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Rapid method for the simultaneous determination of DDTs and PCBs in hair of children by headspace solid phase microextraction and gas chromatography-mass spectrometry (HSSPME/GC-MS)

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The purpose of this study was to develop a rapid and cost efficient hair extraction method, using the headspace solid phase microextraction (HSSPME) technique for the simultaneous determination and biomonitoring of 1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane) (DDT) and its isomers/metabolites and polychlorinated biphenyls (PCBs) in hair samples. A total of 72 head hair samples were collected from children living in urban and rural regions of the island of Crete. Two hundred milligrams of hair were digested under alkaline conditions and thermostated for 30 min at 90°C while a 65 μm PDMS/DVB fibre was exposed into the headspace of the vial. Analytical parameters of the method (time of incubation, agitation speed, recovery, precision, accuracy, carry over, matrix effect, linearity, and selectivity) were examined. Recoveries of the DDTs in the spiked hair samples were calculated from 42.3% for opDDD to 87.1% for opDDE, while recoveries for PCB congeners were from 52.6% for PCB138 to 96.6 % for PCB28. The method was applied for the analysis of authentic hair samples. Significant differences (p=0.001) of the burden to total DDTs (sumDDTs) as well as of the frequencies of detection of positive samples (p=0.020) were observed between the examined regions. Moreover, significant differences in the detected concentrations of PCB congeners were observed for PCB52 ($p<0.001$) and PCB28 (p=0.017) as well for their prevalence between urban and rural regions. Application of HSSPME for the biomonitoring of DDTs and PCBs biomarkers in hair was tested and successfully applied to the analysis of spiked and authentic hair samples. HSSPME was found to be substantially simpler and faster procedure than previous reported sample treatment procedures. Copyright © 2014 John Wiley & Sons, Ltd.

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Introduction

Human hair has mostly been used in forensic and clinical sciences.[1–3] The idea of using human hair as an indicator in assessing exposure to persistent organic pollutants (POPs) has been advancing for the last two decades.[4–9] One of the reasons for the late development of environmental biomonitoring using human hair as a matrix is the low detection limits which need to be achieved.

The first attempt to assess exposure to POPs in hair was for organochlorine substances; this was due to the great sensitivity and low detection limits achieved for these substances using an Electron Capture Detector (ECD) detector. Later, gas chromatography-mass spectrometry (GC-MS) was employed for the detection and identification of POPs using, in most of cases, a low resolution single quadrupole MS detector.

The rapid development of technology and the use of highly advanced and expensive instruments such as tandem mass spectrometers (MS/MS), high resolution mass spectrometers (HR-MS) or double-focusing HR-MS (DFHR-MS) opened new horizons for the determination of POPs in hair matrix. The detection limits achieved using DFHR-MS were 10 times lower than those achieved using low resolution mass spectroscopy (LR-MS). This means a bigger percentage of positive samples and lower mean values for organochlorine substances.[6,10,11]

Due to the high cost of high-resolution instruments, researchers turned to the optimization of sample preparation techniques with a view to achieving lower detection limits at the same time as decreasing sample preparation time. One of the advances in the field of sample preparation was the use of solid phase microextraction (SPME) for extracting POPs from hair samples.[9] SPME represents a substantial advance in terms of handling matrices, which contain low levels of target compounds and high concentrations of interfering substances.[12] Arthur and Pawliszyn used SPME in 1990 as a solvent-free sampling technique that

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reduces the steps of extraction, clean-up, and concentration to a unique step.[13,14]

The SPME method utilizes a fused silica/fiber coated with a liquid or a solid polymer or a combination of both. The two most common SPME modes of absorption of the analytes on fibre take place either by direct immersion or by the head space approach (HSSPME). Application of the head space sampling allowed the SPME technique to be extended to more complex samples that contain solid or high molecular weight materials. The transfer of the analytes from the sample to the headspace is the time-limiting step and thus the extraction can be optimized by heating or stirring. Increasing the sampling temperature was found to have a significant effect on the extraction kinetics of the less volatile compounds.[15,16]

PCBs and DDTs are some of the most known POPs. They are resistant to biological and chemical degradation and have long environmental half-lives.[17] PCBs are highly lipophilic, thermally stable, and low flammable compounds[18] while their degree of lipophilicity increases with increasing chlorination. Since over 100 PCBs have been identified and the analysis of all of them is almost impossible and not cost effective; the most abundant PCB congeners (PCB28, 52, 101, 118, 138, 153 and 180) usually are monitored to assess environmental exposure.[19] On the other hand, DDT is a very effective organochlorine insecticide. It is slowly metabolized to DDE and DDD metabolites (which have similar physical and chemical properties to DDT), both of which are stored in the fatty tissues.[8,20]

Both DDTs and PCBs accumulate in the food chain, in human and animal tissues.[21] As a result, they have potential significant impact on human health and the environment. Since PCBs and DDTs are no longer metabolized or excreted in hair, their quantification could be a biomarker for possible long-time exposure to these pollutants. As has been reported, a ratio of ppDDE/ppDDT lower than 5 indicates recent exposure to parent DDT.[22]

The biomonitoring of DDTs and PCBs for the estimation of human exposure requires complex sample preparation and analytical procedures using sensitive state-of-the-art instrumentation to achieve the desired selectivity and quantification limits. To our knowledge, the first attempt to use SPME in hair was by Salquebre *et al.*[9] by testing protocols involving both direct and headspace approaches to analyze pesticides. In that work, a previous overnight sample preparation was necessary for both protocols.

The purpose of this study was to develop a one-step hair-extraction method, using the HSSPME technique, for the simultaneous determination and biomonitoring of DDTs and PCBs in hair samples.

Materials and method

Sample collection and storage

Hair sampling was scheduled and performed by the staff of the Laboratory of Toxicology and Forensic Sciences, Department of Medicine, University of Crete. The sampling target group was children from urban and rural regions of the island of Crete, in southern Greece. A total of 72 hair samples were collected during 2009–2012 from pre-school and elementary school aged children (4.5 to 12 years old). Fifteen (15) samples were collected from the urban region of Heraklion and 28 and 29 samples were collected from two rural regions (rural A and rural B) close to the city of Heraklion. The sample included 43 females (59.7% of total sample).

Questionnaires were completed that included information about personal details of the individual (gender, weight, and age) and information concerning their personal habits, profession of parents, and their possible association with exposure to pesticides. Parents of the participants were informed about the aim of the study and provided their consent. All hair samples were placed in a paper envelope and kept in the dark at room temperature.

Reagent and materials

The organochlorines opDDD, ppDDD, opDDE, ppDDE, opDDT, and ppDDT were obtained from Chem Service (West Chester, PA, USA). Sodium hydroxide was obtained from Merck, D-6100 (Darmstadt, Germany). The 1,2,3,4-tetrachloronaphthalene (TCN) was purchased from Dr Ehrenstorfer (Augsburg, Germany). PCB congeners (28, 52, 101, and 138) and sodium chloride were purchased from Fluka Analytical-Sigma-Aldrich (St Louis, MO, USA). Ultrapure water was obtained by a Direct-Q 3UV water purification system (Merck, Germany).

Stock solutions of each organochlorine and PCB congener individually were prepared in hexane at a concentration level of 100 ppm. Mixed working solutions of PCB congeners and DDTs were prepared at concentrations 10, 1.0, 0.1, and 0.01 ppm and used for the preparation of the spike hair. All solutions were stored at -20°C.

Sample pretreatment - HSSPME procedure

Approximately 200 mg of hair was washed twice with 5 mL water and once with 5 mL of hexane and dried in an oven set to 40°C and then placed in 8 mL SPME vials. Two (2) mL of NaOH 10 M, 1 mL of ultrapure water, 0.6 gr of NaCl, and 10 ng of TCN [used as an internal standard (IS)] were added. SPME vials were sealed with PTFE/silicon septum caps and placed in the GS-MS tray. Online extraction followed with a PDMS/DVB type extraction fibre at 90°C for 30 min with an agitation speed at 250 rpm.

Whilst the digestion the hair sample was performed, the fibre was dipped in the headspace phase of the sample in order to selectively absorb the chosen substances resulting from the change of the liquid phase to the gas phase. After the absorption process finished, the fibre tip was inserted in the injection port of the GC-MS, where it remained for 3 min until the complete release of the substances.

Instrumentation

Samples were analyzed with a Shimadzu (Kyoto, Japan) QP-2010 GC-MS instrument. The system was equipped with a split/splitless injection inlet, electronic pressure control AFC and an AOC-5000 robotic autosampler with the appropriate functions to operate in SPME mode. GC-MS solution software was used for instrument control and data analysis. GC analysis was conducted on a Supelco Analytical SLB^{tm}-5 ms (Bellefonte, PA, USA) capillary column of 30 m length, 0.25 mm i.d, 0.25 μm film thickness. The AOC-5000 SPME autosampler was equipped with syringe with a 65 um PDMS/DVB Metal Alloy fibre from Supelco (Bellefonte, PA, USA).

The following conditions were used: Helium with a constant flow rate of 1 mL/min as a carrier gas, inlet temperature 270°C, splitless injection for 1 min, MS interface temperature 310°C, ion source temperature 230°C. The column temperature was initially held at 60°C for 3 min, raised at 15°C/min to 180°C and held for

1 min. Then there was a gradual increase at a rate of 4°C/min until 250°C and finally at a rate of 30°C it reached 300°C where it remained for 1 min.

The MS detector was operated at the selected ion-monitoring mode (SIM). The qualification *m/z* ions (Q1 and Q2), the target m/z ion (quantitative ion) and the retention times of each PCB congener and DDTs are listed in Table 1. A typical chromatogram of spiked sample, standards solutions, and authentic sample was presented in Figure 1.

Spiked human samples

Human hair samples with no detected levels of DDTs or PCBs or levels below the LOQ values were used as blank hair. Hair samples were washed as described above and pooled. The pooling samples that provide no detectable levels of the analytes was spiked at different concentrations 0, 2.5, 5, 10, 25, and 50 pg/mg for DDTs and 0, 0.5, 1.0, 2.5, 5.0, and 10.0 pg/mg for PCBs and then analyzed for the preparation of the spiked calibration curves, as well as for the validation of the method.

Table 1. Analytical parameters of analytes

Analytes	Rt (min)	target m/z	Q1 m/z	Q2 m/z	Standard curves r^2	Mean % error	Spiked curves r^2	Mean % error	Mean recovery %	LOD (pg/mg)	LOQ (pg/mg)	Accuracy %	Inter-days precision (% RSD)
PCB28	19.26	256	186	150	0.9952	-0.4	0.9905	8.1	96.6	0.06	0.21	118.7	17.5
PCB52	20.54	292	220	255	0.9955	-0.6	0.9872	11.3	82.5	0.09	0.29	99.9	21.8
PCB101	24.05	326	256	290	0.9950	7.9	0.9932	-0.5	59.9	0.04	0.12	98.8	18.2
PCB138	28.82	360	218	290	0.9840	9.9	0.9987	2.8	52.6	0.11	0.36	100.1	12.5
opDDE	23.84	246	318	176	0.9957	-3.7	0.9992	3.5	87.1	0.27	0.89	96.2	17.0
ppDDE	25.25	246	318	176	0.9913	1.7	0.9903	12.2	68.6	0.29	0.96	92.3	8.5
opDDD	25.58	235	165	199	0.9948	-0.8	0.9944	-17.4	42.3	0.51	1.71	104.1	14.6
ppDDD+opDDT	27.19	235	165	199	0.9947	4.0	0.9834	10.1	66.9	1.50	5.00	102.3	10.5
ppDDT	28.63	235	165	199	0.9999	-0.9	0.9994	20.8	61.3	2.14	7.14	87.3	21.9
IS	21.1	266	194	-	-		-		-		-		

Q1 and Q2: *m/z quanfication ions.*
Standard curves: *curves of standards mix solutions of PCBs and DDTs.*
Spiked curves: *curves of blank hair sample spiked with known amount of each PCB and DDT.*

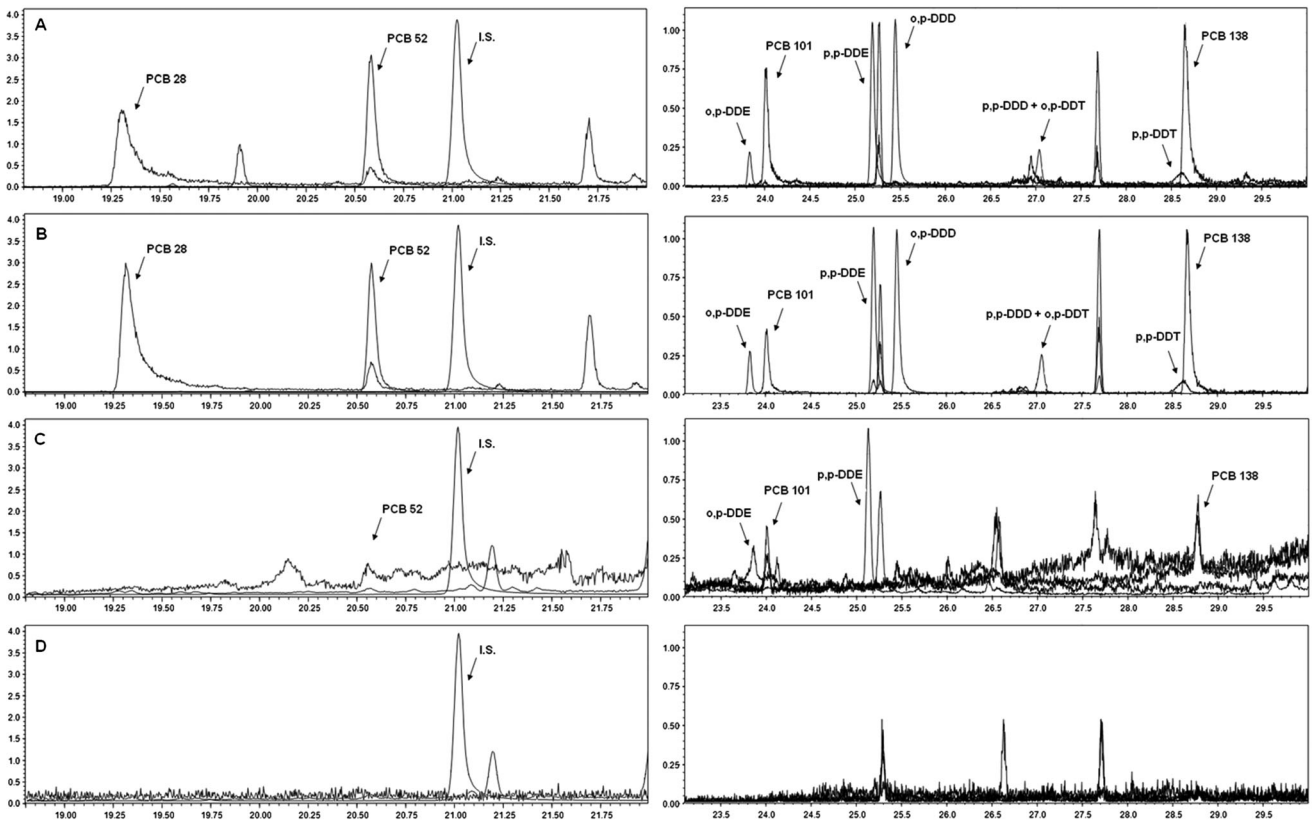

Figure 1. Typical chromatograms of (A) a mix standard solution of PCBs and DDTs, (B) spiked sample at concentration 1 and 10 pg/mg for PCBs and DDTs, respectively, (C) authentic hair sample positive for PCB 52, 101, and 138 and for op and ppDDE and (D) blank hair sample spiked only with IS.

Statistic analysis

Levels of DDTs and PCBs are expressed as mean (SD) and quartiles (1st, median, 3rd), while prevalence of positive samples (>LOD) are expressed as counts and percentages. Differences in prevalence of positive samples are examined using Pearson's chi-square test. Differences in levels between areas are examined using non-parametric tests Mann-Whitney and Kruskal-Wallis. Bar charts and error plots are used for graphical representation of data. IBM SPSS Statistics 20.0 was used for data analysis and a level of 0.050 was set as significant. For the calculation of the mean and median concentrations all negative samples (<LOD) were set as LOD/2.

Results

Method evaluation

The HSSPME/GC-MS method was validated by evaluating the parameters of agitation speed, incubation time, recovery, linearity, carry over, specificity, matrix effect, accuracy, and precision.

Linearity and limits of quantification and detection

Both standard and spiked curves were prepared using the ratio of the area of each analyte to area of IS. The instrument response obtained from the analysis of working solutions were linear with $r^2>0.98$ for all analytes at the concentration range from 0 to 1 ng/mL for both DDTs and PCBs (Table 1). Spiked curves were constructed by analyzing spiked hair samples at concentrations ranging from 0 to 50 pg/mg for DDTs and from 0 to 10 pg/mg for PCB congeners. All spiked curves had an $r^2>0.99$, except for the curve of PCB52 ($r^2=0.9872$) and ppDDD+opDDT ($r^2=0.9834$) (Table 1). Statistical test for the linearity of the curves was applied (using Excel) and presented as % error of the curve. For standard curves the mean % error was -3.7, 1.7, -0.8, 4.0, and -0.9 % for opDDE, pDDE, opDDD, ppDDD+opDDT, and ppDDT, respectively, and for PCB28, PCB52, PCB101 and PCB138 was -0.4, -0.6 7.9 and 9.9%, respectively, for the concentration range 0.1 to 1 ng/mL. For spiked curves the respectively values (% error) were 3.5, 12.2, -17.4, 10.1, and 20.8 for opDDE, ppDDE, opDDD, ppDDD+opDDT and ppDDT, respectively, and 8.1, 11.3, -0.50, 2.8 for PCBs for spiked levels 2.5 to 50 pg/mg and 0.5 to 10 pg/mg, respectively (Table 1).

The limits of detection (LOD) and quantification (LOQ) were calculated by analyzing the lowest spiked level for both PCBs and DDTs and defined as the peaks that gave a signal-to-noise ratio >3 for LOD and >10 for LOQ. The LOD values ranged from 0.04 to 0.11 pg/mg for PCBs congeners and from 0.27 to 2.14 pg/mg for DDTs (Table 1). The lowest LOQ value for PCB congeners was observed for PCB101 (0.12 pg/mg) followed by PCB28, PCB52, and finally PCB138 (0.21, 0.29 and 0.36 pg/mg, respectively). For DDTs, the LOQ values were 0.89, 0.96, 1.71, 5.00 and 7.14 pg/mg for opDDE, ppDDE, opDDD, ppDDD+opDDT, and ppDDT, respectively.

Precision, recoveries and accuracy

The inter-day precision (analysis at three consecutive working days) was evaluated at levels 2.5, 5.0, 10.0, 25.0, and 50.0 pg/mg for DDTs and at 0.5, 1.0, 2.5, 5.0, and 10.0 pg/mg for PCBs and estimated to be lower than 22% for all PCBs and DDTs. The precision values (expressed as % relative standard deviation, %RSD) for each analyte are presented in Table 1.

The recovery of the method was performed on spiked blank hair samples at concentration levels of 0.5, 1.0, 2.5, 5.0, and 10.0 pg/mg for PCB congeners and of 2.5, 5.0, 10.0, 25.0, and 50.0 pg/mg for DDTs. The mean recovery for PCB28 was 96.6% at the above spiked concentrations, for PCB 52 it was 82.5%, for PCB101 59.9% and for PCB138 52.6% (Table 1). The mean recoveries for DDTs varied from 87.1% for opDDE to 42.3% for opDDD (Table 1).

The accuracy of the method was calculated in three spiked levels 10, 25, and 50 pg/mg for DDTs and the mean accuracy (%) values were 96.2%, 92.3%, 104.1%, 102.3%, and 87.3% for opDDE, ppDDE, opDDD, ppDDD+opDDT, and ppDDT, respectively. The mean % accuracy for PCB28, PCB52, PCB101, and PCB138 were 118.7%, 99.9%, 98.8, and 100.1 % respectively, at concentration levels 0.2, 0.5, 1.0, and 2.0 pg/mg.

Matrix effect, carry over, and selectivity

The matrix effect was evaluated by comparing mix standard solution (n=3) of the analytes to spiked blank hair sample at concentration level 2 pg/mg for PCBs and 10 pg/mg for DDTs (n=3). In all solutions or spiked samples 10 ng of IS was added. The matrix effect was evaluated and expressed as % mean value and for PCB28, PCB52, PCB101 and PCB138 were 25.3%, 14.4%, 10.3% and 4.3%. For opDDE, ppDDE, op DDD, pp DDD+opDDT and ppDDT were 25.1, 14.5, 1.0, 7.0, and 59.5 %, respectively. The matrix effect % value for the IS was 8.7%.

Lack of carry over was demonstrated analysing blank sample after of three consecutive analysis of hair samples spiked with PCB congeners at level of 10 pg/mg and DDTs at level 50 pg/mg (n=3). To investigate the selectivity of the method, pooling blank hair containing no analytes was analyzed. No interference of other compound or endogenous compound with the PCB or DDT analytes was observed (chromatogram D, Figure 1).

Incubation time and agitation speed study

The effect of incubation time on the detected levels of the analytes was performed in blank hair spiked with 2 pg/mg of each PCB congener and with 10 pg/mg for each DDT. Then the spiked hair was incubated at 90°C for 30, 35, and 45 min and the area of each analyte was recorded. The results are shown in Figure 2A.

A slight decrease in the detected areas of the sumDDTs (sum areas of DDTs) was observed increasing the incubation time. The same tendency was also noticed for sumPCBs (sum areas of PCBs). Nevertheless, individually, for PCB28 and PCB52 an increase of their area (37% and 36% for PCB 28 and 21% and 32% for PCB 52) was noticed by increasing the incubation time to 35 and 45 min, respectively. The opposite was found for PCB101 and PCB138 (a decrease of the detected area at -41% and -50% for PCB101 and at -56% and -74% for PCB138) by increasing the incubation time to 35 and 45 min. Based on these results and in order to develop a time effective method, we chose a 30 min incubation time for authentic hair.

The effect of agitation was also studied at three speed levels (0, 250, and 350 rpm). The results are shown in Figure 2B. PCB congeners' sum areas seem not to be affected by agitation speed in contrast to those of DDTs for which a slight decrease in the sum areas was observed when the agitation speed was increased (from 0 to 350 rpm). Based on the previous observed finding, we chose an agitation speed of 250 rpm for better homogenization of the hair matrix in the alkaline solution during the incubation step.

Biomonitoring of PCBs in hair samples

The percentages of positive hair for PCBs are shown in Table 2 and Figure 3A. There is a significant difference in positive detected samples for PCB52 between urban and rural regions ($p<0.001$). Samples from rural A and rural B have a high prevalence of 82.1% and 82.8%, respectively, compared to the urban

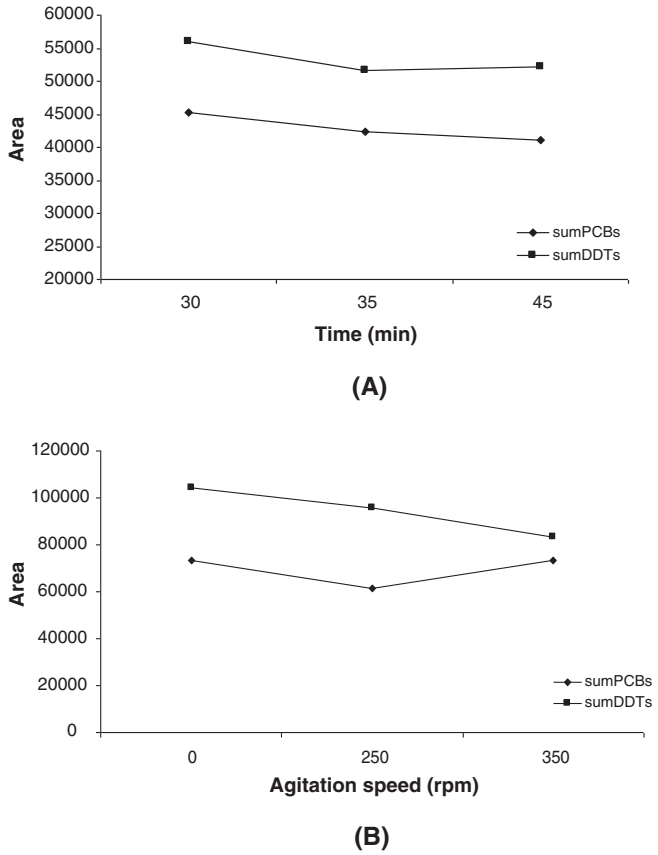

Figure 2. Influence of incubation time (min) (A) and agitation speed (rpm) (B) on the detected area of PCB congeners and DDTs using the HSSPME techniques.

region (13.3%) for PCB52. A higher prevalence was also observed for PCB28 for rural A (75.0%) and for rural B (75.9%) region in comparison with the urban region (46.7%) ($p=0.031$). No significant association was observed on the prevalence of PCB101 ($p=0.817$) and PCB138 ($p=0.647$) (Table 2) between the examined regions. According to the detected concentrations of each PCB congeners, statistical differences exist between the examined regions for PCB28 ($p=0.040$ for all regions and $p=0.017$ between rural and urban regions) and for PCB52 ($p< 0.001$) for all regions. No significant statistical differences were observed for PCB101 and PCb138 (Table 2). The mean±SD and the median values of PCBs concentrations are shown in Table 2. The median values of PCB28 range from 0.03 to 0.73 pg/mg, for PCB52 from 0.05 to 0.43 pg/mg, for PCB101 and PCB138 0.02 and 0.06 pg/mg for all regions, respectively.

Biomonitoring of DDTs in hair samples

In Table 3, the prevalence of DDT metabolites and the detected mean and media concentrations per region are shown. The majority of the samples were found positive for at least one organochlorine pollutant. The compounds which appeared more frequently were ppDDE and opDDE. ppDDE prevalence is 100% in the rural A region and 96.6% in the rural B region, significantly higher ($p=0.027$) than the urban region prevalence (80.0%) (Table 3). Statistical differences in the percentage of positive samples between rural and urban regions were also observed for opDDE ($p=0.045$), opDDD ($p=0.016$) and sumDDTs ($p=0.027$) (Table 3, Figure 3B). Moreover, significant differences of the burden to total DDTs (sumDDTs) ($p=0.001$) were observed in the examined regions. Statistical differences were also recorded for ppDDE ($p=0.040$) and for opDDD ($p=0.020$).

Discussion

In this study, we developed a fast, cost efficient, and precise method for the simultaneous detection and determination of PCBs and DDTs in hair samples using an HSSPME/GC-MS analytical technique. The presented method is simpler and faster than previously reported methods,[6,8] since there is no requirement of any time-consuming matrix pretreatment (e.g. overnight incubation, SPE clean-up step, or ultrasonic extraction). The only step that we adopted from the previously reported methods was the decontamination step for the removal of possible external contaminants.

Children (residents of three different regions of Crete, Greece) were selected as subjects in this study as aforementioned, and hair analysis was performed in order to assess the total burden to PCBs and DDTs. The selection of this population group was based on the estimation of low exposure (past and present) of the children to the targeted pollutants, since the island of Crete is an area with low industrial activity. The only possible source of contamination, mainly for DDTs, could be the extensive past agriculture activity or dietary habits.

The PCB28 and PCB52 congeners were more frequently detected compared to the congeners PCB101 and PCB138. Specifically, for PCB28 detection rates were 46.7% for the urban area, 75.0% for rural area A, and 75.9% for rural area B (in total, 69.4% of all investigated samples were positive for PCB28). For PCB52 detection rates were also relatively high, with detection rates ranging from 13.3% (for the urban area) to 82.8% (for rural area B), while 68.1% of all the investigated samples were positive for this congener. The percentiles of detection for PCB101 and PCB138 were much lower, ranging from 13.3% (PCB138, urban area) to 28.6% (PCB101, rural area A) (Table 2). Significant differences of the % positive samples were observed for PCB28 ($p=0.031$) and PCB52 ($p<0.001$) between urban and rural areas, while for all regions significant differences were observed only for PCB52 ($p<0.001$).

The fact that the PCB28 and PCB52 were detected at higher rates makes these isomers more suitable biomarkers for assessing the health impact on children from these specific environmental pollutants. Significant differences were only observed in the measured levels of PCB28 ($p=0.040$) and PCB52 ($p<0.001$) per region and per urban/rural classification ($p=0.017$ and $p<0.001$, respectively). Based on the literature,[5,23–26] variations have been noticed amongst population groups from various regions that have different industrial development as aforementioned pollutants are considered to originate mainly from industrial factories or areas.[5] Therefore, because the chosen areas of this study had no such industrial development, there are insufficient results, especially for PCB101 and PCB138, and the detection rates were relatively lower than the corresponding rates in the literature. Nonetheless, in a previous study[6] where subjects from similar population groups were examined, the positive % samples for PCB28, 52, 101, and 138 were lower (9.5%, 85.7%, 0%, and 9.5%, respectively) than the reported detection rates in the present study. Therefore, the lower detection limits of the proposed method provided higher detection rates for PCBs, even in non-exposed subjects.

Table 2. Mean, median values, and frequencies (%) of positive samples of PCB congeners in urban and rural regions of Crete

	Region	% positive	p-all regions*	p (urban-rural)*	Mean (pg/mg)	±SD	Median (pg/mg)	Min-Max (pg/mg)	p-all regions**	p (urban-rural)***
PCB28	**Urban**	46.7	0.098	0.031	3.7	13.1	0.03	LOQ -50.9	0.040	0.017
	Rural A	75.0			2.3	4.5	0.73	LOQ -22.5		
	Rural B	75.9			0.8	0.9	0.53	LOQ -3.7		
	Total	69.4			2.0	6.6	0.50	LOQ -50.9		
PCB52	**Urban**	13.3	<0.001	<0.001	0.1	0.1	0.05	LOQ -0.4	<0.001	<0.001
	Rural A	82.1			0.7	0.8	0.43	LOQ -3.3		
	Rural B	82.8			0.6	0.9	0.29	LOQ -2.8		
	Total	68.1			0.6	0.8	0.20	LOQ -3.3		
PCB101	**Urban**	20.0	0.817	0.528	5.4	13.7	0.02	LOQ -51.3	0.826	0.561
	Rural A	28.6			8.6	20.3	0.02	LOQ -93.8		
	Rural B	27.6			5.5	13.2	0.02	LOQ -62.8		
	Total	26.4			6.7	0.02	0.02	LOQ -93.8		
PCB138	**Urban**	13.3	0.647	0.352	1.3	3.6	0.06	LOQ -13.1	0.725	0.435
	Rural A	25.0			1.8	4.2	0.06	LOQ -17.2		
	Rural B	24.1			1.8	6.2	0.06	LOQ -33.0		
	Total	22.2			1.7	4.9	0.06	LOQ -33.0		

* *p-values for percentages were resulted from Pearson's chi-square tests p-values for imputed concentration were resulted from.*

** *Kruskal-Wallis.*

*** *Mann-Whitney tests.*

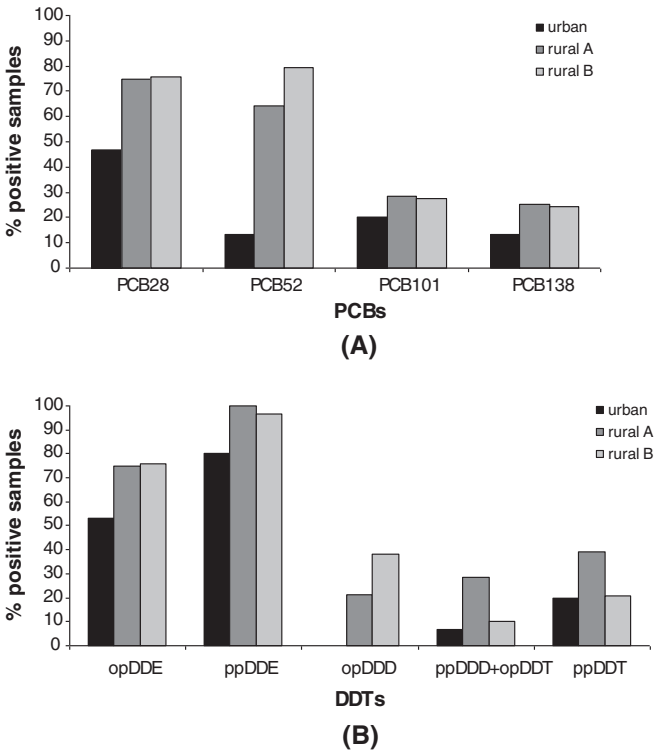

Figure 3. Prevalence of PCB congeners (A) and DDTs (B) in head hair samples collected from urban or rural population of Crete.

The median detected concentration of sumDDTs in rural region A participants' hair was higher (11.7 pg/mg) than urban and rural B regions region participants' samples (4.7 and 4.6 pg/mg, respectively). The majority of hair samples analyzed were positive for DDTs. Specifically 94.4% of hair samples were positive for at least one of the examined DDTs (Table 3). Samples from rural area A were 100% positive for sumDDTs and samples from rural area B were positive by 96.6%. Even for the urban area the detection rates were 80.0%. The organochlorine pollutants with the higher detection rates were ppDDE (94.4%) and opDDE (73.6%), which are considered to be metabolites of DDT. The opDDD, ppDDD+opDDT and ppDDT compounds were detected in lower detection rates for all investigated areas. Briefly, the detection rates for these compounds ranged from 0% (opDDD, urban area) to 39.3% (ppDDT, rural area A). Moreover, significant differences were observed in detection rates for opDDE (p=0.045), ppDDE (p=0.027), opDDD (0.016) and sumDDTs (0.027) per urban/rural classification, while per region significant differences were observed for ppDDE (p=0.020), opDDD (p=0.018) and sumDDTs (p=0.020) (Table 3). These differentiations of the detected positive rate from region to region are probably due to its past agricultural activities, which are mainly crops in greenhouses or open cultivations. Although, DDT has been banned for the last few decades in Greece, the observed differences on the rate of positive samples can be justified by its extensive use in the past. That, in combination with the residue activity from the use of DDTs and its long half-life once it enters living organisms, can justify such variations in the results. Detection rates for sumDDTs of the current study are similar to most of the reported rates in the existing literature[7,23,24] or higher compared to previously published data,[8] where similar population groups were also investigated for DDTs levels. This may also be attributed to the lower detection limits for DDTs that the proposed method provides.

To our knowledge, this is the very first attempt for the simultaneous detection of the aforementioned analytes using the technique of SMPE/GC-MS. The only study close to ours was that of Salquebre *et al.*,[9] where a simultaneous detection and quantification method for 22 pesticides was presented using SPME/GC-MS/MS. Amongst these 22 pesticides, DDTs were also included

Table 3. Mean, median values and frequencies (%) of positive samples of DDTs in urban and rural regions of Crete

		% positive	p-all regions*	p (urban-rural)*	Mean (pg/mg)	SD	Median (pg/mg)	Min-Max	p-all regions**	p (urban-rural)***
opDDE	**Urban**	53.3	0.116	0.045	0.8	0.9	0.54	LOQ-2.9	0.111	0.168
	Rural A	82.1			2.3	4.3	0.98	LOQ -22.5		
	Rural B	75.9			2.7	5.7	0.69	LOQ -23.5		
	Total	73.6			2.1	4.5	0.7	LOQ -23.5		
ppDDE	**Urban**	80.0	0.020	0.027	2.2	2.2	1.43	LOQ -7.6	0.040	0.081
	Rural A	100.0			8.6	15.2	3.57	1.0-65.3		
	Rural B	96.6			6.3	16.2	1.18	LOQ -68.0		
	Total	94.4			6.4	14.1	1.6	LOQ -68.0		
opDDD	**Urban**	0.0	0.018	0.016	0.3	-	-	-	0.020	0.018
	Rural A	21.4			2.4	7.7	0.26	LOQ -41.2		
	Rural B	37.9			4.6	10	0.26	LOQ -38.6		
	Total	23.6			2.8	8.1	0.3	LOQ -41.2		
ppDDD+opDDT	**Urban**	6.7	0.092	0.243	0.9	0.4	0.75	LOQ -2.4	0.100	0.204
	Rural A	28.6			4.7	10.5	0.75	LOQ -41.8		
	Rural B	10.3			2.8	6.4	0.75	LOQ -26.7		
	Total	16.7			3.1	7.7	0.8	LOQ -41.8		
ppDDT	**Urban**	20.0	0.220	0.450	2.2	2.7	1.1	LOQ -9.6	0.189	0.394
	Rural A	39.3			4.5	5.6	1.1	LOQ -25.5		
	Rural B	20.7			4	9	1.1	LOQ -39.2		
	Total	27.8			3.8	6.8	1.1	LOQ -39.2		
SumDDTs	**Urban**	80.0	0.020	0.027	6.3	3.9	4.7	LOQ -13.2	0.001	0.021
	Rural A	100.0			22.4	32.6	11.7	3.9-161.9		
	Rural B	96.6			20.5	44.8	4.6	LOQ -189.3		
	Total	94.4			20.5	44.8	4.6	LOQ -189.3		

* *p-values for percentages were resulted from Pearson's chi-square tests.*

** *p-values for imputed concentration were resulted from Kruskal-Wallis.*

*** *p-values for imputed concentration were resulted from Mann-Whitney tests.*

and provided LODs from 0.02 pg/mg (for ppDDE) to 0.5 pg/mg (for ppDDT). Compared to our detection limits (Table 1), Salquebre *et al.* presented an even lower detection limit with the benefits of a more sensitive mass spectrometric system. On the other hand, we managed to combine a fast treatment method for hair with the HSSPME technique coupled with a simple GC-MS system and we achieved lower detection limits than previously reported for the same system[6,8] for the aforementioned PCBs congeners and for DDTs (except ppDDT where detection limit was still relatively similar). Summarizing, we have presented a cost-effective and low time-consuming method for the simultaneous detection and quantification of PCBs and DDTs in hair, using SPME combined with a simple GC-MS.

Conclusion

Our proposed method includes a rapid HSSPME technique, performed at high temperature (90°C), in alkaline ambient, which allowed particularly low LODs to be achieved and satisfactory validation parameters for all compounds analyzed. The low cost and low time-consumption of the presented method render it very suitable for biomonitoring.

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