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Placental concentrations of xenoestrogenic organochlorine pesticides and polychlorinated biphenyls and assessment of their xenoestrogenicity in the PA-MAMI mother-child cohort

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ABSTRACT

Background: Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCB), they have contributed to the exposure of women to persistent organic pollutants (POPs). These compounds can cross the placental barrier and interfere with the hormonal system of newborns.
Aim: To determine concentrations of OCPs and PCBs and their xenoestrogenic activity in placentas of women from the PA-MAMI cohort of Panama.
Methods: Thirty-nine placenta samples from women in the Azuero peninsula (Panama) were analyzed. Five OCPs [p-p'-dichlorodiphenyldichloroethylene (*p-p'*-DDE), beta-hexachlorohexane (β-HCH), γ-hexachlorohexane (lindane), hexachlorobenzene (HCB) and mirex] and three PCB congeners (PCB-138, PCB-153 and PCB-180) were quantified in placenta extracts. The xenoestrogenic activity of extracts was assessed with the E-Screen bioassay to estimate the total effective xenoestrogen burden (TEXB).
Results: All placental samples were positive for at least three POP residues and >70% for at least six. The frequencies of quantified OCPs ranged from 100% for *p,p'*-DDE and HCB to 30.8% for β-HCH. The highest median concentration was for lindane (380.0 pg/g placenta), followed by *p,p'*-DDE (280.0 pg/g placenta), and HCB (90.0 pg/g placenta). Exposure to *p,p'*-DDE was associated with greater meat consumption, suggesting that animal fat is a major source of exposure to DDT metabolites. The frequency of detected PCBs ranged between 70 and 90%; the highest median concentration was for PCB 138 (17.0 pg/g placenta), followed by PCB 153 (16.0 pg/g placenta). All placentas were positive in the estrogenicity bioassay with a median TEXB-α of 0.91 pM Eeq/g of placenta. Exposure to lindane was positively associated with the xenoestrogenicity of TEXB- α, whereas this association was negative in the case of exposure to PCB 153.
Conclusions: To our best knowledge, this study contributes the first evidence on the presence of POPs and xenoestrogenic burden in placentas from Latin-American women. Given concerns about the consequences of

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prenatal exposure to these compounds on children's health, preventive measures are highly recommended to eliminate or minimize the risk of OCP exposure during pregnancy.

1. Introduction

Persistent Organic Pollutants (POPs) are a group of chemicals that includes the organochlorine pesticides (OCPs) p-p'-dichlorodiphenyldichloroethylene (p-p'-DDE), beta-hexachlorohexane (β-HCH), γ-hexachlorohexane (lindane), hexachlorobenzene (HCB), and mirex, and polychlorinated biphenyls (PCBs). Before awareness of their toxic, persistence and bioaccumulative properties, they were used for agriculture, vector control, and industrial processes. Some POPs in the environment are known to bind to estrogen receptors and induce a physiological response equivalent to the response to the hormone in various organs and tissues. Since the 1970s, a growing list of chemicals has been included in The Stockholm Convention of Persistent Organic Pollutants (amendments made to Directive 76/769/EEC for PCBs and Directive 79/117/EEC available for OCPs), established to prevent the production and discharge of POPs into the environment (76/769/EEC; 79/117/EEC; Stockholm Declaration on the Human Environment, 1972). Nevertheless, the utilization of OCPs continues or only recently ceased in countries affected by malaria and other vector-linked diseases and having extensive agricultural production (PNUMA. VI DESAFIO PARA LA COMUNIDAD MUNDIAL, 2000; WHO Guidelines for malaria, 2022).

Public health surveillance of pesticide exposure and associated diseases is nonexistent or extremely limited in the Latin-American region (Zúñiga-Venegas et al., 2022). Hence, little is known about human exposure to pesticides in Panama, despite their widespread application for public health vector control and agricultural purposes in this country (Tasón and Barba, 2014). After experimental studies on the utility of dichlorodiphenyltrichloroethane (DDT) spraying against mosquitoes in the Panamá Canal Zone (Gahan et al., 1945; Stage, 1946), this pesticide was used to combat malaria in the villages of Guayabalito and Santa Rosa on the banks of the Chagres River, in a pioneering work by the Gorgas Laboratory (Trapido, 1946). An extensive intra-domiciliary spraying program in the main urban and rural centers was then started in 1947 (Trapido, 1952). In 1955, the World Health Organization (WHO) launched a global DDT malaria control program in which 75 countries participated, including Panama (Pérez-Maldonado et al., 2010). Of special interest is the massive and indiscriminate use of OCPs, largely DDT, for sanitary and agricultural purposes in the Panama Canal Zone and in banana plantations in Bocas del Toro/Chiriquí regions (Olson and Tornoe, 2021). Moreover, obsolete pesticides and contaminated containers were abandoned throughout the country after their prohibition, and their storage/disposal and the cleaning up of sites remained a pending environmental challenge. In 2007, the National Pesticide Inventory identified 16 sites that were contaminated with pesticides and other POPs, estimating a total of at least 6.75 tons of OCPs. In the same year, a survey of electric transformers in Central America reported the presence of around 1000 tons of PCB-containing equipment in Panama (Matamoros EA, 2007). In 2020, the Panamanian Ministry of Health (MINSA) started a program to remove more than 325 tons of obsolete pesticides and contaminated soil from circulation (Carranza R and Jiménez AM, 2020), including 130 tons of PCBs taken from equipment or oils and exported for disposal. However, although hundreds of thousands of Panamanians may have been exposed to highly toxic substances in a systematic and prolonged manner, no scientific research has been undertaken on the impact of this exposure on health and the environment (Olson and Tornoe, 2021).

Extensive spraying has led to frequent reports of pesticide residues in the environment, food, and humans (Environmental Hygiene Agence, 1976; Espinosa González and Thiel, 1988; Risebrough et al., 1967). Four decades ago, Espinosa González and Thiel (1987) described the presence of DDT residues in human breast milk in the range of 0.1–7.3 ppm DDT, either as *p,p'*-DDT or its metabolite *p,p'*-DDE. HCB, among other pesticide residues, was also found in composite milk samples collected throughout the USA and Panamá (Trotter and Dickerson, 1993). It has been reported that exposure of the Panamanian general public to OCPs and PCBs largely derives from the intake of contaminated food, although environmental, occupational, and other sources of exposure cannot be ruled out (Garcerán and Castillo, 2019). Lifetime maternal bioaccumulation of these compounds in fat tissue may be a major source of offspring exposure during both pregnancy and breastfeeding. Prenatal exposure to OCPs and PCBs is a critical health issue, because it has been related to fetal vulnerability and lower immune and metabolic capabilities to detoxify these pollutants. Migration of OCPs and PCBs from mother to fetus has been evidenced by their detection in maternal blood, umbilical cord blood, placentas, and newborn tissues (Björvang et al., 2021). Jeong et al. assessed the usefulness of the placenta as a non-invasive matrix for biomonitoring prenatal exposure to POPs in comparison to maternal blood, cord blood, and meconium (Jeong YLee et al., 2018). In addition, it has been demonstrated that the combined effect of complex mixtures of xenoestrogenic OCPs and PCBs can be determined in placentas by estimating the total effective xenoestrogen burden (TEXB), a biomarker of effect for persistent environmental organohalogenated estrogens (Lopez-Espinosa et al., 2009; Rodríguez-Carrillo et al., 2021).

Given the scant research to date on the exposure of pregnant Panamanian women to POPs, the objective of this study was to assess the exposure to OCPs and PCBs of Panamanian mother–infant pairs from the PA-MAMI cohort, analyzing their residues in placentas and estimating the TEXB from the combined effect of xenoestrogens in addition to correlating the measured concentrations of POP in the placenta versus TEXB in the placenta.

2. Material and methods

2.1. Study population and sample collection

The study was conducted between 2016 and 2017 and enrolled 60 women, 30 recruited at the Cecilio Augusto Castillero Hospital (Herrera Province) and 30 at the Joaquín Pablo Franco Sayas Hospital (Los Santos Province). Study inclusion criteria were female sex, age between 20 and 30 years, primiparity, birth and residence in Azuero peninsula (Herrera and Los Santos provinces), normal pregnancy, and natural delivery or cesarean section. Exclusion criteria were positive serology for human immunodeficiency virus (HIV), risk factors for sexual transmitted diseases, the presence of chronic or degenerative disease, temporary residence in the province, high-risk pregnancy, and complicated delivery. Women meeting eligibility criteria were invited to participate in the study. After signing their informed consent to participation in the study, volunteers completed an *ad hoc* questionnaire on sociodemographic and reproductive characteristics, lifestyle habits, and diet. The study was approved by the ethics committees of the University of Panama and the participating hospitals.

The placentas were stored at −80 °C in the hospital where the sample was collected. Next, they were transported to the University of Panama in dry ice, homogenized, and stored at −80 °C. The sample homogenate was sent to the laboratory of the Instituto de Investigación Biosanitaria of Granada (ibs. GRANADA) in Spain, maintaining the cold chain throughout the transfer. Samples were stored there at −80 °C until extraction and analysis. Tubes used to collect samples were pre-tested to ensure that they did not contain or leach any target compound.

2.2. Chemicals and reagents

All reagents were analytical grade unless otherwise specified. p-p'-DDE, lindane, HCB, β-HCH, mirex, PCBs (138, 153 and 180), 4,4′-DDT-D_8 (internal standard for p-p'-DDE, lindane, HCB, β-HCH and mirex) and 4,4′-Dichlorobenzophenone (internal standard) for PCBs, were purchased from Sigma-Aldrich (Madrid, Spain). Solvents for extraction procedures, acetonitrile, and trichloromethane, were purchased from Sigma-Aldrich, and hexane and sodium chloride (NaCl) were supplied by Honeywell (North Carolina, USA). Water (18:2 MX cm) was purified using an in-house Milli-Q® system (Millipore). For chemical analyses, stock standard solutions (100 mg/L) of each compound were prepared in acetonitrile and stored at 4 °C in the dark. Solutions remained stable for at least 2 months. Working standards were prepared immediately before use by dilution with pure acetonitrile. Phosphate buffer saline (PBS), calcium chloride, and magnesium sulfate were purchased from Sigma-Aldrich (Madrid, Spain). Collagenase type-I from *Clostridium histolyticum* was supplied by Sigma-Aldrich. The enzymatic solution was prepared immediately before use by dissolving 1 mg of enzyme powder in 10 mL of PBS medium (0.01 M, pH = 7.4) with the presence of 5 mM Ca^{2+}.

For *in vitro* cell assays (E-Screen), reference standards for 17β-estradiol (E_2), ICI 182780 (henceforth ICI), sulforhodamine B (SRB), and trichloroacetic acid were obtained from Sigma-Aldrich (St Louis, MO). Stock solutions (10 mM) of E_2 and ICI were prepared in ethanol, and successive dilutions were performed in culture medium. Stock solutions were kept at −20 °C, and dilution series were freshly prepared before each experiment. The culture medium and fetal bovine serum (FBS) were supplied by Gibco (Invitrogen, Barcelona, Spain) and all cell culture plastics by Falcon (VWR International Eurolab, Barcelona, Spain).

2.3. Sample extraction and treatment

The extraction protocol was adapted from Vela-Soria et al. (2021). Briefly, placenta samples were completely thawed at room temperature and analyzed in duplicate, adding 0.10 g of collagenase enzyme solution (calcium) to each aliquot (0.50 g). After mixing, 10 μL of a solution in acetonitrile of internal standards (1 mg/L) was added, and the sample was incubated at 37 °C for 4 h, followed by the addition of 5 mL acetonitrile saturated with hexane and 0.40 g NaCl and the immediate manual shaking of this mixture for 4 min. The mixture was centrifuged at 11,357 g for 10 min at 4 °C, and the supernatant was transferred to a glass tube and evaporated under a nitrogen stream to a final volume of 1 mL. The residue was vortexed for 6 s and then transferred to a conical glass tube for dilution with 10.0 mL of 10% NaCl aqueous solution (w/v) at pH 2. After using a syringe to rapidly inject 250 μL of trichloromethane into the aqueous sample, the mixture was centrifuged at 3226 g for 10 min at 4 °C. The entire trichloromethane sediment phase (250 μL) was then transferred to a clean glass vial with insert being prepared for analysis by gas chromatography/tandem mass spectrometry (GC-MS/MS).

2.4. Instrumentation and GC-MS/MS conditions

GC–MS/MS analysis was performed using an Agilent 7890 GC (Agilent Technologies, Palo Alto, CA, USA) with split-splitless inlet and 7693 ALS autosampler. The detector was an Agilent 7000D triple quadrupole mass spectrometer with inert electron-impact ion source, operated in SRM mode. Electron impact (EI) ionization was set at 70 eV. Analytes were separated in a HP–5MS-UI capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from Agilent. The injection port of the GC was set at 250 °C. Samples were automatically injected in splitless-injection mode, using an Ultra Inert Liner 5190–3163 from Agilent. The injection volume was 2 μL, with a 5181–3354 10 μL Syringe supplied by Agilent. The flow of helium carrier gas (99.999% purity) was maintained at 1.2 mL min-1. The initial oven temperature was set at 70 °C and held for 2.0 min, then ramped to 120 °C at 25 °C min-1, held for 0.5 min, to 250 °C at 10 °C min-1 and, finally, to 280 °C at 120 °C min-1, held for 4 min (total time of 22 min). Single reaction monitoring (SRM) mode was performed on the spectrometer, reporting two MS/MS transitions for each analyte, the first for quantification and the second for confirmation. Temperatures of the transfer line, ion source, and quadrupoles were 280 °C, 280 °C, and 150 °C, respectively. The mass spectrometer was auto-tuned weekly.

pH measurements were obtained by means of a Crison 200 digital pH-meter. Enzymatic treatment was conducted with an MS-100 Thermo Shaker (Optimum Ivymen System, Cornecta, Spain). Statgraphics Plus version 5.0 (Manugistics, Inc., MD, USA) was used for statistical and regression analyses.

Limits of detection (LODs) were 0.003 ng/g for PCB 138, PCB 153, and PCB 180; 0.01 ng/g for HCB and mirex; 0.02 ng/g for p-p′-DDE; and 0.05 ng/g for lindane and HCH.

2.5. Quality control for analytical determinations

Background contamination was controlled by analyzing procedural blanks every 10 injections (milliQ water was used as sample for this purpose). No quantifiable concentrations of target analytes were detected. In addition, a pool of placental tissue spiked at 0.2,10 and 20 ng $g^{-1}$was analyzed in triplicate every 10 injections.

2.6. E-screen bioassay: hormone-like activity assessment

This study used MCF7 BUS human breast cancer cells (Soule et al., 1973) gifted by C. Sonnenschein (Tufts University, Boston, MA). The cell line was cultured as previously described (Molina-Molina et al., 2014). Briefly, MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with phenol red supplemented with 10% FBS (seeding medium). Given the hormonal activity of phenol red and FBS, experiments were performed in a test culture medium of phenol red-free DMEM supplemented with 10% dextran-coated charcoal-FBS (10% DCC-FBS) for MCF-7 cells in a 5% carbon dioxide (CO_2) humidified atmosphere at 37 °C.

The E-Screen bioassay was performed as previously reported (Molina-Molina et al., 2019) with some modifications. Briefly, MCF-7 cells were trypsinized and plated in 96-well culture plates at initial concentrations of 4×10^3 cells/well. One day later, the seeding medium was removed and replaced with test culture medium. For agonistic assays, dry extracts of α- and β-fractions were resuspended in 1 mL of test culture medium, vigorously shaken, left at rest for 30 min, filtered through a 0.22 μm filter (PALL® Acrodisc® Syringe Filters with Supor® Membrane, 13 mm), and then tested (200 μL per well) on MCF-7 cells at dilutions of 1:1 to 1:10. Each experiment included a dose-response curve (0.1–1000 pM) for E_2, a negative control of cells treated solely with hormone-free medium (test culture medium), and a solvent control (0.1% ethanol in test culture medium). The bioassay was ended on day 6 (late exponential phase) by removing the media from wells, fixing the cells with trichloroacetic acid (10% w/v at 4 °C, 30 min), and staining them with SRB [0.4% w/v in acetic acid (1% v/v), room temperature, 30 min]. Finally, the bound dye was solubilized using tris(hydroxymethyl)aminomethane (10 mM, room temperature, 30 min, pH 10.4) and the absorbance was read at 492 nm. Next, the ratio of treated to hormone-free (negative control) SRB-stained cells was calculated for each dilution. Tests were done in triplicate, and results were expressed as proliferative effect (PE) [MCF-7 cell proliferation (fold over control)]. Because the PE only yields information on the effect of the extract in the E-Screen bioassay, it was transformed into E_2 equivalent (Eeq) units related to 1 g of placenta by reading from E_2 dose-response curves) to quantify the estrogenicity of the original placenta samples. In this way, the PE of each extract was referred to the maximal PE obtained with E_2 and transformed into E_2eq. The E_2eq values for each placenta extract were recorded as the mean of the three values for the different dilutions.

Values obtained were corrected by the dilution factor and reported as E_2eq/g of the original placenta samples.

We followed a standardized method to assess the total effective xenoestrogen burden (TEXB) in human placentas by the extraction and separation by high-performance liquid chromatography of two fractions apha and beta that effectively separate endogenous steroid hormones from persistent organic pollutants (Lopez Espinosa et al., 2009). In the TEXB-α-fraction mainly elutes lipophilic and persistent organic pollutants such as PCBs and OCPs (lindane, HCB, DDT and DDE), among other organohalogenated compounds. The estrogenicity in the E-Screen bioassay of the beta fraction (TEXB-β) is due to less lipophilic xenoestrogens and endogenous hormones.

2.7. Lipid determinations

Total lipid content was quantified gravimetrically using a previously reported method (Lopez-Espinosa et al., 2007), dissolving 200 mg of placenta homogenate in 5 mL of chloroform:methanol:hydrochloric acid (20:10:0.1) (v/v/v). After repeating the process, 10 mL of 0.1 N HCl were added and centrifuged at 11,357 g for 10 min. The organic phase was then collected. After drying under a nitrogen stream, extracts were weighed, expressing the total lipid in gram of lipid per gram of placenta. Mean lipid content of placentas was determined at 1.11 ± 0.21% g of lipid per gram placental wet weight and was used to calculate concentrations of the more lipophilic OCPs and PCBs on a lipid content basis.

2.8. Independent variables

The *ad hoc* questionnaire was used to gather data from each participant on the following independent variables: age (years), menarche age (years), civil status (single/married), place of residence (Herrera/Los Santos), schooling (university/non-university), occupation (worker at home/worker outside home), working for ≥3 months in previous three years (yes/no), occupation in previous three years (worker at home/worker outside home), weight before pregnancy, weight after delivery, height, newborn weight, use of contraceptives (yes/no), presence of composite filling (yes/no), chemical exposure (yes/no), smoker (yes/no), passive smoker (yes/no), and the frequencies of passive smoking (not exposed/<30min/day), cleaning product utilization (never/5–10 h/week), contact with paper or textiles (never/5–10 h/week), and contact with dust (never/1 h/day).

The questionnaire also included a dietary section on the consumption frequency of the main food groups: fish (lean and fatty), selfish (fresh and frozen), meat, fruit (fresh, fresh juice, processed juice, processed, and dehydrated), vegetables (green salad, fresh, and frozen), cheese (fresh and aged), yogurt, egg, tofu, soya, pulse, canned grain, organic food, chocolate, viscera, alcohol-free beverage (water, coffee, chocolate bar, liquid chocolate, tea, and soft drink), alcoholic beverage (beer, wine, and alcoholic drinks), and alcohol during pregnancy.

2.9. Statistical analysis

Detection frequencies in placenta samples were calculated for *p-p'*-DDE, lindane, HCB, β-HCH, mirex, and PCBs (138, 153 and 180). Concentrations below the LOD were assigned a value of LOD/$\sqrt{2}$. OCP and PCB concentrations were summarized as means with standard deviations (SD), medians, 25th, 75th, and 95th percentiles, and minimum/maximum values. OCP and PCB, which were non-normally distributed (Kolmogorov-Smirnoff test), were log-transformed to minimize the influence of extreme values. Spearman's correlation test was used to evaluate relationships between the studied compounds analyzed. Bivariate and multiple regression analyzes were performed to identify predictors of OCP and PCB concentrations in placenta samples, applying both forward and backward stepwise procedures to ensure model robustness. Linear regression models were constructed for chemicals detected in ≥75% of samples (i.e., *p-p'*-DDE, lindane, HCB, PCB 138, PCB 153, and PCB 180) and logistic regression models for those detected in <75% of samples (i.e., β-HCH and mirex). POP concentrations were assigned to results of E-Screen bioassay alfa and beta-fraction using linear regression models. Statistical significance was set at $p < 0.05$. SPSS v.23.0 (IBM, Armonk NY, USA) was used for statistical analyses.

3. Results

3.1. Characteristics of study participants

Table 1 exhibits the characteristics of participants. The mean (±SD) age was 21.9 (±3.17) years; 66.7% of participants lived in Herrera Province, 64.1% were homeworkers, 56.4% had not completed university education; 66.7% did not use contraceptives pre-pregnancy, 74.4% had no composite fillings, and all were non-smokers. Their dietary habits are summarized in Supplementary Table S1.

3.2. Concentrations of OCP and PCB in placenta samples

Table 2 displays the concentrations of OCPs and PCBs in placenta samples. Residues of at least three OCPs and/or PCBs were detected in

Table 1
Characteristics antrophometrich and sociodemographic of the study population (N = 39).

	N(%)		N(%)
Age = years	21.90 ± 3.17	**Use of contraceptives**	
Menarche age = years	11.3 ± 3.66	*No*	26 (66.7)
Weight before pregnancy	58.88 ± 14.34	*Yes*	13 (33.3)
Weight before delivery	72.16 ± 15.90	**Composite filling**	
Height = m	1.59 ± 6.84	*No*	29 (74.4)
Newborn weight = Kg	3.24 ± 0.45	*Yes*	10 (25.6)
Civil status		**Smoker**	
Single	5 (12.8)	*No*	39 (100.0)
Married	34 (87.2)	*Yes*	0 (0.0)
Residence		**Passive smoker**	
Herrera	26 (66.7)	*No*	17 (43.6)
Los Santos	13 (33.3)	*Yes*	22 (56.4)
Occupational class		**Frequency of passive smoke**	
Work at home	25 (64.1)	*Not exposed*	17 (43.6)
Work outside home	14 (35.9)	<30 min/*day*	22 (56.4)
Work ≥ 3 months in the last 3 years		**Frequency cleaning products utilization**	
No	21 (53.8)	*Never*	23 (59.0)
Yes	18 (46.2)	*5-10 h/week*	16 (41.0)
Occupational class in the last 3 years		**Frequency contact with paper, textile**	
Work at home	14 (35.9)	*Never*	35 (89.7)
Work outside home	25 (64.1)	*5-10 h/week*	4 (10.3)
Educational level		**Frequency contact with dust**	
Non-universitary	22 (56.4)	*Never*	27 (69.2)
Universitary	17 (43.6)	*1 h/day*	12 (30.8)
Chemical exposure (work or home)			
No	35 (89.7)		
Yes	4 (10.3)		

Table 2
Concentrations of organochlorine pesticides in human placenta (pg/g placenta) (N = 39).

	N > LOD (%)	Mean	SD	Min.	Percentiles				Max
					25	50	75	95	
p-p'-DDE	39 (100)	595.4	930.6	90.0	179.9	280.8	580.0	2130.9	5520.7
HCB	39 (100)	110.0	128.7	<LOD	60.1	90.0	120.4	230.7	820.9
β-HCH	12 (30.8)	53.1	33.9	<LOD	<LOD	<LOD	91.2	95.0	100.0
Lindane	35 (89.7)	413.6	342.6	<LOD	200.1	380.0	480.4	1309.9	1602.3
Mirex	18 (46.2)	15.7	13.5	<LOD	<LOD	<LOD	19.9	50.2	70.0
PCB 138	35 (89.7)	18.8	15.8	<LOD	<LOD	17.0	24.7	66.4	67.0
PCB 153	34 (87.2)	18.7	12.7	<LOD	12.0	16.1	26.8	36.3	65.3
PCB 180	28 (71.8)	25.5	47.6	<LOD	<LOD	<LOD	<LOD	13.1	240.0
Estrogenicity*									
Placenta TEXBα	31 (79.5)	1.24	1.13	0.00	0.31	0.91	2.01	3.77	3.82
Placenta TEXBβ	39 (100)	55.67	95.25	1.05	4.82	10.81	64.15	353.92	358.62

100% of samples and at least six in 70% (Fig. 1). All compounds were detected in more than 75% of samples except for mirex and β-HCH, which were detected in 46.2 and 30.8% of samples, respectively. Among OCPs, *p,p'*-DDE and HCB were present in 100% of samples, with median concentrations of 280.8 pg/g placenta (mean of 56.70 ng/g of lipid (lw)) and 90.0 pg/g placenta (mean of 11.6 ng/g lw), respectively. Lindane was detected in 89.7% of samples, with a median concentration of 380.0 pg/g placenta (mean 37.6 ng/g lw). Mirex and β-HCH were detected in 46.2% and 30.8% of samples, respectively, with median concentrations < LOD (means of 5.06 and 1.49 ng/g lw, respectively). The detection frequency of PCBs ranged from 89.7% for PCB 138, with a median concentration of 17.0 pg/g placenta (mean 1.63 ng/g lw), to 71.8% for PCB 153, with a median concentration of 16.0 pg/g (mean 1.7 ng/g lw). The median concentration of PCB 180 was below the LOD (mean 2.83 g/g lw).

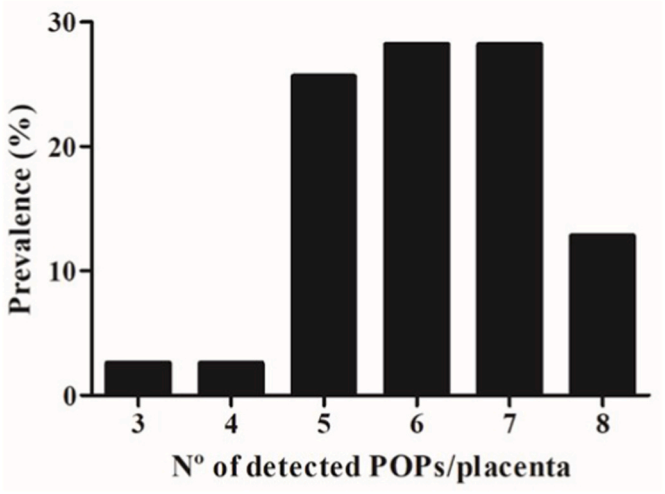

Fig. 1. Prevalence of OCPs and PCB_s in placenta samples.

Significant positive correlations were found between mirex and PCB-153 (Spearman's rho = 0.34, p < 0.050), between isomers β-HCH and lindane (rho = 0.497; p < 0.010), and between PCB 138 and PCB 153, PCB 138 and PCB 180, and PCB 153 and PCB 180 (Spearman's rho >0.50, p < 0.010) (Table 3).

3.3. Estrogenicity of placentas

Estrogenicity was detected in all placenta samples (Table 2). The median xenoestrogenicity of the alpha chromatographic fraction (TEBX-α), in which OCPs and PCBs are eluted, was 0.91 pM E_2eq/g of placenta, with a mean value of 1.24 pM E_2eq/g/g of placenta. The estrogenicity of the beta fraction (TEXB-β), in which less lipophilic xenoestrogens and endogenous hormones elute, had a much higher median value of 10.81 pM E_2eq/g of placenta (mean 55.67 pM E_2eq/g). Significant positive correlations were found between TEXB-α and lindane (Spearman's rho = 0.505, p < 0.05) and negative correlations between TEXB-α and PCB-153 (Spearman's rho = −0.413, p < 0.05) (Table 3).

3.4. Factors related to OCP and PCB concentrations in placenta samples

Bivariate associations between potential predictors of exposure and placenta concentrations of OCP and PCB are summarized in Supplementary Tables S2 and S3. Some statistically significant associations were observed between sociodemographic/dietary habits and placenta POP concentrations in multiple regression models (Table 4). Thus, a positive association was found between meat consumption and *p,p'*-DDE and negative associations between meat consumption and PCB-138 and PCB-153, although statistical significance was not reached for PCB-153 (p-value = 0.056). Chocolate intake was positively associated with HCB concentrations and coffee drinking with PCB-138 concentrations. Pulse intake was associated with lower concentrations of PCB-153 and PCB-180. Inverse associations were also found between tea drinking and lindane and between processed juice and β-HCH. Associations close to statistical significance were observed between fresh fruit juice intake and lower PCB-153 concentrations (p-value = 0.058).

Table 3
Intramatrix correlation coefficients between selected EDCs.

	p-p'-DDE	Lindane	HCB	β-HCH	MIREX	PCB 138	PCB 153	PCB 180	*Placenta TEXBα*	*Placenta TEXBβ*
p-p'-DDE	–	0.197	0.216	0.000	−0.029	−0.117	0.097	0.010	−0.710	−0.259
Lindane		–	0.194	**0.497****	0.243	0.192	0.045	0.310	**0.505***	**−0.572****
HCB			–	0.002	−0.065	−0.006	−0.311	−0.108	0.038	**−0.416***
β-HCH				–	0.180	0.295	0.210	0.053	0.275	**−0.619****
MIREX					–	0.263	**0.344***	0.189	0.093	0.091
PCB 138						–	**0.652****	**0.542****	−0.216	−0.084
PCB 153							–	**0.500****	**−0.413***	0.223
PCB 180								–	−0.235	−0.008
Placenta TEXBα									–	−0.130
Placenta TEXBβ										–

4-4′-DDE: p-p'-Dichlorodiphenyldichloroethylene; Lindane: γ-hexachlorohexane; HCB: Hexachlorobenzene; β-HCH: Beta-hexachlorohexane; *p < 0.05; **p < 0.01.

Table 4
Factors associated with placenta samples levels of organochlorine pesticides and polychlorinated biphenyls (N = 39). Multivariate regression analyses.

	p-p'-DDE (R2 = 0.100)*				Lindane (R2 = 0.109)*				HCB (R2 = 0.108)*				β-HCH (R2 = 0.157)**			
	exp(β)	95%	CI	p-value	exp(β)	95%	CI	p-value	exp(β)	95%	CI	p-value	OR	95%	CI	P-value
Frequency of meat consumption = 1–3 times/week [a]	1.79	0.00	1.16	0.050	–	–	–	–	–	–	–	–	–	–	–	–
Consumption of tea = Yes[b]	–	–	–	–	0.79	−0.46	−0.01	0.040	–	–	–	–	–	–	–	–
Consumption of chocolate bar = Yes[b]	–	–	–	–	–	–	–	–	1.1	0.00	0.18	0.041	–	–	–	–
Frequency of processed juice consumption = 4–6 times/week[c]	–	–	–	–	–	–	–	–	–	–	–	–	0.10	0.01	0.87	0.037
	PCB 138 (R2 = 0.256)*				**PCB 153 (R2 = 0.221)***				**PCB 180 (R2 = 0.106)***							
	exp(β)	**95%**	**CI**	**p-value**	**exp(β)**	**95%**	**CI**	**p-value**	**exp(β)**	**95%**	**CI**	**p-value**				
Residence = Los Santos [d]	0.99	−0.02	0.00	0.030	0.99	−0.02	0.00	0.028	–	–	–	–				
Frequency of meat consumption = 1–3 times/week[a]	0.99	−0.02	0.00	0.033	0.99	−0.02	0.00	0.056	–	–	–	–				
Consumption of chocolate bar = Yes[b]	0.99	−0.02	0.00	0.043	–	–	–	–	–	–	–	–				
Consumption of coffee = Yes [b]	1.01	0.00	0.02	0.019	–	–	–	–	–	–	–	–				
Frequency of pulse consumption = 4–6 times/week [c]	–	–	–	–	0.99	−0.02	0.00	0.018	0.97	−0.06	0.00	0.043				
Frequency of green salad consumption = 4–6 times/week [c]	–	–	–	–	0.99	−0.02	0.00	−0.015	–	–	–	–				
Frequency of fresh fruit juice consumption = 1–3 times/week [c]	–	–	–	–	0.99	−0.02	0.00	0.058	–	–	–	–				

4-4'-DDE: p-p'-Dichlorodiphenyldichloroethylene; Lindane: γ-hexachlorohexane; HCB: Hexachlorobenzene; β-HCH: Beta-hexachlorohexane; CI: confidence intervals; * = Linear regression analyses; ** = Logistic regression analyses a reference category = 1–3 times/moths b reference category = No c reference category = R/N d reference category = Herrera.

4. Discussion

To the best of our knowledge, this is the first study to assess OCP and PCB concentrations in placenta samples from South/Central American women and to quantify the hormonal activity generated by the combined effect of estrogenic organochlorinated compounds. Xenoestrogenic activity was widely observed in samples from Panamanian mothers and was associated with the simultaneous presence of multiple OCPs (*p,p'*-DDE, lindane, HCB) and/or PCBs (PCB-138, -153, −180) in more than two-thirds of samples.

Placentas have been recommended as a valuable and readily available source of human tissue for biomonitoring (Dodd-Butera et al., 2017), including assessment of the exposure of mother-child pairs. Nevertheless, studies on placenta exposure in South/Central America have more frequently used blood samples from the mother and umbilical cord for POP assessment (Calderón-Garcidueñas et al., 2022; Díaz et al., 2002; García Salcedo et al., 2022; Levario-Carrillo et al., 2001). In the present study, multiple OCPs and PCBs were detected in Panamanian placentas, in line with the findings of Björvang et al. (2021). They described HCB, *p,p'*-DDE, PCB-138, and PCB-153 as the POPs most frequently detected worldwide in placenta, maternal serum, and at much higher concentrations, unexpectedly, in fetal adipose tissue, liver, heart, lung and brain.

In general, it has been observed that DDT metabolites are the main contributors to placental OCP exposure (Fernández-Cruz et al., 2020; Nanes et al., 2014). However, despite the scientific interest in prenatal exposure to OCPs, studies have not followed a standard protocol for the selection of participants, timing of sampling, or expression of concentrations (i.e., wet/dry weight, lipid weight in placenta, plasma, serum, or blood), hampering comparison of their results (Müller et al., 2019). In addition, reported POP concentrations have varied widely as a function of geographic location, year of mother-child recruitment, and local utilization of OCP in agriculture and public health programs (Pérez-Maldonado et al., 2010). For instance, the Mexican Institute of Health and Labor reported that 189 tons of DDT were used in Panama from 1946 through 1971, much lower than the amount used in neighboring countries (Pérez-Maldonado et al., 2010). Regrettably, DDT continues to be utilized in around 25 countries for vector control against leishmaniosis or malaria under the Stockholm Convention on POP exemptions (POP, 2009). Unfortunately, however, information on mother-child exposure in these countries is only available from Saudi Arabia (Al-Saleh et al., 2012), India (Dewan et al., 2013; Tyagi et al., 2015), and South Africa (Channa et al., 2012). In the present investigation, *p,p'*-DDE was present in 100% of Panamanian placenta at the second highest median concentration (200 pg/g of placenta), comparable to previous findings in Europe (Bergonzi et al., 2009; Shen et al., 2008), Asia (Jeong YLee et al., 2018; Man et al., 2014; Ren et al., 2011; Zhang et al., 2018), and Africa (Müller et al., 2019). Highly similar values were reported by a study of placentas collected in 2011–2012 from several regions of the USA (Nanes et al., 2014), which detected p, p'-DDE in 100% of samples at a mean concentration of 205 pg/g of placenta. In contrast, much higher placental *p,p'*-DDE concentrations (from 17,700 pg/g to 263,000 pg/g of placenta) have been observed in countries with a history of substantial OCP utilization in agriculture and/or at hot-spot pollution sites (Galassi et al., 2008; Hura et al., 1999; Toichuev et al., 2018). Elevated concentrations (from 4000 to 61,000 pg/g of placenta) have also been reported in countries that had recently implemented vector control programs (Al-Saleh et al., 2012; Dewan et al., 2013; Tyagi et al., 2015). The proximity of mothers to OCP-treated sites would be of particular importance, and a South African study recorded maternal plasma *p,p'*-DDE concentrations of 22,613 pg/mL in recently treated provinces versus 179 pg/mL in those that had never received treatment (Channa et al., 2012).

Lindane was detected in almost 90% of the placenta samples. Information on HCH use in Panama is very limited. It was banned in 2009 but previously used in agriculture (Espinosa González and Thiel, 1988)

and to control scabies (Taplin et al., 1991). HCH isomer concentrations in Panamanian placentas are similar to those reported in China (Ren et al., 2011; Zhang et al., 2018) and Spain (Lopez-Espinosa et al., 2007) and higher than those described in Denmark/Finland (Shen et al., 2008), Croatia (Želježić et al., 2018), or Korea (Jeong YLee et al., 2018). Much higher concentrations of lindane and other HCH isomers have been found in areas with intensive application of this pesticide (Hura et al., 1999; Toichuev et al., 2018; Tyagi et al., 2015).

HCB was detected in all the present placenta samples at similar concentrations to those reported in previous studies in Europe (Bergonzi et al., 2009; Fernández-Cruz et al., 2017; Lopez-Espinosa et al., 2007; Vizcaino et al., 2014; Želježić et al., 2018) and Asia (Ando et al., 1985; Ren et al., 2011). Utilization of this OCP was prohibited in Panama in 1999. Finally, mirex, which was definitively banned in Panamá in 2011, was detected in almost half of the placentas. Mirex and its homologue chlordecone were used intensively for pineapple and banana cultivation in the past, but its utilization has been heavily restricted or prohibited in most countries (Faroon et al., 1995). Mirex concentrations in the present placenta samples are similar to those reported in Denmark, Finland, and Spain (Lopez-Espinosa et al., 2007; Puertas et al., 2010; Shen et al., 2008).

Despite being banned for a shorter period, PCBs showed lower placental concentrations in comparison to OCPs. Three PCB isomers were detected in more than 90% of the present placentas at comparable concentrations to those reported in the USA (Kappil et al., 2016) and Europe (Bergonzi et al., 2009; Fernández-Cruz et al., 2020; Reichrtová et al., 1999; Vizcaino et al., 2014). Nevertheless, caution should be taken in comparing PCB frequencies and concentrations among studies, given the different congeners considered and the variations in detection limits and data quality. In general, placental PCB concentrations appear to be higher in North America and Europe than in Asia. This may be related to the fact that the USA and Europe are responsible for around 80% of global PCB production (Breivik et al., 2002).

Data obtained from this Panamanian cohort can serve to identify sources of exposure to OCPs and PCBs and to establish reference values for chemical biomonitoring. For instance, p,p'-DDE was associated with the consumption of meat, which would be related to persistent OCPs accumulated in fatty tissues. An association was also found between HCB exposure and chocolate consumption, which is strikingly high in comparison to neighboring countries. This reflects a long history of cocoa production and, until their prohibition in the 1990s, the wide use of HCH and other OCPs for its cultivation (Bateman, 2015).

Placentas from Panamanian mother-child pairs can serve to monitor the effectiveness of governmental programs for controlling exposure to chemicals. In this way, human biomonitoring programs (e.g., NHANES in the USA and HBM4EU in Europe) have described several hundreds of man-made chemicals in human biological samples, both "legacy" chemicals and an increasing number of emerging chemicals. The present findings point to the need for assessment of the cumulative or cocktail effects on health of complex mixtures of chemicals (Demeneix and Slama, 2019; Drakvik et al., 2020). In this study, the TEXB was calculated by using a validated procedure to measure the combined *in vitro* estrogenicity of chemical mixtures extracted from placentas (Lopez-Espinosa et al., 2009). All placentas in the cohort elicited estrogenicity at similar values to those previously reported in Spanish birth cohort placentas (Lopez-Espinosa et al., 2009; Vilahur et al., 2013). Earlier studies demonstrated the absence of endogenous steroid hormones in the TEXB-α-fraction, which mainly elutes lipophilic and persistent organic pollutants such as PCBs and OCPs (lindane, HCB, DDT and DDE), among other organohalogenated compounds. The present study identified a positive association between the presence of lindane and the estrogenicity of TEXB-α that is in agreement with the abundance of this pesticide in the placentas of Panamanian mothers and the estrogenic effect attributed to the pesticide in vitro bioassays. Interestingly, the study has also identified an association, in this case a negative one, between PCB 153 exposure and the estrogenicity of TEXB-α, which could be explained by the anti-estrogenic effect shown by PCB 153 and other nondioxin-like PCBs (138 and 180) in MCF7 breast cancer cells used in the E-Screen (Bonefeld-Jørgensen et al., 2001). The placental TEXB-α burden has been associated with negative health outcomes in the offspring, including higher risks of urogenital malformations (cryptorchidism and/or hypospadias) (Fernandez et al., 2007), increased birth weight (Vilahur et al., 2013), and more behavioral problems in boys (Vilahur et al., 2013). The use of this combined approach together with additional biomarkers of effect (e.g., hormone levels, biochemical parameters, etc.) would increase the weight of evidence linking chemical exposures to health outcomes in observational studies.

This study had a relatively small sample size, reducing the ability to establish associations with many of the maternal characteristics considered. Furthermore, testing was limited to five OCPs and three PCBs, although the TEXB-α offers a good representation of the most lipophilic and persistent compounds. The main strength of this study is that it is the first to evaluate human exposure to chemical mixtures in Central/South America by synergizing toxicology and epidemiology. *Ex-vivo* bioassays can play a crucial role in the assessment of human exposure to complex mixtures by determining the joint activity that they elicit through a specific mode of action.

5. Conclusions

This preliminary study of the PA-MAMI cohort contributes the first report on POP concentrations and combined xenoestrogenic activity in placentas from South/Central American women. These data can serve as reference values for the biomonitoring of chemical exposure in Panamanian provinces. Research is under way into the effects of exposure during pregnancy on offspring health and into the combined effect of environmental pollutants on sex hormone balance.

Author contributions

Iribarne-Durán LM: Investigation, Formal analysis, Writing – original draft; Castillero-Rosales I: Investigation, Formal analysis; Peinado FM: Investigation; Artacho-Cordón F: Investigation, Formal analysis, Writing-Review; Molina-Molina JM: Investigation; Medianero E: Investigation; Nicolás-Delgado SI: resources; Sánchez-Pinzón L: resources; Núñez-Samudio V: resources; Vela-Soria F: Investigation; Olea N: Conceptualization, Methodology, Supervision, Formal analysis, Writing-Review & Editing; Alvarado-González NE: Conceptualization, Funding acquisition, Supervision, Writing-Review & Editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117622.

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