997) *Pesticids: R. Ecotoxicol e Meio Ambiente* 7, 123-135.
5. Carvalho DP, Guimarães MT, Ribeiro TS, Campina NN, Lobarinhas MR, Lopes ALJ, Cunha MG, Souza LB, Oliveira VLF, Braga E Braga L, Martins LC, Gomes A, Pereira AA and Braga A. (2014) *Envron Sci Pollut Res.*
6. Covaci A, Tutudaki M, Tsatsakis AM and Schepens P. (2002) *Chemosphere* 46, 413-418.
7. Covaci A, Hura C, Gheorghe A, Neels H and Dirtu AC. (2008) *Chemosphere* 72, 16-20.
8. Wielgomas B, Czarnowisk W and Jansen EHJM. (2012) *Chemosphere* 89, 975-981.
9. Meire RO, Lee SC, Yao Y, Targino AC, Torres JPM and Harner T. (2012) *Atmos Environ* 59, 108-116.
10. Saldanha GC, Torres JPM, Bastos WR, Claudio L, Henkelmann B, *Schramm KW. (2009) Organohalogen Compd* 71, 754-757.