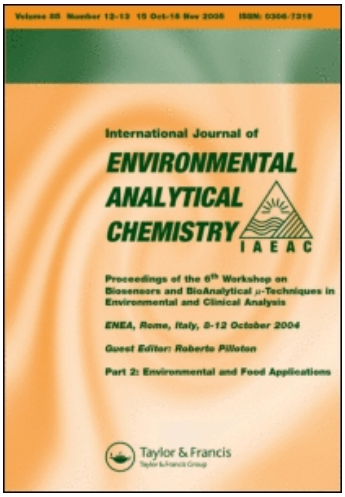

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Serum organochlorine pesticide and polychlorinated biphenyl levels measured in delivering women from different locations in Tunisia

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Blood serum concentrations of polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) such as dichlorodiphenytrichloroethane and its metabolites (DDTs), hexachlorobenzene (HCB) and hexachlorocyclohexane isomers (HCHs) were measured in maternal serum ($n = 82$) from mothers living in different regions in Tunisia. Gas chromatography with electron capture detection was used to quantify residue levels on a volume basis of the organochlorine compounds (OCs). The pattern of OCP in human serum showed that DDTs was consistently the prevalent OC in blood. p,p′-DDE, the major metabolite of p,p′DDT, and HCB were found in all serum samples with mean concentrations of 1.69 and 0.42 ng mL^{-1}, respectively. PCB congeners 138, 153 and 180 were the predominant congeners measured in all serum samples with a detection frequency of 80%, 72% and 83%, respectively. In general, results found in the investigated group are lower than the concentrations measured in serum blood in other countries.

Keywords: human blood; organochlorine pesticides; polychlorinated biphenyls; gas chromatography analysis; Tunisian delivering women

1. Introduction

Polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) were detected in all environmental and biological matrices due to their widespread use. The production and use of these compounds were banned in industrialised countries during the 1970s [1] or subjected to restrictions in use in many other countries, in response to their effects as seen in wildlife and humans.

Due to their high lipophilicity and the resistance to biodegradation, the organochlorine compounds (OCs) bioaccumulate along the food chain, especially in fatty food, and thus within the food chain bring on a high degree of contamination in high trophic organisms [2]. Humans, who occupy the top position in the trophic levels, are exposed to higher levels of these pollutants and become more vulnerable to the toxic effects. The concentration of fat-soluble contaminants like OCs is expected to be considerably higher in breast milk than in whole blood [3]. However, many investigations conducted on human milk and blood serum analysis showed similar organohalogen compound levels and significant correlations between human milk and maternal serum pair samples concentrations when OCs are expressed on lipid basis. Equilibrium can be reached in organism

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in PCB lipid levels of maternal serum, breast milk, cord serum and adipose tissue [4–7]. The PCB levels expressed on serum basis in maternal serum before delivery are around 5 times higher than in cord serum, because the lipid content of maternal serum (before delivery) is around 5 times higher than in cord serum.

OCs have been detected in human lipid rich tissues such as in human breast milk [8–10], umbilical cord and maternal serum [1,11], and maternal adipose tissues [12]. The elevated concentrations of OCs found in human samples have caused concerns about human health effects. However, assessing the human health impacts of exposure to OCs is a very difficult task. Humans are always exposed to mixtures of OCs, and never to a single compound. Toxic effects of these compounds are of major public concern, especially for the foetus and for breast-fed infants [13,14]. Several epidemiological studies have reported an association between exposure to organochlorine compounds and several health problems such as cancer of the breast [15]; brain [16,17], thyroid and soft tissue carcinomas [18]; and development disorders among infants [19].

For the general population, food (especially fish from contaminated waters), ambient and indoor air, dust, water and soil are considered as the main sources of exposure to PCBs and OC pesticides [20–22].

The determination of maternal body burden as well as of pre- and post-natal exposure of neonates for OCs in humans has been conducted in serum, human milk, adipose tissue and follicular fluid. Collection of adipose tissue involves surgical removal of tissues. Therefore blood and breast milk have become common tissues for measuring OC levels in humans [10,23,24]. However, serum is usually preferred over whole blood due to the minor complexity of the matrix and because it is a more homogenous material [25].

The present study was performed, for the first time in Tunisia, in order to investigate the concentration of DDTs, HCHs, and HCB and the congener-specific distribution of PCBs in human blood from healthy delivering women, and to provide useful data on contamination levels in delivering women from different regions of Tunisia.

2. Experimental

2.1 *Sample collection*

The target population ($n = 82$) was delivering women (average age of 32 years old), healthy, non-smoking and living in Tunisia during at least the preceding 3 years. The number of primipara mothers was 45/82 and the number of multipara mothers was 37/82. The selection of the subjects was random. The women were interviewed about occupational hazards. None of the subjects had any known occupational exposure to OCs. The enrolment and sampling were performed in the period February 2003–January 2005. Blood samples were taken by medical professionals, 3–8 days after the delivering process finished, at the normal morning procedure between 8.00 h and 12.00 h. An amount of 10 mL whole blood was taken from each volunteer. The blood samples were transported frozen to the laboratory, centrifuged at 2000 rpm for 5 min to separate the serum from the blood cells and immediately stored at -20°C.

2.2 *Reagents and materials*

Analytical reference standard solutions of 15 OCPs in hexane (SRM 2261:HCB, γ-HCH, heptachlor, aldrin, heptachlor epoxide, 2,4′-DDE, cis-chlordane, *trans*-nonachlor,

dieldrin, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, 2,4′-DDT, 4,4′-DDT and mirex) and 20 PCB congeners in 2,2,4-trimethylpentane (SRM 1493: PCB 8, 18, 28, 44, 52, 66, 77, 101, 105, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206 and 209) were purchased from the National Institute of Standard and Technology (USA). For β-HCH, certified analytical standard (purity > 99%) was obtained from Supelco (Bellefonte, USA) and stock solution ($1\,mg\,mL^{-1}$) was prepared in acetone. These standard solutions were further diluted by *n*-hexane to obtain mixed fortifying and GC calibration standard solutions for all OCs. The solvents used in this study (*n*-hexane, ethanol, acetonitrile and dichloromethane) were pesticide quality and were obtained from Fluka (Buchs, Switzerland). Florisil (60–100 mech) was obtained from Merck (Darmstard, Germany), activated at 650°C and re-treated at 130°C for 5 h. Thereafter, it was stored in a desiccator until use. Anhydrous sodium sulfate suitable for use in pesticide analysis was purchased from Fluka, heated at 300°C and stored in a 130°C oven.

2.3 *Extraction procedure*

Sample analysis was performed according to the method previously described [26] with some modifications. Blood serum (3 mL) was passed to a 50 mL tube with a stopper, the formic acid (3 mL) at 98% was added and mixed with an ultra-turrax to hydrolyse and liberate OCs from complexes with endogenous substances of blood. Thereafter, OC residues were extracted with 15 mL hexane by centrifugation at 2000 rpm for 5 min. The extraction was repeated with 10 mL of hexane and the extracts were collected in a 100 mL separatory funnel and left for 30 min to separate the organic phase. The hexanic phase was rotary evaporated to about 1 mL. The extract was purified with activated florisil (2 g) contained in a chromatographic mini-column (40 cm × 0.5 cm ID) and topped up with 1 g of anhydrous sodium sulphate. The extract was eluted with 30 mL of dichloromethane and *n*-hexane (1 : 9; v/v). The eluate was evaporated in a Kuderna-Danish to 1 mL and was ready for gas chromatography (GC) analysis.

2.4 *GC-ECD analysis*

Total PCBs, DDTs, HCHs, HCB and dieldrin in serum were identified and quantified using an Agilent gas chromatograph equipped with a split/splitless injection port, coupled with a ^{63}Ni electron capture detector (ECD) and operated by HP Chemstation Software.

An amount of 2 μL of extract was injected in splitless mode into a PTE-5 30 m × 0.32 mm ID, 0.32 μm film thickness capillary column, using nitrogen carrier gas with a 1.5 mL/min flow rate and the following oven temperature programme: 50°C initial (2 min) to 160°C at 5°C/min and to 260°C at 2°C/min, hold 10 min. The temperature of the detector and injector were 300°C and 250°C, respectively. The PTE-5 column was used as the primary analytical column. The data presented in this paper were obtained using this column. A second HP1 fused silica column 30 m × 0.32 mm ID, 0.25 μm film thickness was used as a confirmatory column.

Quantitative and qualitative analysis were done by comparison with external standard. The ECD response linearity was assessed with respect to the peak height for all studied compounds in a concentration span $0.5–30\,ng\,mL^{-1}$ with 2 μL injection. The correlation coefficients of the corresponding calibration curve are satisfactory ($R > 0.99$). The limit of detection (LOD) was calculated from the expression: $LOD = a + 3\sigma_{x/y}$ where a is

the intercept of the calibration curve and $\sigma_{x/y}$ is the standard deviation of the fitting. The LOD are within the range of 0.2 to 0.5 ng mL^{-1} for OCPs and from 0.01 to 0.4 ng mL^{-1} for PCB. The limits of quantification (LOQ) for an actual sample were determined in the same way but using a chromatogram background of non-spiked blood sample. The estimated values are 2 to 3 times higher than the LOD. Full method precision and recovery were determined by spiking blank cow blood serum which presented contamination levels below the detection limits at two levels: 1 and 10 ng mL^{-1} with $n = 10$ per level. The fortification study showed mean values ranged from 70% to 97%. The standard deviation and coefficient of variation were below 10%, indicating adequate repeatability of the method.

2.5 *Statistical analyses*

All statistical analysis was conducted with the SPSS package (release 11.0; SPSS, Chicago, IL, USA). Concentrations of OCs were expressed as arithmetic means ± standard deviations. The result obtained on OCs were calculated using analysis of variation (ANOVA), determining the signification of categorical factors on OC levels after removing any differences caused by the variability among groups. A p-value ≤ 0.05 was considered to indicate statistical significance.

3. Results and discussion

The arithmetic mean and standard deviations of the detected PCBs are given in Table 1 and the results of OC levels in maternal serum in Table 3. Both are expressed on serum volume. Values below the LOD are treated as being zero for calculating total chemical group concentrations (i.e. 'Total DDT' etc.).

To our knowledge, this study represents the most comprehensive survey of organochlorine chemicals in humans in Tunisia in at least the last 20 years, and includes the first report of PCBs in human blood serum samples from the general population in Tunisia.

Table 1. Mean PCB congener concentrations (μg L^{-1} serum basis), relative standard deviation (RSD), frequency of detection and range in maternal blood serum collected in 12 locations from Tunisia.

PCB congeners	Structure	Mean	%RSD	Max	Frequency of detection (%)
8	2,4′	0.034	12.7	0.0366	52
28	2,4,4′	0.040	24.0	0.0694	31
52	2,2′,5,5′	0.031	15.4	0.0812	62
44	2,2′,3,5′	0.032	11.9	0.0580	52
101	2,2′,4,5,5′	0.043	6.3	0.0481	33
105	2,3,3′4,4′	0.027	13.5	0.0567	42
118	2,3′4,4′,5	0.45	23.4	0.620	57
138	2,2′,3,4,4′,5′	0.65	16.8	0.743	80
153	2,2′,4,4′,5,5′	0.66	26.7	0.732	72
170	2,2′3,3′,4,4′,5	0.15	18.8	0.598	64
180	2,2′,3,4,4′,5,5′	0.59	10.7	0.902	83

3.1 *PCB levels*

In all analysed maternal blood serum the presence of PCB 18, 66, 77, 126, 128, 195, 206 and 209 were below the limit of quantification and therefore, were not taken into further calculations. The low levels of these congeners can be justified by the fast enzymatic degradation usually observed in animals for those PCBs [27].

Among analysed congeners, seven prevalent indicator PCBs mainly PCB Nos. 28, 52, 101, 118, 138, 153 and 180, were studied. These compounds were considered to be primary indicators of biological PCB burdens [28] and are used as marker compounds to monitor occurrence and distribution [29]. PCB 138, 153 and 180 were the most dominant congeners, contributing to more than 33% to the total PCB concentration. These congeners were detected in 80, 72 and 83% of analysed samples with mean concentrations of 0.065, 0.066 and 0.059 $\mu g\,L^{-1}$ serum basis, respectively. These PCBs are considered as mid-chlorinated congeners (hexa- and hepta-PCB) with high K_{ow} and are likely to be accumulated in lipid rich tissues such as human blood. A similar PCB congener composition was observed in a recent published study concerning contamination of maternal breast milk by PCB congeners in Tunisia [8]. In this work, the authors have found that the PCB 138, 153 and 180 were the most detected and quantified congeners in human breast milk.

In Table 2, we present a comparison of our data with recent published results on the presence of these congeners in human blood serum and it shows a good agreement in both concentrations and distribution patterns.

3.2 *Organochlorine pesticides in serum*

The present study reports the presence of organochlorine pesticide residues in mothers living in different areas of Tunisia (agricultural areas, industrial areas and costal areas). In all analysed samples, aldrin, dieldrin, heptachlor, heptachlor epoxide, cis-chlordan, trans-nonachlor and mirex were not detected, thus they are not discussed. Concentrations of DDT and its metabolites, HCH isomers and HCB are reported in Table 3. p,p′-DDE and HCB were detected as the most ubiquitous contaminants in all analysed blood serum samples (100%) with mean concentrations of 1.69 and 0.42 $ng\,mL^{-1}$ serum basis, respectively. Among the other remaining DDTs, p,p′DDT was found at a concentration of 0.91 $ng\,mL^{-1}$ serum basis followed by p,p′-DDD with mean level of 0.42 $ng\,mL^{-1}$

Table 2. Concentrations of the seven prevalent indicator PCBs in maternal blood serum from some countries ($\mu g\,L^{-1}$ serum basis).

PCB	28	52	101	118	138	153	180	References
USA	0.015	0.014	0.054	1.131	2.871	2.110	1.506	[30]
Germany	Ne	Ne	Ne	Ne	0.45	0.53	0.22	[31]
Spain	0.31	Ne	Nd*	0.38	1.25	1.46	1.80	[32]
Norway	Ne	0.11	0.05	0.34	0.53	0.49	0.17	[33]
Belgium	Nd*	Nd*	Nd*	0.185	0.445	0.670	0.310	[34]
Tunisia	0.048	0.031	0.043	0. 45	0. 65	0. 66	0. 59	Present study

Ne: not evaluated; Nd*: not detected.

Table 3. Mean concentrations ($\mu g\,L^{-1}$ serum basis) of organochlorine pesticide residues in maternal blood serum from different areas in Tunisia.

Compounds	Mean	%RSD	Minimum	Maximum	Frequency
HCB	0.42	15.4	Nd	77.0	100
β-HCH	0.30	19.6	Nd	89.7	23
γ-HCH	0.22	22.5	Nd	54.8	16
Σ-HCH	0.49	20.2			
p,p′-DDE	1.69	10.7	Nd	141.2	100
p,p′-DDT	0.91	11.9	Nd	73.6	51
p,p′-DDD	0.42	9.9	Nd	45.7	12
Σ-DDT	4.33	13.2			

Nd: not detected; $n = 82$; RSD: relative standard deviation.

Table 4. Average levels ($\mu g\,L^{-1}$) of OCPs in maternal blood serum samples from different regions worldwide from recent studies.

	HCB	β-HCH	γ-HCH	p,p′-DDE	p,p′-DDT	References
Tunisia	0.42	0.30	0.22	1.69	0.91	Present study
Belgium	0.205	Ne	Ne	2.16	Ne	[34]
India	Ne	1.44	0.31	1.41	2.69	[35]
Mexico	1	1.4	0.2	14.5	1.8	[36]
Norway	0.57	3.59	0.16	5.42	1.61	[33]
Spain	36.7	9.2	Nd	9.61	0.94	[32]

Ne: not evaluated.

serum basis. Detectable concentrations of β–HCH and γ-HCH were found in 23 and 16% of blood serum samples.

The detection of DDT and its metabolites in Tunisian blood serum, despite their prohibition, reflects either a relatively recent exposure or a cumulative past exposure. DDT was extensively used in Tunisia in the years from the end of the 1940s to the 1980s for insect control, sanitary purposes and agricultural protection. In fact, p,p′DDE, the major metabolite of DDT, was present in all analysed human blood serum and with high concentrations. Interestingly, a recent study conducted in Tunisia [8] investigated mean level of OCPs in human breast milk and shows similar results concerning p,p′-DDE contamination. These findings are consistent with worldwide reports that around 90 to 100% of the populations have a detectable concentration of DDT [33,34].

The mean level of p,p′-DDE in human blood serum of the present study ($1.69\,ng\,mL^{-1}$ serum basis) is lower than in Norway (mean $5.42\,ng\,mL^{-1}$ serum basis), in Spain (mean $9.61\,ng\,mL^{-1}$ serum basis) and in Mexico (mean $14.5\,ng\,mL^{-1}$ serum basis) (Table 4). This result shows that DDT is still used or has been used until recently in these countries.

The estimation of the p,p′-DDT to p,p′-DDE ratio, which may indicate whether the exposure was recent or distant, was at 0.54. This value suggests the influence of the restriction and prohibition of DDT and the decrease in exposure to this compound over the past few decades.

From the organochlorine pesticides, HCB was found as one of the most ubiquitous (detected in all analysed samples), reaching a value of $0.42\,ng\,mL^{-1}$ serum basis in

maternal blood serum. This concentration was somewhat lower than that found in Norway in 2003 or Spain in 1999.

Among HCH isomers, β-HCB and γ-HCH were determined at 0.30 and 0.22 ng mL^{-1} serum basis in blood serum. In fact, HCH is a mixture of isomers; the most dominants are α, β and γ isomers. The β-isomer is the most persistent and bioaccumulative form. Similar findings are observed in some other studies (Table 4) and in other biological matrixes such as human milk [8,40], honey and falcon eggs [37–39].

No significant differences were detected between studied locations ($p < 0.05$) in terms of DDT, HCHs and HCB, showing uniform exposure of the Tunisian population to organochlorine pesticides.

4. Conclusion

This study was, as far as we know, the first survey of maternal blood serum concentrations of PCBs and organochlorine pesticides in Tunisia. The results produced may be seen as an indicator of human levels of this range of chemicals in the Tunisian population in general. The mean concentrations of pollutants in human blood samples are situated at the low end of the concentration range measured in some developed nations for PCB congeners but higher than those detected in some developing countries. No significant differences were observed between all studied regions in terms of PCBs and OCPs.

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