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FULL PAPER

Determination of Environmental Exposure to DDT by Human Hair Analysis in Santos and São Vicente Estuary, São Paulo, Brazil

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Abstract:

In 2009, human hair samples were collected in Brazilian sites historically contaminated with organochlorine to assess the level of contamination with DDT and its metabolites in local human populations (Santos and São Vicente Estuary - São Paulo State). 122 hair samples from members of four contaminated community were collected (Pilões and Água Fria, Cubatão Center, Continental São Vicente and Guarujá), along with one non-contaminated sample (Bertioga). DDT and its metabolites were detected in approximately 70.0% of the hair samples from all areas, and its concentrations ranged from 50.3 ng.g^{-1} to 141.8 ng.g^{-1} in Cubatão Center and Pilões and Água Fria, respectively. The highest p,p'-DDT concentration was detected in Pilões and Água Fria (134 ng.g^{-1}) and, Cubatão Center (43.9 ng.g^{-1}) exhibited the lowest concentration. There was not a clear association between the occurrence of evaluated diseases and DDT detection in hair. None of the locally produced groceries appeared to be risk factors for the presence of DDT in hair. However, DDT concentrations found in this study showed an increase of DDT available for human exposure. These results strengthen the evidence of current exposure routes between DDT and the population of the estuarine region; therefore, this issue deserves further investigation.

Keywords: dichlorodiphenyltrichloroethane (DDT); environmental contamination; human exposure; persistent organic pollutants (POPs); risk assessment

1. Introduction

Dichlorodiphenyltrichloroethane (DDT) has been produced in large volumes since 1945. In Brazil, large quantities of DDT were produced and used in agriculture and the prevention of animal parasites, as a parricide and insect repellent, and in tropical disease control programs. In 1971, restrictions on commercialization and production were established, but DDT application was still allowed for public health programs. DDT was used especially to control malaria, Leishmania and dengue vectors. DDT use in public health programs was prohibited in 1998. In 2009, DDT was completely prohibited in Brazil [1-4].

The Santos and São Vicente Estuary, located on the São Paulo state shore, is a region with high levels of environmental degradation. Cubatão's industrial pole installation during the 1950s and Santos' port presence have turned

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this region into an important industrial center in Brazil. The industrial pole has received new chemical plants as fertilizers and petrochemicals. Over the last 40 years, industries have used the cities within Santos and São Vicente Estuary to dump tons of hazardous wastes that contained persistent organic pollutants (POPs) into the environment [5].

During this period, researchers identified POPs within the areas, although there was no production of DDT, and its last application in public health programs in São Vicente region was in 1997. For all these years, DDT has been highlighted as one of the most common organic contaminants present both in the environment and in human tissues [5-11].

Pereira and Tommasi (1985) detected DDT in sea water in the estuarine area and concluded that sea contamination originated from rivers from the estuary region [6]. Magalhães (2005) observed DDT in fishes (*Paralonchurus brasiliensis*, *Trichiurus lepturus* and *Cathorops spixii)* captured from different points within Santos and São Vicente Estuary [11]. In 2001, the São Paulo state environmental company (CETESB) published a document indicating the contaminated areas, and the same points in the estuary highlighted in the past were still present as contaminated areas [5]. After fourteen years, a document published in 2015 by the statal company still included the same contaminated areas with POPs in the region [12]. Magalhães et. al. (2012) detected DDT concentrations in Santos Bay Crabs and p,p'-DDE was the most present metabolite [13]. Lailson-Brito et. al. (2011) and Leonel et.al. (2012) revealed DDT concentrations in franciscana dolphins and spotted dolphins, respectively, and DDE was the most present metabolite, which was suggestive of past contamination [14, 15]. In 2016, Taniguchi et.al. detected DDT concentrations in pellets from sand from São Vicente, Guarujá and Bertioga beaches. In these pellets, DDT concentrations were higher than DDE and DDD concentrations, which was suggestive of recent contamination [16].

During the 1990's and 2000's, DDT was detected in blood and breast milk from residents of Continental São Vicente and in the blood from adults and children from Cubatão. Despite these studies, there is still a lack of information about affected populations' health status in Santos and São Vicente Estuary [5, 7, 9, 10, 12].

Inhalation and ingestion are the main pathways to DDT exposure in humans. As a lipophilic compound, DDT and its metabolites can attach to air particles or food, undergo body absorption, and accumulate in fat tissues. Human exposure to this compound is associated with many diseases, such as liver diseases, neurological problems, congenital malformations, miscarriage, problems in reproductive organs and cancer [1, 17, 18]. Although the use of POPs is illegal in Brazil, exposure routes from which the population can contract these contaminants still remain [19].

Hair samples are used to assess exposure to POPs because it is a non-invasive matrix, easy to collect, transport and store, and presents a high lipid level (1–9% lipids). Several chemicals from DDT present in the blood are carried through the bloodstream to the hair follicle, where they are strongly linked to hair structure (internal exposure). Moreover, hair samples could display key information about the deposition of air pollutants, which is directly related to external environmental pollution (external exposure). Regarding its feasible usage in biomonitoring, hair analysis can reveal internal, external, short and long-term exposure without restrictions in any biologic population [20-24].

This study aimed to measure the concentration of DDT in hair samples of exposed community members from contaminated areas in Santos and São Vicente Estuary and Bertioga and to evaluate the potential risk factors for DDT in hair samples and its association with possible health problems.

2. Results and Discussion

Hair samples were collected in 67 households (Pilões and Água Fria = 7; Cubatão Center = 10; Continental São Vicente = 20; Guarujá = 14 and Bertioga = 16). DDT was present in 74.6% of them. From the 122 participants, 93.4% of them were female (Pilões and Água Fria = 100%; Cubatão Center = 100%; Continental São Vicente = 87.8%; Guarujá = 90% and Bertioga = 100%). The mean age in the five studied areas were 27.6 years old (SD= 20.0; min =1-

max=79). Among these areas, Cubatão Center was the area that revealed the highest mean age (mean=34.8±23.5; min=1-max=79), followed by Continental São Vicente (mean=28.2±20.5; min=1-max=76), Pilões and Água Fria (mean=27.8±21.2; min=9-max=76), Guarujá (mean=25.7±18.1; min=1-max=70) and the youngest area was Bertioga (mean=25.7±19.9; min=1-max=64). DDT was detected in almost ¾ of the participants' hair. Table 1 shows the distribution of DDT in participants' hair from the five studied areas.

Table 1. Distribution of DDT in participants' hair per area.

	DDT Presence N (%)					
	Pilões and Água Fria	**Cubatão Center**	**Continental São Vicente**	**Guarujá**	**Bertioga**	**Total**
Absence	3 (27.3)	2 (15.4)	9 (22.0)	13 (43.3)	11 (40.7)	38 (31.1)
Presence	8 (72.7)	11 (84.6)	32 (78.0)	17 (56.7)	16 (59.3)	84 (68.9)
Total	11 (100.0)	13 (100.0)	41 (100.0)	30 (100.0)	27 (100.0)	122 (100.0)

Cubatão Center is the area that revealed the highest prevalence of participants with DDT in hair. There was no association between the presence of DDT in hair and living in one of the five areas (Pearson's $\chi 2$: z = 6.4; p = 0.169), and there were no significant differences between areas. Table 2 shows DDT (ΣDDT) and each metabolite concentrations per area.

Pilões and Água Fria exhibited the highest ΣDDT concentrations, followed by Continental São Vicente, Guarujá, and Bertioga. The lowest DDT concentration was detected in Cubatão Center. There were no significant differences between the five areas (ANOVA p=0.461).

The concentrations of o,p-DDT; p,p'-DDE; o,p'DDD and p,p'-DDD were extremely low in studied areas, and o,p'DDE was not detected in any of the studied areas. The p,p'-DDT metabolite was present in the five areas. Pilões and Água Fria revealed the highest concentration of p,p'-DDT in participants' hair, and Cubatão Center showed the lowest value. There were no significant differences in the p,p'-DDT concentrations between the studied areas (ANOVA p=0.482). The contribution of p,p'-DDT in the ΣDDT concentration in all five areas was 94.3%. Figure 1 shows the contribution of each DDT metabolite to ΣDDT per area.

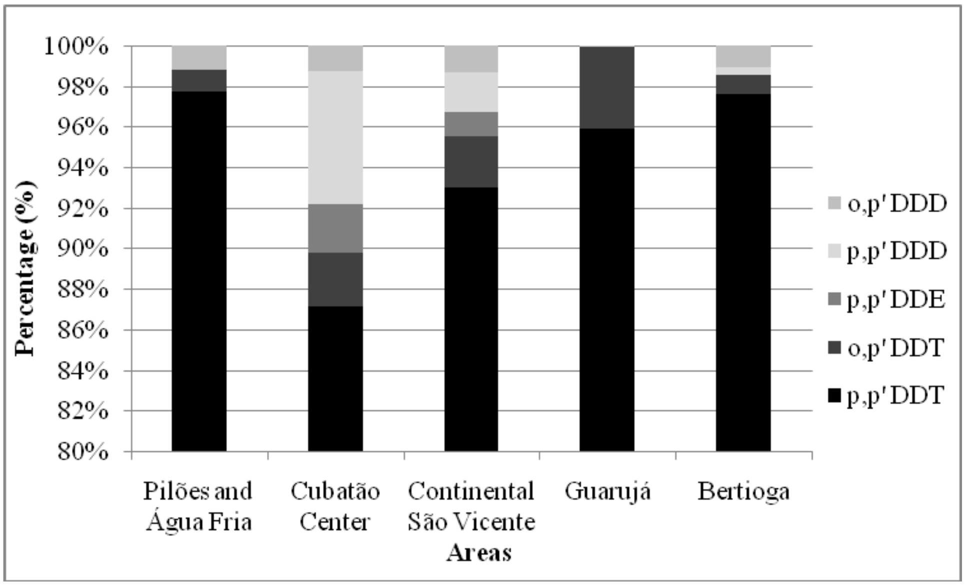

Figure 1. Contribution (%) of each DDT metabolite to ΣDDT in hair samples (n=122) per area.

Table 2. Concentration of DDT metabolites (ng.g^{-1}) in participants' hair from the five studied areas.

	Mean	SD*	SE**	1ºQ+	Median	3º Q+	Minimum	Maximum
p,p'-DDT								
Pilões and Água Fria	134.0	186.4	56.2	ND	53.7	193.9	ND	522.0
Cubatão Center	43.9	43.1	12.0	7.9	30.8	72.8	ND	128.3
Continental São Vicente	88.4	126.7	19.8	ND	48.7	108.1	ND	562.7
Guarujá	85.5	137.3	25.1	ND	49.2	96.9	ND	593.9
Bertioga	63.1	133.1	25.6	ND	ND	69.0	ND	615.6
o,p'-DDT								
Pilões and Água Fria	1.5	3.3	1.0	ND	ND	ND	ND	8.8
Cubatão Center	1.4	3.4	0.9	ND	ND	ND	ND	10.2
Continental São Vicente	2.4	4.2	0.6	ND	ND	6.9	ND	13.8
Guarujá	3.6	15.3	2.8	ND	ND	ND	ND	82.3
Bertioga	0.6	2.1	0.4	ND	ND	ND	ND	8.7
p,p'-DDE								
Pilões and Água Fria	ND	ND	0.0	ND	ND	ND	ND	ND
Cubatão Center	1.2	3.0	0.8	ND	ND	ND	ND	9.2
Continental São Vicente	1.1	4.0	0.6	ND	ND	ND	ND	22.0
Guarujá	ND	ND	0.0	ND	ND	ND	ND	ND
Bertioga	ND	ND	0.0	ND	ND	ND	ND	ND
o,p'-DDD								
Pilões and Água Fria	1.7	3.8	1.1	ND	ND	ND	ND	10.7
Cubatão Center	0.6	2.3	0.6	ND	ND	ND	ND	8.4
Continental São Vicente	1.2	4.1	0.6	ND	ND	ND	ND	19.3
Guarujá	ND	ND	0.0	ND	ND	ND	ND	ND
Bertioga	0.7	2.2	0.4	ND	ND	ND	ND	9.0
p,p'-DDD								
Pilões and Água Fria	ND	ND	0.0	ND	ND	ND	ND	ND
Cubatão Center	3.3	8.1	2.2	ND	ND	ND	ND	24.1
Continental São Vicente	1.9	7.6	1.2	ND	ND	ND	ND	41.8
Guarujá	ND	0.1	0.2	ND	ND	ND	ND	0.6
Bertioga	0.3	1.3	0.3	ND	ND	ND	ND	6.8
ΣDDT								
Pilões and Água Fria	141.8	199.0	60.0	ND	53.7	211.9	ND	573.6
Cubatão Center	50.3	41.1	50.3	17.1	44.9	79.3	ND	128.3
Continental São Vicente	96.9	130.1	20.3	7.0	49.4	125.8	ND	562.7
Guarujá	89.1	149.0	27.2	ND	49.2	98.7	ND	676.2
Bertioga	64.6	132.7	25.5	ND	9.0	69.0	ND	615.6

*SD= Standard deviation; **SE= Standard Error; Q+= Quartiles; ND=Non Detected.

The participants' age distribution was as follows: 47.0% were up to 20 years, 26.5% were between 21 and 40 years, 21.4% were between 41 years and 60 years and 5.1% were more than 60 years. Table 3 shows the distribution of ΣDDT, p,p'-DDT and p,p'-DDE concentrations by age group.

The age group with the highest ΣDDT and p,p'-DDT concentrations was the group between 21 to 40 years, followed by the group up to 20 years. p,p'-DDT showed higher concentrations than p,p'-DDE in all age groups. There were no statistically significant differences between age groups (ΣDDT - ANOVA: p=0.633; p,p'-DDT - ANOVA: p=0.597; p,p'-DDE - ANOVA: p=0.914).

Table 3. Distribution of ΣDDT, p,p'-DDT and p,p'-DDE concentrations ($ng.g^{-1}$) by age group.

Age (years)	ΣDDT (average±SD*)	p,p'-DDT (average±SD*)	p,p'-DDE (average±SD*)
≤20 (n=54)	97.27 (± 160.54)	91.15 (± 153.27)	0.61 (± 3.32)
21 a 40 (n=31)	102.75 (± 146.08)	98.22 (± 143.63)	0.37 (± 1.44)
41 a 60 (n=24)	57.15 (± 83.85)	51. 85 (± 72.95)	0.00 (± 0.02)
≥60 (n=8)	70.17 (± 73.98)	66.02 (± 72.23)	0.96 (± 2.72)

*Standard Deviation

Table 4. ΣDDT, p,p'-DDT and p,p'-DDE concentrations ($ng.g^{-1}$) distribution by the time of residence in the region.

Residence time (years)	ΣDDT (average±SD*)	p,p'-DDT (average±SD*)	p,p'-DDE (average±SD*)
1-3 (n=11)	64.71 (±137.58)	64.71(±137.58)	0.00 (±0.00)
3-5 (n=14)	87.34 (±117.53)	84.29 (±119.14)	0.78 (±2.92)
5-10 (n=27)	100.69 (±161.05)	93.61 (±169.57)	0.20 (±1.03)
10-15 (n=25)	110.14 (±178.42)	102.28 (±153.66)	1.19 (±4.61)
15-20 (n=13)	85.25 (±132.40)	82.77 (±129.25)	0.00 (±0.00)
Over 20 (n=28)	70.62 (±82.68)	64.80 (±73.86)	0.22 (±1.17)

*Standard Deviation

Most of the participants had lived in the region for more than 20 years (23.5%); 22.7% of participants had lived in region for 5-10 years, 21.8% for 10-15 years, 11.8% for 3-5 years, 10.9% for 15-20 years and 9.2% for 1 to 3 years. Table 4 shows the distribution of ΣDDT, p,p'-DDT and p,p'-DDE concentrations by the time of residence in region.

Residents from the 10-15 years group exhibited the highest DDT concentrations in hair. Residents from the 1-3 years group showed the lowest DDT concentrations in hair. There were no statistically significant differences between the residence time groups (ΣDDT - ANOVA: p=0.902; p,p'-DDT - ANOVA: p=0.924; p,p'-DDE - ANOVA: p=0.581).

Among the participants whose hair samples exhibited DDT (exposed group), a total of 57 participants (67.9%) reported having/had some of the screened diseases. Approximately half of them (50.9%; n=29) reported having/had one disease, 22.8% revealed having/had two diseases (n=13), 10.5% (n=6) revealed having/had 3 or 4 diseases, and 5.3% (n=3) mentioned having/had five diseases.

The most mentioned disease in the DDT-exposed group (participants whose hair samples contained DDT) in the five areas was respiratory disease (36.5%; n=19), and Continental São Vicente was the area with the highest prevalence (56.3%; n=9). Blood disease (26.4%; n=19) was the second most prevalent in the five areas, and Cubatão Center was the area that revealed the highest prevalence (55.6%; n=5). Hypertension was reported by 27.8% of the participants (n=15), and Cubatão Center was the area that revealed the highest prevalence (42.9%; n=3). Depression was reported by 17.6% (n=12) of the participants, and Guarujá was the area with the highest prevalence (25.0%; n=4). Approximately 16% of the participants (n=10) reported learning problems, and Guarujá was the area with the highest prevalence (12.5%; n=3). There were no statistically significant differences between the exposed group and the not exposed group to DDT with regard to disease prevalence.

Six cases of cancer were reported: 2 cases in Continental São Vicente, 2 cases in Guarujá and 2 cases in Bertioga. In Guarujá, one case of reproductive system cancer and one case of gastro-intestinal cancer were reported. There was one case of childhood tumor in Bertioga. There were 2 cases of breast cancer: one in Continental São Vicente and one in Bertioga. There were 2 cases of leukopenia in the five areas: one in Continental São Vicente and the other in Guarujá. Seven cases of skin disease were reported: 5 in Continental São Vicente and 2 in Guarujá. There was only 1 case of mental disability, 2 cases of liver disease and 1 case of neurological problems in Continental São Vicente. Only 1 case of congenital malformation was reported (Bertioga), and 4 cases of miscarriage were reported: 1 in Cubatão Center, 2 cases in Continental São Vicente and 1 case in Guarujá.

At least one of the locally produced food items was consumed in 57 of the investigated households (Pilões and Água Fria = 5; Cubatão Center = 10; Continental São Vicente 16; Guarujá = 10 and Bertioga = 16). There was no report of the consumption of locally produced milk or fruit. Finally, we found no evidence that locally produced food was a risk factor for the occurrence of DDT in hair samples.

Occupational exposure in the region has already been evaluated, but there is little information available regarding the environmental exposure of vulnerable populations to POPs pollutants. In this study, DDT was detected in all studied areas. The metabolite with the highest concentration and the one that most contributed to the ΣDDT concentration in participants' hair was p,p'-DDT. There was no significant difference between DDT concentrations in hair and age groups and DDT concentrations and the time of residence. Although almost seventy percent of the subjects positive for DDT mentioned having/had some disease, there was no statistical association between disease prevalence and residing in the areas under study. Respiratory diseases, blood diseases, hypertension, depression and learning problems were the diseases most cited by the participants of this study. None of the locally produced groceries was shown to be a risk factor for the presence of DDT in hair.

DDT can persist in soil for a long time period and contaminate food and the water supply or humans. It may also evaporate or get carried by dust, affecting people's health through inhalation and direct contact. Furthermore, this organic compound can be metabolized to DDE in organisms and in the environment [17, 11, 25, 14].

DDT use has been mentioned since the 1970s in Santos and São Vicente Estuary. It has been detected in sediment and fishes; contamination from within the estuary reaches the bay system. Although concentrations have been decreasing over the years, the areas mentioned in the present work are still classified as POP-contaminated by the São Paulo State Environmental Agency [5, 6, 12].

As Santos and São Vicente Estuary has been contaminated for over 40 years, it was expected that DDE concentrations were higher. For this reason, the age and time of residence are important to evaluate participants' exposure. The studied population was young (up to 20 years), but the highest concentrations were in 21- to 40-year-old participants. The oldest age range was the group that revealed the lowest DDT concentrations in hair, and DDE concentrations were extremely low in all age groups. Regarding the time of residence, 10-15 year local residents exhibited the highest concentrations. It was expected that the group of residents for over 20 years would reveal the higher DDT concentrations in hair. Furthermore, the group of residents for 1-3 years revealed DDT concentrations in hair as well. Considering that all the participants in this study had no past or current occupational exposure to chemicals, these facts suggest the recent exposure to DDT in Santos and São Vicente Estuary region.

When these study DDT concentrations were compared with DDT concentrations revealed in other countries, DDT concentrations in hair samples in this study were lower than DDT concentrations in hair samples from European countries from the 1960s to 2000s. However, they were higher than the DDT concentrations revealed in hair samples in other countries from 2000 to 2008, including in farm workers in Cretan, as shown in Table 5.

The concentrations in all mentioned studies showed a reduction in the availability of DDT to human exposure and revealed past exposure due to p,p'-DDE concentrations. Even in farm workers in Cretan, a group that should be more exposed (occupational exposure) to these substances, revealed hair DDT levels lower than the concentrations detected in selected estuary populations that did not have past or present occupational exposure to POP pollutants.

Table 5. Comparison between DDT concentrations in hair samples in this study and DDT concentrations in hair samples from other countries.

Country	Year	ΣDDT (ng.g^{-1})	p,p'-DDT (ng.g^{-1})	p,p'-DDE (ng.g^{-1})	Reference
Poland	1968	1658.0	374.2	673.9	Wielgomas et al., 2012 [24]
Poland	1989	143.9	39.5	10.7	Wielgomas et al., 2012 [24]
Greece	1996	101.0	22.0	37.6	Covaci et al., 2002 [20]
Romania (adolescents)	2002/2003	394.0	192.0	127.0	Covaci et al., 2008 [21]
Estuary region	2009	141.8-64.6	134.0-63.1	N.D.-1.2	this study
Romania	2000	52.2	7.9	37.5	Covaci et al., 2002 [20]
Poland	2009	36.5	11.2	17.3	Wielgomas et al., 2012 [24]
Belgium	2000	18.8	5.9	10.5	Covaci et al., 2002 [20]
Cretan (farming works)	2008	----	23.2	----	Tsatsakis et al., 2008 [26]

Studies on human contamination carried out in the Santos and São Vicente Estuary region showed DDT concentrations in blood and breast milk much lower than those found in hair, as shown in Table 6.

Table 6. Comparison of DDT concentrations in other studies carried on in Santos and São Vicente Estuary and concentrations revealed in this study.

Estuary city	Tissue	ΣDDT (ppb)	p,p'-DDT (ppb)	p,p'-DDE (ppb)	N	Reference
Continental São Vicente	Blood	0.13	-----	-----	9	Silva et al., 1997 [7]
Continental São Vicente	Breast milk	40.62	-----	-----	23	Silva et al., 1997 [7]
Pilões (Cubatão)	Blood	3.71	-----	3.65	222	Santos Filho et al., 2003 [10]
Pilões – children (Cubatão)	Blood	0.06	-----	0.85	242	Santos Filho et al., 1993 [9]
Estuary region	Hair	141.8-64.6	134.0-63.1	N.D.-1.2	122	this study

The last DDT application in São Vicente was in 1997, and these studies were conducted when the use of DDT in public health programs was still allowed. The authors considered this concentration of p,p'-DDE to be high and suggested that they were not due to a recent DDT exposure. These concentrations are much lower than the ΣDDT concentrations observed in the present study, which points to a different conclusion [7-10].

Our results suggested a recent exposure to DDT due to the high p,p'-DDT concentrations and presented similar DDT patterns to those found in the Amazon region. Saldanha et al. (2009) conducted a study in the Amazon region with a history of the widespread use of DDT against the malaria vector. ΣDDT and p,p'-DDT concentrations in the hair samples from the Amazon riverside population were much higher (ΣDDT=760.26; p,p'-DDT=1017.9 ng.g^{-1}; p,p'-DDE=526.11) than the concentrations revealed in the present study. The results from the Amazon region also showed a recent exposure to DDT [27].

There are three main pathways by which DDT in the environment can contaminate humans: ingestion, inhalation and skin contact. In this study population, ingestion and inhalation were the most likely exposure routes. Because environmental sources, such as soil, sediment and sea and river water from the estuary region, are contaminated, ingestion would be a route of exposure to DDT. However, this study showed that affected populations do not consume locally produced groceries, and none of the evaluated groceries was shown to be a risk factor for the presence of DDT in hair.

In Santos and São Vicente Estuary, no malaria cases have been found; the region has suffered from dengue epidemics, but there is no record of DDT use against the dengue vector since its last application in 1997. Another substance that could improve the availability of DDT is dicofol. In Brazil, dicofol is used in cotton, apple and citrus crops. However, there are no agriculture areas where dicofol could be used in the region, and dicofol is not produced in the industrial pole. The resuspension of soil contaminated particles could be another exposure route; however, DDT was present in Bertioga with no history of contamination. According to the Agency for Toxic Substances and Disease Registry (ATDRS), DDT in tropical soil takes less than one year to disappear [8, 17, 28].

Even though there was no significant difference in disease prevalence between the exposed group and the not exposed group in this study, health problems related to DDT exposure were reported by participants. The prevalence of respiratory diseases in the exposed group in the five study areas (36.5%) was higher than the prevalence of admissions in the public hospital system in each city in 2009 (Cubatão: 8.7%; São Vicente: 10.4%; Guarujá: 6.4% and Bertioga: 13.4%) and in southeast Brazil, where Santos and São Vicente Estuary are located (12.1%) [29].

Blood diseases presented the same pattern as respiratory diseases. Its prevalence in the exposed group in this study was much higher in this study than the prevalence of admissions to the public hospital system in each city in 2009 (Cubatão: 0.6%; São Vicente: 0.7%; Guarujá: 0.6% and Bertioga: 0.8%). This prevalence (26.4%) was more than eight times higher than the prevalence of blood disease (3.1%) revealed in another study conducted in 2010 in the same population [29, 30].

The prevalence of hypertension (27.8%) in this study was higher than that in Brazil (20%), São Paulo city (23%), and in the metropolitan region where Santos and São Vicente Estuary are located (Baixada Santista – 23.7%); it was higher than the prevalence revealed in another study conducted in the same population (24%) [31-34].

The prevalence of learning problems in this study (15.9%) was higher than the prevalence in Brazil's population (2-10%). Only the prevalence of depression in this study (17.6%) was lower than the prevalence (54.1%) revealed by another study conducted in Baixada Santista, where Santos and São Vicente Estuary are located [35, 36].

These results show that vulnerable populations were exposed to DDT and confirm that exposure routes to DDT are still present in the region over 40 years from the first alerts of estuary contamination. However, it was not possible to define these routes. Furthermore, the fact that DDT, mainly p,p' – DDT, is present in human tissue indicates that special attention is necessary for prevention in all five areas. Local governments must improve the public health system to be able to identify and treat future health problems related to environmental exposure to pollutants.

3. Material and Methods

3.1 Sampling

Five areas were selected in four different cities in Santos and São Vicente Estuary (São Paulo State - Brazil). The selection was based on previous information about environmental contamination in Santos and São Vicente Estuary [5]. All the studied areas have similar social and economic profiles. Four estuarine areas located near hazardous waste zones were selected: Area 1: Pilões and Água Fria (Cubatão city) are located near a closed industrial landfill. Area 2: Cubatão Center (Cubatão city) is located next to the industrial park. Area 3: Continental São Vicente (São Vicente city) is located near or above contaminated sites with chemical substances. Area 4: Guarujá (Pae Cara neighborhood in Guarujá city) is located between the Santos harbor channel and an intense traffic road. A control area outside the estuary region and without a history of contamination was also selected, called Area 5: Bertioga (Bertioga city). Figure 2 shows the five selected areas.

To evaluate the health status of these vulnerable populations, in 2007 and 2008, a questionnaire was distributed to approximately 820 households in each one of the five areas to assess information about the socioeconomic status, occupational exposure, and diagnosed disease. A description of the questionnaire structure, data collection, criteria for acceptance of referred disease and questionnaire validation have been reported in Carvalho et al. [19]. In 2009, in the second step of this study, 103 families (Pilões and Água Fria n=20, 19.5%; Cubatão Center n=20, 29.5%; Continental São Vicente n=19, 18.4%; Guarujá n= 23, 22.3% and Bertioga n=21, 20.3%) without a history of chemical occupational exposure were selected in the five areas to perform clinical tests and provide hair samples to assess the exposure to environmental chemicals. A total of 122 hair samples of the studied populations were collected. The number of hair samples collected per area was as follows: 11 in Pilões and Água Fria; 13 in Cubatão Center; 41 in Continental São Vicente; 30 in Guarujá and 27 in Bertioga. There were four factors that result in lower samples in Pilões and Água Fria and Cubatão Center: some families have changed address, small families with one or two members only, refusal by some of families members to have their hair cut and some selected families were composed by, mainly, men members with very short hair which made impossible the sample collection. The hair samples were cut close to the root from the occipital region and stored in plastic bags. Samples from colored, short (2 days) or recently washed hair were not collected.

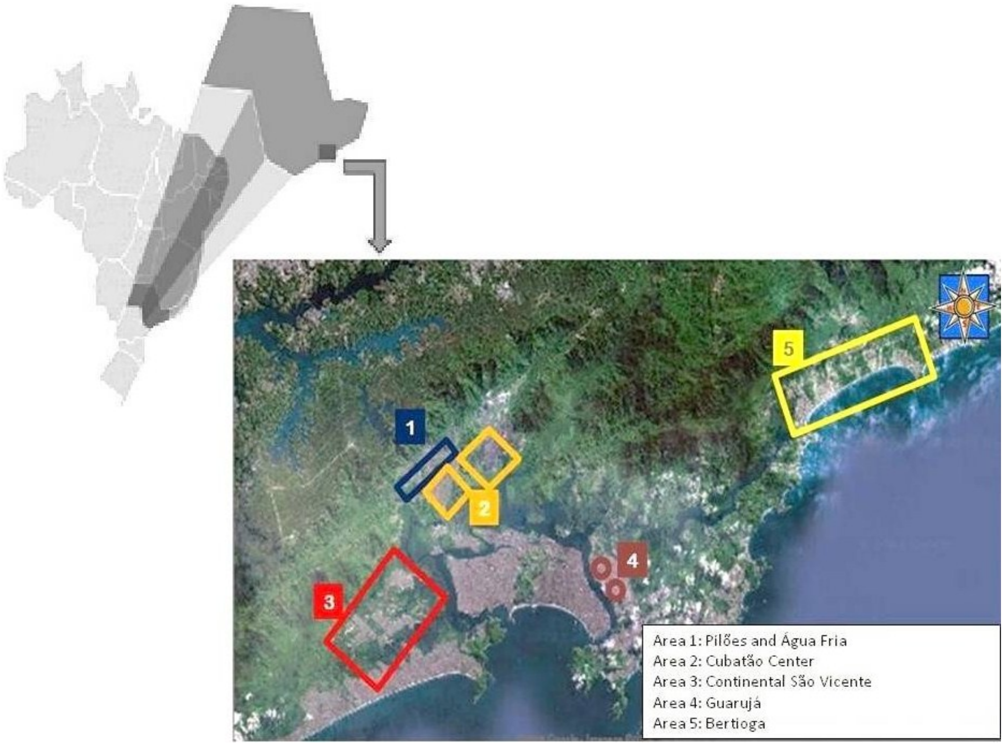

Figure 2. The five studied areas.

3.2 Chemical analysis

Details on the sample preparation have been well described in Wielgomas et al. [24]. Briefly, approximately 100 mg of human hair was cut into small fragments and spiked with PCB surrogate standards (PCB-103 and 198, 200 ng.g^{-1}). Then, 4 mL of HCl (4 M) and 4 mL of a hexane:acetone mixture (4:1vv) were added. The samples were incubated overnight at 40 °C. Then, the extracted samples were agitated and centrifuged; the organic phase was transferred to another tube (three times). The cleanup was performed with a column of 1 g of silica, 1 g of acidified silica (1%), and 1 g of anhydrous sodium sulfate. Samples were then eluted with 10 mL of hexane and 10 mL of acetone. Cleaned eluates were transferred to a vial and concentrated to a volume of 50 µL. Lastly, 2-4-5-6 tetrachlorometaxylene – TCMX (200 ppb) was added to all samples as an internal standard for volume correction prior to analysis. The instrumental analyses of the extracts were 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 spectrometer (Agilent 5975CMS) using negative chemical ionization (NCI). The conditions of NCI analysis and the selection of ions have been previously described [37]. Injection was splitless at 265 °C, and the oven program was as follows: 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 analyzed for 1,1-dichloro-2(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethylene (o,p'-DDE), 1,1-dichloro2,2-bis(*p*-chlorophenyl)ethylene (p,p'-DDE), 1,1-dichloro-2-(*o*-chlorophenyl)2-(*p*-chlorophenyl)-ethane (o,p'-DDD), 1,1-dichloro-2,2bis(*p*-chlorophenyl)-ethane (p,p'-DDD), 1,1,1-trichloro-2(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (o,p'-DDT), and 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane (p,p'-DDT).

3.3 Quality assurance/quality control

To analyze the efficacy of the methodology adopted, validation tests were performed. A batch was performed with two blanks, two blanks spiked with DDT standards, two hair samples and two hair samples spiked with DDT standards. In the spiked samples, we added a solution containing the DDT metabolites and the internal standard (PCB 103 and PCB 198). This solution was prepared from dilutions of commercial standards. The mean recovery value of DDT and its metabolites (ΣDDT) at the tests for method validation was 74.4%. Calibration solutions were composed of five different concentrations that ranged from 5 to 100 ng.g^{-1} prepared from commercial standards. The calibration curve was constantly checked and to be accepted, the coefficient of Pearson's correlation test was required to be higher than 99.5% (r = 0.995).

Blanks (total=14) were performed during each analytical batch to evaluate interferences. The limit of detection (LOD) of each DDT metabolite was defined as three times the standard deviations of the average of blanks. The limit of detection (LOD) for each DDT metabolite was as follows: p,p'- DDT, 11.5 ng.g^{-1}; o,p' - DDT, 6.8 ng.g^{-1}; p,p' - DDE, 4.8 ng.g^{-1}; o,p' – DDE, 3.9 ng.g^{-1}; p,p'- DDD, 8.3 ng.g^{-1}, and for o,p' - DDD, 5.9 ng.g^{-1}. Concentrations lower than LOD were assigned a value of zero. The internal standard recovery mean in the samples was 79% for PCB 103 and 92% for PCB 198. No recovery correction was used in our study.

3.4 Statistical test

The distribution of DDT in the five studied areas was statistically tested with a one-way ANOVA followed by Tukey's test to compare the five areas. The association between the presence of DDT in hair and the area of residence and between the presence of DDT in hair and diseases were estimated using Pearson's chi-square test, incorporating Yates' correction for continuity or Fisher's exact test and the two proportions difference test. The difference in disease prevalence between the exposed group and the unexposed group were tested using the difference of two proportions test. Diseases included in the analysis were based on the International Classification of Disease, version 10 from the World Health Organization – (ICD-10/WHO): respiratory diseases (J00-J99), bronchitis (J20; J40; J41; J42; J68), asthma (J45), liver diseases (K70–K77), hepatitis (B15–B19; K72–K73, K75), cancer (C00–D48), reproductive system cancer (C51–C58; C60-63), gastrointestinal cancer (C15–C26), childhood tumors, breast cancer (C50), blood disease (D50–D89), leukopenia (D72), skin disease (L00–L99), arterial hypertension (I10), mental disability (F00–F99), depression (F32–F33), neurological problems (G00–G99), learning problems (F80–F89), congenital malformations (Q00–Q99) and miscarriage (O06). Logistic regression models were used to evaluate the possible risk factors for the presence of DDT, and the considered variables were: street pavement, income, tap and natural local water consumption, local and non-local milk consumption and the consumption of local fruits, vegetables, fish, meat, pork, egg, chicken, mollusks, and crustaceans. We adopted a statistical significance level of 5%. All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) 17.0 for Windows.

4. Conclusions

Forty years have passed since the first

historical records of contamination in Santos and São Vicente Estuary, and 17 years after the prohibition of DDT in Brazil, the contamination in this region has not changed. The results presented in this study suggest an increase in the availability of DDT. The consumption of local groceries was not common in the region, and there is no record of the production or use of substances containing DDT for the last twenty years. Diseases related to pollutant exposure are present in the region. This scenario reaffirms the existence of current exposure routes between Santos and São Vicente estuarine populations and this organic compound, but these exposure routes are not well defined. Therefore, this issue deserves further investigation to improve public health programs in contaminated areas and to reduce future health problems in these vulnerable populations.

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