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Organochlorine pollutants' levels in hair, amniotic fluid and serum samples of pregnant women in Greece. A cohort study.

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Highlights

- The levels of HCB in amniotic fluid were positively correlated with those in hair.

- The levels of PCB101 in hair were positively correlated with those in serum and amniotic fluid samples.
- Smoker pregnant women presented higher levels of DDDs, DDTs and PCB28 in hair samples compared to non-smokers
- Significant higher levels of PCB101 were detected in the group of pre-term gestation women compared to full-term group.

Abstract

Persistent organic pollutants are synthetic chemicals highly resistant to degradation with strong tendency to bioaccumulation. Assessment of human exposure to these compounds is crucial for public health protection, especially during vulnerable periods.
The aim of the present cohort study was to evaluate the level of contamination to PCBs, o,p'- and p,p'-DDE, o,p' and p,p'-DDD, o,p' and p,p'-DDT and HCB in pregnant women. Hair, amniotic fluid and serum samples were collected and analyzed by HS-SPME-GCMS.
The most detected analytes in amniotic fluids were p,p'-DDE, p,p'-DDD, o,p'-DDE and PCB101, in serum p,p'-DDE, HCB and PCB101 and in hair p,p'-DDE, HCB and PCB101. The levels of HCB and PCB101 in amniotic fluids were positively correlated with those in hair. Higher levels of DDDs and DDTs in hair samples and PCB28 in amniotic fluids were observed in smoker pregnant women. Gestation age was inversely proportional with the detected levels of PCB101 in all tested samples.

Abréviation list

TCN: 1,2,3,4-tetrachloronaphthalene, **HS-SPME:** head space solid phase micro - extraction, **GC - MS:** gas chromatography - mass spectrometry, **PDMS/DVB:** polydimethyl siloxane/divinyl benzene, **PTFE:** polytetrafluoroethylene, **POPs:** persistent organic pollutants, **PCBs:** polychlorinated biphenyls (PCB 28, 52, 101, 118, 138, 153, 180), **DDTs:** o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT and p,p'-DDT, **OCPs:** Organochlorine pesticides, **LOD:** limit of determination, **LOQ:** limit of quantification

Keywords: hair, amniotic fluid, serum, PCBs, DDTs, HCB

1 Introduction

Persistent organic pollutants (POPs) are organic chemical substances with a particular combination of physical and chemical properties. Through biochemical cycles they are introduced into the food chain, with higher concentration in the fatty tissues of the organisms belonging to the upper levels of the food chain. Organochlorine pesticides (DDTs), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are included in POPs (Iatrou et al., 2019).

Organochlorine pesticides (as HCB and DDT) belong to the group of chlorinated hydrocarbon derivatives known for their high toxicity, slow degradation and bioaccumulation (Ravindran et al., 2016). HCB is a fungicide formerly used as a seed treatment and as wood preserving agent, considered one of the 12 most persistent organic pollutants and has been banned globally under the Stockholm Convention on Persistent Organic Pollutants. This compound is highly persistent in the environment and has an approximate half-life of 2 years in the air and 6 years in water and sediments (Barber et al., 2005). HCB acts as an endocrine disruptor in thyroid, uterus and mammary gland and is considered as probable human carcinogen (Group 2B) (Miret et al., 2019).

DDTs are organochlorine pesticides that had been used from 1940 to 1970 in fungus and pest control, with neurotoxic properties. The intake route is food since they can be found in meat, fish, and milk products (Mahalingaiah S et al., 2012). Several studies have been done to determine their effect on the reproductive system. The exposure in DTT seems not to have any association between oocyte, fertilization, and implantation parameters in women undergoing in vitro fertilization (IVF) but there is an association with increased time of pregnancy and early clinically detected fetal lose (Al-Saleh I et al. 2009, Longnecker et al. 2005).

PCBs are synthetic chlorinated organic compounds extensively used until 2004 which can influence gene expression of neuronal proliferation and maturation (Sazonova et al., 2011). They have been used as plasticizers in rubber and resins, in hydraulic fluids, wax extenders, inks, lubricants and carbonless copy paper. It has been shown that PCBs can affect the fertility of both genders and they are also associated with decreased fecund ability in utero and reduced sperm motility (Brehm et al., 2019). Prenatal exposure to PCBs seems to be associated with increased chances of attention-deficit/hyperactivity disorder (ADHD)-like beha-viors (Neugebauer et al., 2015) and lower intelligence levels (Lai et al., 2002) in children, probably because of the disruption that they can cause in central nervous system(Roylandetal.,2008).

In the current study, blood, amniotic fluid and hair samples were collected from pregnant women who underwent amniocentesis for diagnostic reasons (fetal karyotype, genetic diseases or related infections) in the second trimester of their pregnancy (16^{th}-21^{st} weeks). The aim of our study was to investigate the concentration of POPs in amniotic fluid and consequently fetus exposure. The determination of POPs levels in blood samples and the correlation of their concentrations in amniotic fluid will provide conclusions about the permeability of placental barrier, as well as the effects on embryos development, pregnancy outcome and neonatal morbidity. The detection of these agents in amniotic fluid could be also used as biomarker for fetus exposure since amniotic fluid is the product of its metabolic activities.

2. Materials and Method

2.1 Sample collection and storage

Hair, blood and amniotic fluid samples were collected at the Alexandra Maternity Hospital, Athens, Greece from women during amniocentesis in the 2^{nd} trimester of their pregnancy. Totally, 120 women participated in the current study. The women were informed for the purpose of the study and were asked to complete questionnaires from which information about personal details (gender, weight and age), personal habits and medical history were collected. Each hair sample was placed in a paper envelope and kept in the dark at room temperature. Serum and amniotic fluid samples were stored at -20^{o}C until their analysis.

2.2 Reagent and materials

HCB, o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT and p,p'-DDT were purchased from Chem Service, West Chester. TCN (1,2,3,4-tetrachloronaphthalene) was used as internal standard (Dr Ehrenstorfer, GmbH D86189, Augsburg, Germany). The congeners PCB 28, PCB 52, PCB 101, PCB118, PCB 138, PCB153, PCB180 and sodium chloride were obtained from Fluka analytical-Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). Sodium hydroxide was obtained from Merck (Darmstadt, Germany).

2.3 Stock and working solution

The stock solution of PCB congeners was prepared in hexane (10 μg/ml) while the stock solutions of each individually HCB and DDT congeners were also prepared in hexane at concentration level of 1000 μg/ml. Further dilutions in hexane provided multi mix working solutions of all the target compounds at concentrations 10, 1.0, 0.1 and 0.01 μg/ml. The working standards were used for the construction of calibration curves and for the preparation of the spiked samples. All solutions were stored at -20ºC.

2.4 Spiked samples

Pooled human hair samples with detected levels below the LOQ values were spiked with all the target analytes at concentration levels 0, 5, 10, 25, 50 and 100 pg/mg while for both blood and amniotic fluid the levels were 0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 ng/ml. In any case, the spiked samples were used for the construction of spiked curves and the determination of the concentration of each compound in the authentic collected samples.

2.5 Samples extraction procedure

The samples extraction procedures have been published previously and applied with slightly modifications (Tzatzarakis et al. 2014; Vinceti et al. 2017; Iatrou et al. 2019).

Hair: An amount of hair (100 mg), had been previously washed twice with 5 ml water and once with 5 ml of hexane, placed in 8 ml SPME vials containing 2 ml of NaOH 10 M, 1 ml of ultrapure water, 0.3 gr of NaCl and 10 ng of internal standard (TCN).

Blood and amniotic fluid: One ml of serum or three ml of amniotic fluid, previously centrifuged at 4000 rpm for 4 min, were placed in 8 ml SPME vials with 0.3 gr of NaCl and 10 ng of internal standard (TCN).

SPME vials were sealed with PTFE/silicon septum caps and placed in the GS-MS tray. Online extraction followed by a PDMS/DVB type extraction fiber at 90ºC for 30 min with an agitation speed at 250 rpm. During agitation the fiber 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 that, it was inserted in the injection port of the GC-MS, where it remained for 5 min until the complete release of the substances in splitless mode.

2.6 Instrumentation

The analysis of the biological samples was performed on gas chromatography mass spectrometry system (Shimadzu QP-2010, Kyoto, Japan) equipped with an AOC-5000

robotic autosampler. The separation of the compounds was achieved on a SLB^{tm}-5ms capillary column of 30 m x 0.25 mm x 0.25 μm (Supelco Bellefonte PA, USA). A 65 μm PDMS/DVB type Metal Alloy fiber was used for the absorption of the analytes (Supelco Bellefonte PA, USA).

Helium was used as a carrier gas (1 mL/min) while the inlet, MS interface and ion source temperatureswere set up at 270^{o}C, 310^{o}C and 230^{o}C, respectively. The initially temperature of the column was 60^{o}C stable for 5 min, then was raised to 180^{o}C (15^{o}C/min) stable for 1 min and finally to 300^{o}C with a rate of 30^{o}C/min where remained for 1 min. The determination of the target compounds was made by selected ion-monitoring mode using one or two qualification m/z ions and a target m/z ion for the quantification of each compound.

2.6 Statistical Methods

Continuous variables were expressed in the form of mean and standard deviation. Especially for PCBs and DDTs and HCB concentrations medians and quartiles were additionally applied to describe the hair exposure. Imputed concentration values (=LOD/2) were used in non-detected concentrations. Comparisons of concentrations between two groups (e.g. full vs pre term) were made using independent samples t-test or Mann-Whitney. Spearman's rho coefficients were applied to establish bivariate correlations. IBM SPSS Statistics 24.0 was used for data analysis and graphical representation of data.

3. Results

The response of the analytical system was found to be linear both for the standard and the spiked curves. In total, good sensitivity with low LOD and LOQ values was obtained with the exception of p,p'-DDT which was not detectable at levels lower than 100 pg/mg for the analysis of hair samples and lower than 5 ng/ml for the blood samples. The limits of determination (LOD) and quantification (LOQ) were calculated from the signal-to-noise (S/N) ratio (S/N>3 and S/N>10, respectively) and are depicted in the Table 1.

3.1 Medical reported history and demographic data of the participants

A total of 120 women who underwent, for different reasons, amniocentesis were aged from 27 to 43 years old. The mean age of the participants was 37.7±2.8 years old while the age distribution revealed that 36-39 years old age group was the most frequent (n=70,

61.4%). Most of the participants were of Greek nationality (103 women, 85.8%) and 70 women (64.8%) had a working status (Table 2).

Smoking habits showed that the 69.2% (n=83) were not smokers before current pregnancy while 37.1% (13/35) kept smoking during pregnancy. Alcohol consumers (1-2 drinks/day) were the 10.8% (n=13) before current pregnancy while the 10 of the 13 stopped consume alcohol when getting pregnant. Cannabis use reported only in 1 case (0.8%) before pregnancy but not during it (Table 2).

Thyroid diseases and diabetes I or II were the most frequent diseases in the studied women. A total of 24.2% (n=29) and 5.8% (n=7) of the total sample have reported thyroid diseases and diabetes, respectively. Gastrointestinal diseases and arterial pressure were reported in 2 cases (1.7%) while immunological, urinary and cardiorespiratory diseases in 0.8% of the participants. The majority of the women (66.7%) have no referred diseases in their medical history.

In Table 3 is shown the number of pregnancies and the way of childbirth. For 64 women (53.3%) the studied pregnancy was their first pregnancy, while the rest 46.7% had at least one past pregnancy. Caesarian deliveries were present in 30.0% of the cases (n=36) in contrast to 70.0% that was normal. Problematic deliveries (abortions, miscarriages etc), in past pregnancies were reported in 27 cases (22.5%).The main reason for undergoing a amniocentesis was age (>=35) in 86.0% of the participating women, while the rest had former problems in pregnancy or an abnormal ultrasound.

3.2 Monitoring data

Of the total of 120 women, 104, 118 and 83 women provided enough amounts of hair, amniotic fluid and serum for the monitoring of the target compounds. The % frequencies of detection of each analyte are given in Table 4. For PCBs, the most detected congener was PCB101 (from 10.8% for serum to 16.9% for amniotic fluid) compared to others for which the frequencies of detection were lower than 10%. No positive hair samples were found for PCB118 while PCB153 was not detected at all in hair and amniotic fluid samples.

Concerning HCB, 22.9 % of the serum samples provided detectable levels. p,p'-DDE was the most frequently detected (77.9%, 94.9% and 85.5 % in hair, amniotic fluid and serum samples, respectively), in contrast to p,p'-DDT which was not detectable in any sample.

The levels of PCB congeners, HCB, o,p' and p,p'-DDD (DDDs), o,p' and p,p'-DDE (DDEs) and o,p' and p,p'-DDT were monitored in amniotic fluid, serum and hair samples and are shown in Table 5. Mean concentrations of DDEs were 8.2 ± 14.7 pg/mg, of DDDs

were 28.6 ± 58.2 pg/mg, of sum DDTs (DDE, DDD and DDT congeners) were 36.5 ± 61.3 pg/mg, of PCBs 6.9 ± 2.7 pg/mg and of HCB was 1.0 ± 0.7 pg/mg in hair samples. Same pattern was found for amniotic fluid and serum samples. Serum samples were found to have higher burden to PCBs (0.429 ± 0.0.459 ng/ml) compared to DDEs (0.092 ± 0.107 ng/ml) and HCB (0.029 ± 0.026 ng/ml). The corresponding values of DDEs, DDDs and sum DDTs in amniotic fluids were 0.060 ± 0.271 ng/ml, 0.109 ± 0.641ng/ml and 0.170 ± 0.905 ng/ml, respectively. Lower mean concentration values compared to sum DDTs were detected for HCB (0.013 ± 0.068 ng/ml) and PCBs (0.027 ± 0.041ng/ml).

4. Discussion

During pregnancy, lipophilic chemicals, such as organochlorines, are stored in maternal adipose tissue and can be mobilized to the blood stream reaching the fetus through the placenta (Lopez-Espinosa et al., 2007). In our study, PCBs, HCB and DDEs were measured in hair, serum and amniotic fluid and the correlation estimates are shown in Table 6. HCB and PCB101 levels in amniotic fluid were positively correlated with those in hair levels (r_s=0.337, p<0.001 and rs=0.664, p<0.001, respectively). The PCBs congeners seem to show different distributions patterns in body tissues, probably due to their chemical structure, the lipid content of the compartment and the overall toxin burden of the individual (van der Ven et al., 1992). Regarding the PCB101 detected levels, hair-serum and amniotic fluid - serum samples showed statistically significant correlations (rs=0.280, p=0.022 and rs=0.473, p<0.001), respectively.

Between variables of demographic interest such as age, occupation, smoking (no, not during pregnancy, yes), nationality (Greek nationality or not) and alcohol consumption (no, not during pregnancy), only smokers provided statistical higher detected levels for DDDs and sum DDTs compared to non-smokers in hair samples. More specific, the mean DDDs level in non-smoker participants was 20.9 ± 10.9 pg/mg compared to 47.2 ± 103.9 pg/mg for smokers (p=0.036) while the sum DDTs levels for the same participants groups were (28.4 ± 13.8 pg/mg and 55.9 ± 107.8 pg/mg, p=0.023, respectively) (Table 7). This difference can be explained from the presence of DDT congeners in tobacco (Quadroni et al., 2019). The detected levels of DDTs nowadays are below the guidance residue limits (GRLs) provided by Cooperation Centre for Scientific Research Relative to Tobacco but active and passive smokers are still exposed to (CORESTA, 2018). In a recent published study, DDT isomers were detected in hair of French pregnant women in frequencies 1% for the o,p'- and p,p'DDD, 4 and 9% for o,p'-DDE and p,p'-DDE respectively, while slightly higher detection

rates were depicted for o,p'-DDT and p,p'-DDT (3 and 10%) respectively (Béranger et al. 2018). The above mentioned % detection frequencies of DDTs are similar to those presented in the current study with the exception of the p,p-DDE which was detected in 77.9% of the hair samples.

PCBs concentrations in amniotic fluids were not significantly different between smokers and non-smokers, except for those of PCB28 (smokers 0.011 ± 0.026 ng/ml compared to non-smokers 0.003 ± 0.014 ng/ml) with a p value 0.036 (Table 7). Although significant differences were observed, conclusions must be carefully expressed, due to the small sample size and the existence of outliers/extremes, a common issue on biomonitoring studies. In supplementary Table 1, the levels of HCB, PCB congeners and sum DDTs for smokers and non-smokers participants are depicted.

Pregnant women were divided into two groups, the pre-term group (≤36 weeks of gestation) (n=9) and full-term group (>36 weeks of gestation) (n=79). The statistical analysis revealed differences in the detected levels of PCB101 in hair, serum and amniotic fluid. Higher levels of PCB101 were detected for the group of pre-term gestation women compared to full-term group (Table 8). The differences were statistically significant (p=0.020 for the hair samples, p=0.013 for the amniotic fluid and p=0.025 for the serum samples). The corresponding correlations for all analytes are presented in supplementary Table 2. These findings can be explained by the ability of PCBs to affect the expression of proteins considered viable or potential biomarkers for preterm birth (Geer et al., 2015). The association between PCBs and birth outcomes seems to differ by the molecular weight of the PCB, more specific, low-chlorinated PCB congeners (such as PCB101) were negatively associated with gestational age and head circumference (Tang et al., 2018). Similar study by Taylor (Taylor et al., 1989) found a significant relation between increased estimated serum PCB levels and decreased gestational age (p=0.03) in 200 women who had held jobs with direct exposure in PCBs versus 205 women who had never been exposed. Our results are also in agreement with the findings of Tewari's experiment (Tewari et al., 2009) who used PCBs as environmental triggers and IL-10/ mice as the host and have shown that pregnant mice who were exposed to a PCB mixture experienced preterm delivery.

Moreover, a positive correlation of pre-term gestation and elevated concentrations of HCB in amniotic fluid samples was depicted. Elevated concentrations of HCB were found by Ribas-Fito in cord serum and were correlated with reduced intrauterine physical linear growth (Ribas-Fito et al., 2002). This finding was also confirmed by Torres-Arreola who reported that elevated p,p'-DDE and HCB levels in maternal serum may pose a risk to preterm birth in countries that continue to use such insecticides for malaria control (Torres-Arreola et al., 2003).

5. Conclusions

In the current study, we attended to collect information about the exposure of foetus in POPs. We found that elevated levels of HCB and PCB101 in amniotic fluid are strongly correlated with pre-term gestation. The detected levels of POPs in amniotic fluid maybe could be considered as potential biomarkers for the prevention of pre-term labor.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Al-Saleh I, Coskun S, El-Doush I, Billedo G, Mashhour A, Jaroudi K, et al. 2009. Outcome of in-vitro fertilization treatment and DDT levels in serum and follicular fluid. Med SciMonit 15(11):BR320–BR333.

Barber J., Sweetman A., JonesK..Hexachlorobenzene - Sources, Environmental Fate and Risk Characterisation(2005) Science Dossiers, Euro Chlorhttp://www.eurochlor.org/

Barbounis EG, Tzatzarakis M, Alegakis A, Kokkinaki A, Karamanos N, Tsakalof A, Tsatsakis AM. Assessment of PCBs exposure in human hair applies double focusing high resolution mass spectrometry and single quadrupole mass spectrometry. Toxicology Letters, 210 (2), 225-231, 2012.doi: 10.1016/j.toxlet.2011.07.031.

Basterrechea M, Lertxundi A, Iñiguez C, Mendez M, Murcia M, Mozo I, Goñi F, Grimalt J, Fernández M, Guxens M; INMA project. Prenatal exposure to hexachlorobenzene (HCB) and reproductive effects in a multicentre birthcohort in Spain.Sci Total Environ. 2014 Jan 1;466-467:770-6.doi: 10.1016/j.scitotenv.2013.07.053. Epub 2013 Aug 25.

Béranger R, Hardy EM, Dexet C, Guldner L, Zaros C, Nougadèree A, Metten MA, Chevrier C, Appenzeller BMR. Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort. Environment International 120 (2018) 43–53

Brehm E, Flaws JA. Transgenerational effects of endocrine disrupting chemicals on male and female reproduction.Endocrinology.2019 Apr 18.pii: en.2019-00034.doi: 10.1210/en.2019-00034.

CORESTA Guide No. 1 - the Concept and Implementation of CPA Guidance Residue Levels - July 2016 (+ Additional CPA June 2018)

Dong X, Wang Q, Peng J, Wu M, Pan B, Xing B. Transfer of polycyclic aromatic hydrocarbons from mother to fetus in relation to pregnancy complications. Sci Total Environ. 2018 Sep 15;636:61-68.doi: 10.1016/j.scitotenv.2018.04.274.

Duran Robert, Cravo-Laureau Cristiana. Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. FEMS Microbiol Rev. 2016 Nov; 40(6): 814–830.doi: 10.1093/femsre/fuw031.

Geer LA, Pycke BF, Sherer DM, Abulafia O, Halden RU.Use of amniotic fluid for determining pregnancies at risk of preterm birth and for studying diseases of potential environmental etiology.Environ Res. 2015 Jan;136:470-81.doi: 10.1016/j.envres.2014.09.031.

Lopez-Espinosa MJ, Granada A, Carreno J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from Southern Spain and some related factors. Placenta. 2007 Jul;28(7):631-8.

Quadroni S, Bettinetti R. An unnoticed issue: Organochlorine pesticides in tobacco products around the world.Chemosphere. 2019 Mar;219:54-57.doi: 10.1016/j.chemosphere.2018.12.009

Ram CharitraSah, Keshab Raj Joshi. Fact Sheets of 22 Persistent Organic Pollutants (POPs) under Stockholm Convention.Center for Public Health and Environmental Development (CEPHED), 2011.

RavindranJayaraj, PankajshanMegha, PuthurSreedev. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment.InterdiscipToxicol. 2016 Dec; 9(3-4): 90–100. doi: 10.1515/intox-2016-0012.

Ribas-Fitó N, Sala M, Cardo E, Mazón C, De Muga ME, Verdú A, Marco E, Grimalt JO, Sunyer J. Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth.Pediatr Res. 2002 Aug;52(2):163-7.

Tang M, Yin S, Zhang J, Chen K, Jin M, Liu W. Prenatal exposure to polychlorinated biphenyl and umbilical cord hormones and birth outcomes in an island population.Environ Pollut. 2018 Jun;237:581-591. doi: 10.1016/j.envpol.2018.02.044. Epub 2018 Mar 15.

Taylor PR, Stelma JM, Lawrence CE.The relation of polychlorinated biphenyls to birth weight and gestational age in the offspring of occupationally exposed mothers.Am J Epidemiol. 1989 Feb;129(2):395-406.

Tewari N, Kalkunte S, Murray DW, Sharma S. The water channel aquaporin 1 is a novel molecular target of polychlorinated biphenyls for in utero anomalies. J Biol Chem. 2009 May 29;284(22):15224-32. doi: 10.1074/jbc.M808892200.

Torres-Arreola L, Berkowitz G, Torres-Sánchez L, López-Cervantes M, Cebrián ME, Uribe M, López-Carrillo L. Preterm birth in relation to maternal organochlorine serum levels.Ann Epidemiol. 2003 Mar;13(3):158-62.

IatrouEI, TsygankovV, SeryodkinI, TzatzarakisMN, VakonakiE, BarbounisE, ZakharenkoAM, ChaikaVV, SergievichAA, TsatsakisAM, GolokhvastK. Monitoring of environmental persistent organic pollutants in hair samples collected from wild terrestrial mammals of PrimorskyKrai, Russia. Environ SciPollut Res Int. 2019 Mar;26(8):7640-7650.doi: 10.1007/s11356-019-04171-9.

Kanavouras K, Tzatzarakis MN, Mastorodemos V, Plaitakis A, Tsatsakis AM. A case report of motor neuron disease in a patient showing significant level of DDTs, HCHs and organophosphate metabolites in hair as well levels of hexane and toluene in blood. Toxicology and Applied Pharmacology, 256(3), Special Issue, 399-404, 2011. doi: 10.1016/j.taap.2011.07.022.

Lai, T., Liu, X., Guo, Y.L., Guo, N., Yu, M., Hsu, C., et al., 2002.A cohort study of behavioral problems and intelligence in children with high prenatal polychlorinatedbiphenylexposure. Arch. Gen. Psychiatry 59, 1061–1066

Langlois Peter H., Hoyt Adrienne T., Desrosiers Tania A., Lupo Philip J., Lawson Christina C., Waters Martha A., Rocheleau Carissa M., Shaw Gary M., Romitti, Paul A. Gilboa Suzanne M., Malik Sadia, the National Birth Defects Prevention Study. Maternal occupational exposure to polycyclic aromatic hydrocarbons and small for gestational age offspring.Occup Environ Med. 2014 Aug; 71(8): 529–535. doi: 10.1136/oemed-2013-101833.

Longnecker MP, Klebanoff MA, Dunson DB, Guo X, Chen Z, Zhou H, et al. 2005. Maternal serum level of the DDT metabolite DDE in relation to fetal loss in previous pregnancies. Environ Res 97(2):127–133.

Mahalingaiah S, Missmer SA, Maity A, Williams PL, Meeker JD, Berry K, Ehrlich S, Perry MJ, Cramer DW, Hauser R. Association of hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) with in vitro fertilization (IVF) outcomes.Environ Health Perspect. 2012 Feb;120(2):316-20. doi: 10.1289/ehp.1103696. Epub 2011 Aug 29.doi: 10.1289/ehp.1103696.

Michalakis M, Tzatzarakis MN, Kovatsi L, Alegakis KA, Xairetis G, Tsatsakis A. Hypospadias in offspring is associated with chronic exposure of parents to

organophosphate and organochlorine pesticides. Toxicology Letters, 230, 139–145, 2014.doi: 10.1016/j.toxlet.2013.10.015.

Miret NV, Pontillo CA, Zárate LV, Kleiman de Pisarev D, Cocca C, Randi AS. Impact of endocrine disruptor hexachlorobenzene on the mammary gland and breast cancer: The story thus far. Environ Res. 2019 Jun;173:330-341.doi: 10.1016/j.envres.2019.03.054.

Neugebauer, J., Wittsiepe, J., Kasper-Sonnenberg, M., Schöneck, N., Schölmerich, A., Wilhelm, M., 2015. The influence of low level pre-and perinatal exposure to PCDD/Fs, PCBs, and lead on attention performance and attention-related behavior amongGerman school-aged children: results from the Duisburg Birth Cohort Study. Int. J.Hyg. Environ. Health 218, 153–162.doi: 10.1016/j.ijheh.2014.09.005.

Pashin Y. V.; Bakhitova L. M. Mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons. Environ. Health Perspect. 1979, 30, 185–189.

Royland JE, Kodavanti PR. Gene expression profiles following exposure to a developmental neurotoxicant, Aroclor 1254: pathway analysis for possible mode(s) of action.ToxicolApplPharmacol. 2008 Sep 1;231(2):179-96.doi: 10.1016/j.taap.2008.04.023.

Sazonova NA, DasBanerjee T, Middleton FA, Gowtham S, Schuckers S, Faraone SV. Transcriptome-wide gene expression in a rat model of attention deficit hyperactivity disorder symptoms: rats developmentally exposed to polychlorinated biphenyls.Am J Med Genet B Neuropsychiatr Genet. 2011 Dec;156B(8):898-912.doi: 10.1002/ajmg.b.31230.

Tzatzarakis M, Barbounis EG, Kavvalakis M, Vakonaki E, Renieri E, Vardavas AI, Tsatsakis AM. 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).Drug Testing and Analysis 6, 85–92, 2014.doi: 10.1002/dta.1631.

Van der Ven K, van der Ven H, Thibold A, Bauer O, Kaisi M, Mbura J, Mgaya HN, Weber N, Diedrich K, Krebs D. Chlorinated hydrocarbon content of fetal and maternal body tissues and fluids in full term pregnant women: a comparison of Germany versus Tanzania. Hum Reprod. 1992 Jun;7Suppl 1:95-100.

Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A, et al. 2005. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. Am J Epidemiol 162(8):709–716.

Vinceti M, Violi F, Tzatzarakis M, Mandrioli J, Malagoli C, Hatch E, Fini N, Fasano A, Rakitskii V, Kalantzi OI, Tsatsakis A. Pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in cerebrospinal fluid of amyotrophic lateral sclerosis patients: a case-control study. Environmental Research, 155, 261-267, 2017.doi: 10.1016/j.envres.2017.02.025.

Wang Bin, Jin Lei, Ren Aiguo, Yuan Yue, Liu Jufen, Li Zhiwen, Zhang Le, Yi Deqing, Wang Lin-lin, Zhang Yali, Wang Xilong, Tao Shu, Finnell Richard H. Levels of Polycyclic Aromatic Hydrocarbons in Maternal Serum and Risk of Neural Tube Defects in Offspring. Environ Sci Technol. 2015 Jan 6; 49(1): 588–596. doi: 10.1021/es503990v.

Table captions

Table 1. Limits of determination (LOD) and quantification (LOQ) for each analyte in hair, serum and amniotic samples.

Table 2. Demographic characteristics and personal habits of participating pregnant women

Table 3. Medical history of past pregnancies and deliveries

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Table 5. Descriptive statistics of HCB, specific DDTs, PCBs in hair, amniotic fluid and serum samples

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Table 7. Statistically different levels of DDDs and PCB28 congener according the smoking status of the pregnant women in amniotic and hair samples.

Table 8. Statistically significant detected levels of PCB101 congener in preterm compared to full-term gestation.

Table 1. Limits of determination (LOD) and quantification (LOQ) for each analyte in hair, serum and amniotic samples.

	HCB	o,p'-DDE	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT
Hair (pg/mg)							
LOD	0.104	0.190	0.635	0.825	4.544	6.093	-
LOQ	0.348	0.633	2.117	2.750	15.147	20.309	-
Serum (ng/ml)							
LOD	0.012	0.008	0.014	0.028	0.248	0.268	-
LOQ	0.04	0.03	0.05	0.09	0.83	0.89	-
Amniotic fluid (ng/ml)							
LOD	0.002	0.001	0.001	0.001	0.006	0.002	0.011
LOQ	0.006	0.002	0.002	0.004	0.018	0.007	0.036

	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180

Hair (pg/mg)							
LOD	0.101	0.098	0.485	0.612	1.250	1.792	1.06
LOQ	0.337	0.328	1.618	2.039	4.166	5.972	3.54
Serum (ng/ml)							
LOD	0.005	0.005	0.029	0.011	0.062	0.016	0.086
LOQ	0.02	0.02	0.10	0.04	0.21	0.05	0.29
Amniotic fluid (ng/ml)							
LOD	0.000	0.001	0.004	0.003	0.003	0.005	0.011
LOQ	0.001	0.003	0.014	0.011	0.011	0.018	0.038

Table 2. Demographic characteristics and personal habits of participating pregnant women

		Before pregnancy		During pregnancy	
		n	**%***	**n**	**%***
Nationality	**Greek**	103	85.8		
	Other	17	14.2		
Age groups	**≤ 35**	17	14.9		
	36 - 39	70	61.4		
	40+	27	23.7		
Working status	**No**	38	35.2		
	Yes	70	64.8		
Smoking (cigarettes)	**No**	83	69.2	22	62.9
	1-10	28	23.3	13	37.1
	>10	9	7.5		
Alcohol (drinks/day)	**No**	108	89.2	10	76.9
	1-2	13	10.8	3	23.1
Drugs	**No**	119	99.2	1	100.0
	Yes	1	0.8	0	0.0

***** *Percentages were expressed on valid cases*

Table 3. Medical history of past pregnanciesand deliveries

Number of			**0**	**1**	**2**	**3**	**4**	**5**
Pregnancies		**n**	64	27	18	8	2	1
		%	53.3	22.5	15.0	6.7	1.7	0.8
Deliveries	**Normal**	**n**	81	26	9	3	1	
		%	67.5	21.7	7.5	2.5	0.8	
Caesarean		**n**	84	29	6	1		
		%	70.0	24.1	5.0	0.8		
Problematic		**n**	93	17	7	2	1	
		%	77.5	14.2	5.8	1.6	0.8	

Table 4. The % frequencies values of detection of each analyte in hair, amniotic fluid and serum samples.

	PCB28	**PCB52**	**PCB101**	**PCB118**	**PCB138**	**PCB153**	**PCB180**
hair	6.7	5.8	12.5	ND*	1.9	ND	7.7
amniotic fluid	8.5	2.5	16.9	0.8	0.8	ND	1.7
serum	2.4	1.2	10.8	1.2	3.6	3.6	2.4
	HCB	**o,p'-DDE**	**p,p'-DDE**	**o,p'-DDD**	**p,p'- DDD**	**o,p'-DDT**	**p,p'-DDT**
hair	18.3	9.6	77.9	4.8	14.4	ND	ND
amniotic fluid	5.1	23.7	94.9	4.2	46.6	0.8	ND
serum	22.9	4.8	85.5	1.2	ND	ND	ND

** Not Detected*

Table 5. Descriptive statistics of HCB, specific DDTs, PCBs in hair, amniotic fluid and serum samples

				Quartiles			
	Minimum	**Mean**	**±SD**	**1st**	**Median**	**3rd**	**Maximum**
	Hair samples (pg/mg)						
o,p'-DDE	<2.4	2.3	9.6	<2.4	<2.4	<2.4	107.7
p,p'-DDE	<3.0	5.9	7.6	<3.0	4.5	7.0	62.2
DDEs	<5.4	8.2	14.7	2.7	6.2	8.2	150.2
o,p'-DDD	<3.0	1.8	2.6	<3.0	<3.0	<3.0	23.8
p,p'-DDD	<34.0	27.2	58.5	<34.0	<34.0	<34.0	589.1
DDDs	2.9	28.6	58.2	18.4	18.4	18.4	590.5
DDTs*	5.2	36.5	61.3	21.2	24.8	27.8	604.6
PCB28	<0.4	0.4	1.6	<0.4	<0.4	<0.4	17.7
PCB52	<2.2	1.4	1.8	<2.2	<2.2	<2.2	15.3
PCB101	<2.0	1.3	1.2	<2.0	<2.0	<2.0	7.2
PCB180	<7.4	3.8	0.3	<7.4	<7.4	<7.4	7.5
PCBs	6.0	6.9	2.7	6.0	6.0	6.0	23.5
HCB	<1.6	1.0	0.7	<1.6	<1.6	<1.6	7.5
	Serum (ng/ml)						
o,p'-DDE	<0.026	0.015	0.015	<0.026	<0.026	<0.026	0.172
p,p'-DDE	<0.046	0.077	0.104	<0.046	0.050	0.085	0.936
DDEs	0.036	0.092	0.107	0.036	0.036	0.099	0.949
PCB28	<0.016	0.008	0.002	<0.016	<0.016	<0.016	0.027
PCB52	<0.018	0.009	0.003	<0.018	<0.018	<0.018	0.045
PCB101	<0.096	0.067	0.078	<0.096	<0.096	<0.096	0.600
PCB 118	<0.038	0.023	0.042	<0.038	<0.038	<0.038	0.484
PCB138	<0.206	0.112	0.062	<0.206	<0.206	<0.206	0.659
PCB153	<0.052	0.040	0.105	<0.052	<0.052	<0.052	1.071
PCB180	0.143	0.171	0.296	0.143	0.143	0.143	3.405
PCBs	0.355	0.429	0.459	0.355	0.355	0.355	5.273
HCB	<0.042	0.029	0.026	<0.042	<0.042	<0.042	0.189
	Amniotic fluid (ng/ml)						

o,p'-DDE	<0.002	0.004	0.018	<0.002	<0.002	<0.002	0.195
p,p'-DDE	0.001	0.057	0.254	0.009	0.021	0.040	2.796
DDEs	0.002	0.060	0.271	0.01	0.024	0.042	2.991
o,p'-DDD	0.002	0.003	0.003	0.002	0.002	0.002	0.020
p,p'-DDD	0.0071	0.107	0.641	0.01	0.010	0.052	6.8961
DDDs	0.0091	0.109	0.641	0.012	0.012	0.058	6.8981
DDTs	0.011	0.170	0.905	0.025	0.052	0.103	9.8891
PCB28	<0.0005	0.005	0.019	<0.0005	<0.0005	<0.0005	0.090
PCB52	<0.0005	0.002	0.008	<0.0005	<0.0005	<0.0005	0.080
PCB101	<0.014	0.019	0.029	<0.014	<0.014	<0.014	0.110
PCBs	0.009	0.027	0.041	0.009	0.009	0.009	0.181
HCB	<0.006	0.013	0.068	<0.006	<0.006	<0.006	0.715

Note: < number refers to non-detected levels (<LOD)

*: DDTs=sum of o,p'and p,p'-DDE, o,p'and p,p'-DDD and o,p'and p,p'-DDT

Table 6. Spearman's correlation estimates of HCB, PCB28, PCB52, PCB101, o,p'-DDE and p,p'-DDE between the amniotic, hair and serum samples

			Spearman's (rs)	**p**
HCB	**hair**	**amniotic**	0.337	**<0.001**
	hair	**serum**	-0.076	0.406
	amniotic	**serum**	0.117	0.198
PCB28	**hair**	**amniotic**	-0.074	0.420
	hair	**serum**	-0.032	0.728
	amniotic	**serum**	-0.039	0.673
PCB52	**hair**	**amniotic**	-0.036	0.696
	hair	**serum**	-0.021	0.821
	amniotic	**serum**	-0.014	0.875
PCB101	**hair**	**amniotic**	0.664	**<0.001**
	hair	**serum**	0.208	**0.022**
	amniotic	**serum**	0.473	**<0.001**
DDEs	**hair**	**amniotic**	0.063	0.489
	hair	**serum**	-0.063	0.490
	amniotic	**serum**	-0.009	0.924
o,p'-DDE	**hair**	**amniotic**	-0.094	0.303
	hair	**serum**	0.104	0.252
	amniotic	**serum**	-0.097	0.287
p,p'-DDE	**hair**	**amniotic**	0.107	0.243
	hair	**serum**	-0.054	0.554
	amniotic	**serum**	0.013	0.890

Table 7. Statistically different levels of DDDs and PCB28 congener according the smoking status of the pregnant women in amniotic and hair samples.

		Smoking status						
		No (n=83)			**Yes (n=37)**			
		Mean	**SD**	**Median**	**Mean**	**SD**	**Median**	**p**
Hair	**o,p'-DDD**	1.5	0.2	1.5	2.6	4.7	1.5	0.034**
	p,p'-DDD	19.7	10.8	17.0	44.6	104.0	17.0	0.033**
	DDDs	20.9	10.9	18.4	47.2	103.9	18.4	0.036*
	DDTs^{+}	28.4	13.8	24.8	55.9	107.8	24.5	0.023**
Amniotic	**PCB28**	0.003	0.014	0.001	0.011	0.026	0.001	0.036*

*estimated using independend * Mann-Whitney ** independent samples t-test*

$^{+}$: DDTs=sum of o,p'and p,p'-DDE, o,p'and p,p'-DDD and o,p'and p,p'-DDT

Table 8. Statistically significant detected levels of PCB101 congener in preterm compared to full-term gestation.

	Gestation weeks						
	Preterm (<=36 weeks) (n=9)			**Full (>36 weeks) (n=79)**			
PCB101 levels	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**p**
Hair (pg/mg)	2.31	2.66	0.97	1.26	1.00	0.97	0.020**
Amniotic fluid (ng/ml)	0.04	0.04	0.01	0.02	0.03	0.01	0.013*
Serum (ng/ml)	0.12	0.18	0.05	0.06	0.05	0.05	0.025*

estimated using independend * Mann-Whitney ** independent samples t-test