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Association between pre/perinatal exposure to POPs and children's anogenital distance at age 4 years: A study from the INMA-Asturias cohort

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ABSTRACT

Background: Prenatal exposure to endocrine-disrupting chemicals may impair genital development and alter reproductive tract anatomy. Anogenital distance (AGD) is a useful biomarker of exposure to chemicals that act as endocrine disruptors. We evaluated associations between prenatal and perinatal exposure to several persistent organic pollutants (POPs) and AGD in 4-year-old children.

Methods: Data were drawn from the INMA-Asturias cohort. Pediatricians measured the anofourchetal distance in female children and anoscrotal distance in male children. The anogenital index (AGI) was defined as the AGD divided by the child's weight at age of examination. We measured the levels of two hexachlorocyclohexane isomers, hexachlorobenzene, dichlorodiphenyltrichloroethane (DDT) and its metabolites, six polychlorinated biphenyl (PCB) congeners, and six polybrominated diphenyl ether (PBDE) congeners in maternal serum at 12 gestational weeks (n = 155) and in cord blood serum (n = 229). Anthropometric and parental socio-demographic variables were collected via face-to-face interviews. Linear regression models were used to evaluate the relationship between exposure to POPs and AGI, adjusted for confounders and stratified by sex.

Results: In male children, we found inverse associations between AGI and maternal concentrations of PCB-138 (ß = −0.041, 95% confidence interval [CI]: −0.074, −0.008, second tertile), PCB-153 (ß = −0.052, 95% CI: −0.085, −0.020, second tertile), PCB-180 ß = −0.065, 95% CI: −0.096, −0.035, second tertile; ß = −0.042, 95% CI: −0.073, −0.011, third tertile), PBDE-209 (ß = −0.031, 95% CI: −0.058, −0.006), cord serum concentrations of PCB-153 (ß = −0.029, 95% CI: −0.059, −0.000, second tertile; ß = −0.047, 95% CI: −0.085, −0.008, third tertile), and PCB-180 (ß = −0.041, 95% CI: −0.078, −0.005, third tertile). In female children, AGI was positively associated with maternal serum concentrations of PCB-101 (ß = 0.039, 95% CI: 0.002, 0.076, second tertile), and higher cord serum levels of 4,4′-DDT (ß = 0.032, 95% CI: 0.003, 0.061, third tertile) and 4,4′-DDE (ß = 0.040, 95% CI: 0.011, 0.069, third tertile).

Conclusions: Our findings provide evidence of associations between specific POPs and AGI in boys and girls aged 4 years, and suggest that pre/perinatal exposure to POPs has a feminizing effect in males and a masculinizing effect in females.

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1. Introduction

Anogenital distance (AGD) is the distance from the anus to the genital tubercle, and is an anthropometric parameter that has been validated in animal models as a sensitive marker of intrauterine exposure to estrogens, androgens, and anti-androgens (McIntyre et al., 2001; Wolf et al., 2004). AGD is sexually dimorphic across numerous animal species, and males typically have a longer AGD than females at a 2:1 ratio (Barrett et al., 2014; Dean and Sharpe, 2013; McIntyre et al., 2001). Male genital anomalies (e.g., hypospadias, cryptorchidism, testicular cancer, and declining semen quality) are features of testicular dysgenesis syndrome (Skakkebaek et al., 2001). These characteristics have been related to a shorter AGD (Drake et al., 2009; Singal et al., 2016; Thankamony et al., 2014) and prenatal exposure to endocrine-disrupting chemicals (EDCs) that act as anti-androgens during the masculinization programming window. This refers to a period that occurs at 9–12 gestational weeks, within which androgen action can be affected (Dean and Sharpe, 2013; Skakkebaek et al., 2001). It has also been suggested that female infants with congenital adrenal hyperplasia (Callegari et al., 1987), and young adult women with higher testosterone levels (Mira-Escolano et al., 2014) or multifollicular ovaries (Mendiola et al., 2012) may have a longer AGD. Therefore, AGD may be a biomarker of prenatal exposure to EDCs and a potential predictor of reproductive sequelae in females (Barrett et al., 2017).

Extensive toxicological literature supports the relationship between environmental EDCs (e.g., phthalates, dioxins, and herbicides) and shortened AGD in male animals (Hass et al., 2007; Jin et al., 2008; Rosenberg et al., 2008; Saillenfait et al., 2009). In female rodents, in utero exposure to 2,3,7,8-tetrachlorodibenzeno-p-dioxin can alter reproductive morphology and function (Gray and Ostby, 1995). In addition, estrogen has a role in the development of external genitalia (Yang et al., 2010), and when androgens were administered to a pregnant dam it was observed that female offspring had a longer, more masculine AGD than controls (Hotchkiss et al., 2007). Female genital anomalies (e.g. endometriosis) have been related to an intrauterine origin, and the role of early-life influences, such as hormonal environmental exposure to estrogens in the uterus, are consider as risk factor for endometriosis in adulthood (Sánchez-Ferrer et al., 2017).

A key aspect of assessing the adverse health effects of EDCs is understanding their biological mechanisms of action, especially in early gestation. A few epidemiological studies have investigated the association between fetal exposure to chemicals with estrogenic or anti-androgenic properties, including non-persistent organic pollutants such as phthalates (Huang et al., 2009; Swan et al., 2015, 2005; Wenzel et al., 2018), bisphenol A (BPA) (Barrett et al., 2017; Miao et al., 2011), and non-persistent pesticides (Dalsager et al., 2018) and the AGD of male newborns and children. In contrast, little attention has been paid to the effects of persistent organic pollutants (POPs) on genital development. POPs include several chemical families, such as polybrominated diphenyl ethers (PBDEs), organochlorine (OC) pesticides, and polychlorobiphenyls (PCBs). Although the synthesis of these POPs was banned by the Stockholm Convention in 2009 (SCOPS, 2009), they have persisted in the environment and remain present in human tissues because of their high lipophilicity and biomagnifying properties (Carrizo et al., 2006; Casas et al., 2015; Vizcaino et al., 2014a, 2014b). All POPs are environmental toxicants previously used as flame retardants, dielectrics in transformers, or pesticides (De Wit, 2002; Vizcaino et al., 2010), and some exhibit anti-androgenic or estrogenic properties (Gray, 2001; Kaya et al., 2002; Portigal et al., 2002). It is important to note that androgen and estrogen receptors are needed differentially in males and females for the development of male or female external genitalia morphology (Yang et al., 2010). Only four studies have evaluated the effect of prenatal exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) on human AGD; these studies reported inconsistent results (Bornman et al., 2016; Longnecker et al., 2007; Loreto-Gómez et al., 2018; Torres-Sanchez et al., 2008). To date, only one human study has evaluated the association between PCB concentrations and AGD in both sexes, and reported an inverse association between AGD and levels of PCB-28, PCB-74, and PCB-170 in boys during the first year of life (Loreto-Gómez et al., 2018). In addition, a birth cohort study in China reported an inverse association between cord plasma concentrations of PBDE-47 and AGD measured in boys over the first 48 months of life (Luan et al., 2019). Another study from Denmark reported an inverse association between perfluoroalkyl substances and AGD in girls (Lind et al., 2017).

Early exposure to POPs may have estrogenic or anti-androgenic effects, but there is no clear scientific evidence linking exposure to OC compounds or PBDE to changes in AGD. We previously reported an inverse association between prenatal exposure to PBDEs and AGD among boys aged 18 months (García-Villarino et al., 2018). In the present study, we evaluated the association of AGD and prenatal and perinatal exposure to two hexachlorocyclohexane (HCH) isomers, hexachlorobenzene (HCB), DDT and its derivates, six PCB congeners, and six PBDE congeners in 4-year-old boys and girls.

2. Methods

2.1. Study design and population

The INMA (INfancia y Medio Ambiente [Environment and Childhood]) Asturias study included a population-based cohort of women from nine municipalities in Asturias, northern Spain. Details of this cohort have been published elsewhere (Fernández-Somoano et al., 2011; Fernández-Somoano and Tardon, 2014; Riaño-Galán et al., 2017). Briefly, 494 pregnant women were recruited for the cohort between May 2004 and June 2007. The inclusion criteria were: age ≥16 years, singleton pregnancy, enrolment at 10–13 weeks of gestation, no assisted conception, delivery scheduled at the reference hospital (Hospital San Agustin in Aviles), and no communication disability. The women completed two detailed questionnaires on anthropometric and sociodemographic characteristics and lifestyle information, and 308 blood samples were collected. Deliveries took place between October 2004 and February 2008. In total, 485 children were born, and 326 cord blood samples were successfully collected. Follow-up was conducted at 4 years for 453 children via questionnaires on environmental health data, diet, and sociodemographic characteristics. Pediatricians examined the children, collected blood samples, and recorded anthropometric measures and sexual development. AGD was measured in 382 children at the 4-year follow up; from those children, and POPs concentrations were measured in 155 maternal and 229 cord serum samples (Fig. 1). The background characteristics of excluded children did not differ markedly from those included in this study (Table S1). The Asturias Regional Clinic Research Ethics Committee approved the research protocol. All parents provided written informed consent before inclusion, and signed a second consent to enroll the children in the INMA-Asturias cohort.

2.2. Exposure measurement

2.2.1. Sample collection

Whole cord blood samples (72.16% recovery rate) were collected using venipuncture of cord vessels before the placenta was delivered. Maternal blood samples (95.14% recovery rate) were collected during the first trimester of gestation (median: 12 weeks; range: 10–13 weeks) from 308 pregnant women by trained nurses following the INMA protocol. Five milliliters of venous blood were collected from each woman using a glass syringe and Vacutainer blood collection tubes. Blood samples were stored in the dark at 4 °C 1–2 h after collection, and then centrifuged for 15 min at 2,500–3,000 rpm. The serum obtained was aliquoted into 2-ml glass cryotubes and stored at −80 °C. We measured the concentrations of β-HCH, γ-HCH (lindane), HCB, 4,4′-DDT, 4,4′-

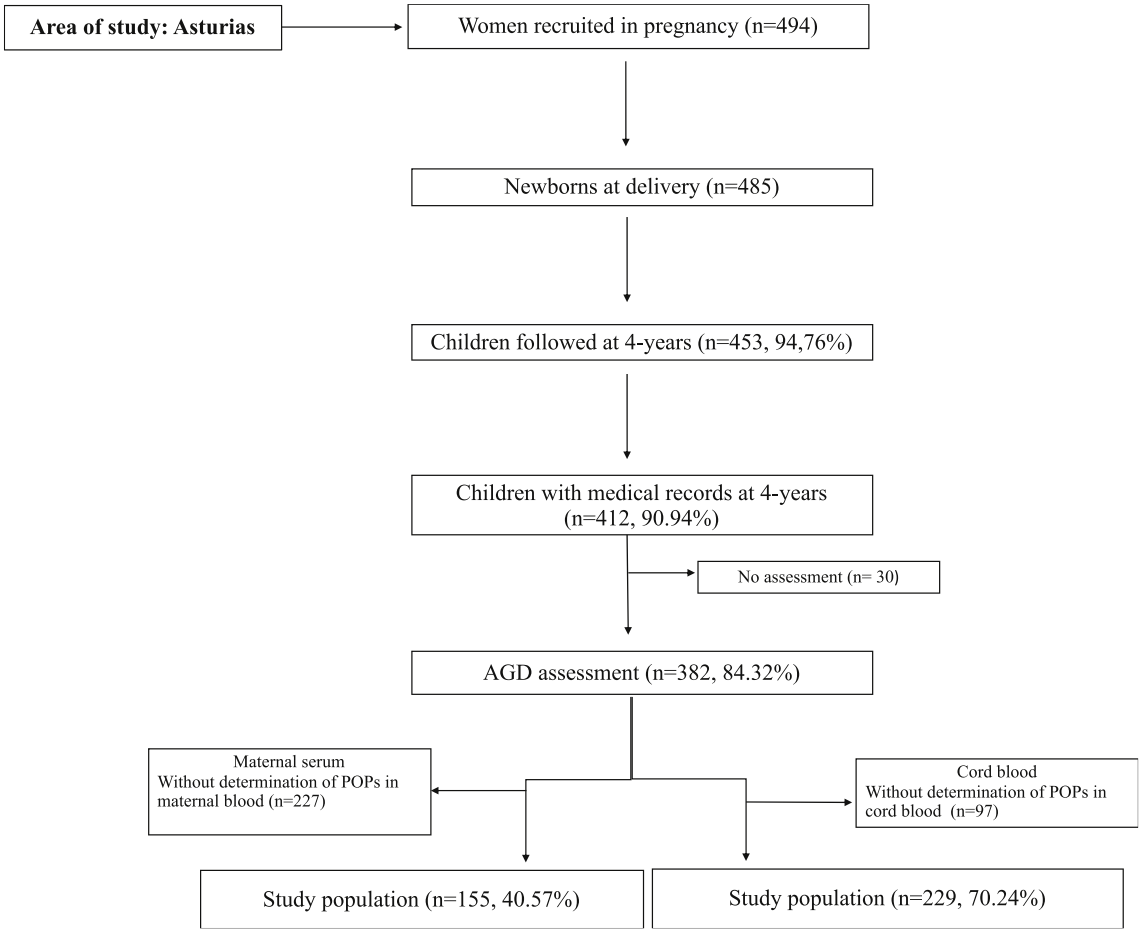

Fig. 1. Flowchart of the participant selection process from the INMA Asturias cohort to be included in the present study.

DDE, 4,4′-dichlorodiphenyldichloroethane (DDD), six PCB congeners (PCB-28, -101, −118, −153, −138, and −180), and six PBDE congeners (PBDE-28, -47, −99, −153, −154, and −209) in maternal and cord serum samples. Samples were analyzed at the Barcelona Institute of Environmental Assessment and Water Research (IDAEA–CSIC) as previously described (Gari and Grimalt, 2010; Grimalt et al., 2010b; Vizcaino et al., 2009).

2.2.2. Laboratory analyses

One milliliter of serum was spiked with the surrogate standards tetrabenzene and decachlorobiphenyl and vortex-stirred for 30 s at 2,000 rpm. *n*-Hexane (3 ml) was added, followed by concentrated sulfuric acid (2 ml). After reacting, the mixture was stirred for 30 s and the supernatant *n*-hexane phase was separated by centrifugation. The extract was transferred to gas chromatography vials using four 25-µl isooctane rinses for instrumental analysis. PCB-142, BDE-118 (20 µl), and [^{13}C]-BDE-209 (10 µl) were added as internal standards before injection. Compound analysis was performed by gas chromatography with electron capture detection (Agilent Technologies 6890N gas chromatograph; Santa Clara, CA, USA), using a DB-5 column protected by a retention gap (60 m × 0.25 mm I.D., 0.25-µm film thickness; J&W Scientific, Folsom, CA, USA). Helium was the carrier gas at a constant flow rate of 1.5 ml/min. Injection (2 µl) was performed in splitless mode at 280 °C. Nitrogen (60 ml/min) was the makeup gas. The electron capture detection was set at 310 °C. The oven temperature program was held at 90 °C for 2 min, and then increased to 130 °C/min and 290 °C at 4 °C/min, with a final holding time of 20 min. The total run time was 60.67 min. The limits of detection (LOD) of OC pesticides and PCBs were 0.010–0.035 ng/ml.

PBDE congeners were analyzed by gas chromatography coupled with mass spectrometry in chemical ionization mode and negative ion recording (LOD: 0.001–0.025 ng/ml). The laboratory methods and quality control procedures have been previously described (Gari and Grimalt, 2010; Grimalt et al., 2010a). Total cholesterol and triglycerides were determined in serum samples using colorimetric enzymatic methods in the General Biochemistry Laboratory of San Agustin Hospital (Asturias). The samples were processed using a Roche Diagnostics COBAS C711 analyzer (Basel, Switzerland). Total serum lipid concentrations were calculated as previously described (Phillips et al., 1989), using the following formula.

$$\text{Total lipids} = (2.27 \times \text{total cholesterol}) + \text{triglycerides} + 62.3 \text{ mg/dl}$$

Analytical results (including POPs and total lipid concentrations) were validated by analysis of reference material obtained from the Arctic Monitoring and Assessment Program.

2.3. Anthropometric measurements

AGD measurements were collected according to the protocol developed by Salazar-Martinez and colleagues (Salazar-Martinez et al., 2004). The child was placed in the dorsal decubitus position; both hips were flexed and light pressure was exerted on the thighs until the pediatrician's hand touched the subject's abdomen. All measurements were performed with Vernier calipers in increments of 0.1 mm by two pediatricians (IRG and CRD) previously trained in the technique. The examiners stood in front of the children and made independent measurements of AGD using the same digital caliper. To evaluate inter-examiner variability, two pediatricians took independent

Table 1
Characteristics of mothers and children participating in the INMA-Asturias cohort study.

Variables	Sub-sample with available data on maternal serum (n = 155)			Sub-sample with available data on cord serum (n = 229)		
Maternal characteristics	*N*	*%*	Mean (SD)	*N*	*%*	Mean (SD)
Age (years)			31.50 (4.45)			31.63 (4.14)
Gestational age (week)			39.81 (1.39)			39.68 (1.37)
Pre-pregnancy BMI						
Underweight (< 18.5 kg/m^2)	4	2.6		7	3.1	
Normal (18.5–24.9 kg/m^2)	106	68.4		158	69.0	
Overweight (25.0–29.9 kg/m^2)	33	21.3		47	20.5	
Obese (≥30 kg/m^2)	12	7.7		17	7.4	
Height (cm)			162.62 (5.60)			162.48 (5.61)
Education						
Primary	27	17.4		35	15.3	
Secondary	66	42.6		93	40.6	
Universitary	62	40.0		101	44.1	
Gravidity						
One	79	50.9		117	51.1	
Two	41	26.5		70	30.5	
Three or more	35	22.6		42	18.4	
Smoking at 1st trimester of pregnancy						
Yes	43	27.7		61	26.6	
No	110	71.0		161	70.3	
Maternal passive smoking						
Yes	72	46.5		98	42.8	
No	81	52.3		124	54.1	
Smoking at week 12 of pregnancy						
Yes	21	13.5		32	14.0	
No	132	84.5		189	82.5	
Smoking at week 32 of pregnancy						
Yes	20	12.9		30	13.1	
No	133	85.8		192	83.8	
Paternal characteristics						
Age (years)			33.10 (5.65)			33.98 (1.37)
BMI						
Underweight (< 18.5 kg/m^2)	-	-		-	-	
Normal (18.5–24.9 kg/m^2)	47	30.3		71	31	
Overweight (25.0–29.9 kg/m^2)	77	49.7		109	47.5	
Obese (> 30 kg/m^2)	26	16.8		40	17.4	
Child characteristics						
AGD at 4 years (mm)						
Male	74	47.8	33.95 (10.65)	116	50.7	33.09 (11.38)
Female	81	52.2	16.48 (9.25)	113	49.3	16.00 (9.21)
AGI at 4-years (mm/kg)						
Male	74	47.8	1.81 (0.58)	116	50.7	1.79 (0.62)
Female	81	52.2	1.07 (0.72)	113	49.3	1.04 (0.62)
Birth weight (kg)			3.35 (4.39)			3.32 (4.39)
Birth length (cm)			49.92 (1.90)			49.91 (1.86)
Height at 4 years (cm)			106.31 (4.47)			106.03 (4.44)
Weight at 4 years (kg)			18.57 (2.94)			18.28 (2.93)
BMI at 4 years (kg/m^2)			16.36 (1.77)			16.20 (1.82)

SD, standard deviation; BMI, body mass index; AGD, anogenital distance; AGI, anogenital index.

measurements of 10% of the entire sample following the same procedure. The outputs did not substantially differ; therefore, only one measurement was noted. AGD was measured from the center of the anus to the posterior convergence of the fourchette (anofourchetal distance, anus to fourchette) in female children, and from the center of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum (anoscrotal distance, anus to scrotum) in male children.

2.4. Potential confounders

Participating women completed two detailed questionnaires during their pregnancy (at 10–13 and 28–32 gestational weeks) and at the 4-year follow-up. The questionnaires were administered by a trained interviewer and covered sociodemographic, environmental, and lifestyle factors. The maternal variables used as potential confounders in this study were age, pre-pregnancy weight (self-reported), gestational age (weeks) at delivery, gravidity (1, 2, or ≥3), education (primary, secondary, or university), and pre-pregnancy body mass index (BMI). Pre-pregnancy BMI was obtained by dividing self-reported pre-pregnancy weight in kg by current height in meters squared, and was categorized as underweight (< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese (≥30 kg/m^2). Paternal covariates included age and BMI. The questionnaires also included an assessment of tobacco exposure: prenatal exposure to tobacco smoke (yes/no), smoking at the beginning of pregnancy (yes/no), smoking at week 12 of pregnancy (yes/no), and smoking at week 32 of pregnancy (yes/no). Child covariates assessed for confounding included length/height, weight at birth, and BMI at age 4 years.

2.5. Statistical analysis

Descriptive statistics were calculated for demographic and clinical characteristics by maternal sample (n = 155) and cord sample

Table 2
Detection rate and levels of persistent organic pollutants (POPs) (ng/g lipid) in maternal and cord blood serum, INMA-Asturias cohort.

POP levels	LOD (ng/ml)	Maternal serum					Cord serum				
		%(> LOD)	Median	5th	95th	CV	%(> LOD)	Median	5th	95th	CV
HCB	0.0151	100	75.05	24.48	216.49	0.75	98.69	50.33	17.68	175.89	0.74
β-HCH	0.001	100	24.95	7.96	88.94	1.08	93.01	17.37	0.29	68.20	1.08
γ-HCH	0.0183	10.32	2.44	1.81	7.60	0.71	4.37	5.01	3.83	6.90	0.49
44-DDD	0.003	40.00	0.36	0.23	3.38	1.25	13.97	0.63	0.48	4.46	2.40
44-DDE	0.0081	99.35	254.67	83.83	1071.43	1.21	99.13	178.82	52.31	824.71	1.07
44-DDT	0.0247	94.19	19.53	4.29	55.67	2.24	96.51	33.38	10.72	130.91	11.26
PCB-28	0.0064	74.84	3.72	0.38	16.72	1.03	14.41	0.93	0.71	5.11	1.39
PCB-101	0.0225	58.71	5.23	1.26	24.32	1.00	62.88	33.12	2.99	59.14	0.52
PCB-118	0.0286	94.84	10.33	2.56	27.74	0.65	89.52	6.76	2.26	20.57	1.09
PCB-138	0.0173	100	37.31	15.92	85.02	0.56	100	32.55	12.04	91.14	2.34
PCB-153	0.0215	100	66.07	32.05	131.52	0.49	100	47.7	18.64	116.42	0.62
PCB-180	0.0103	100	48.28	13.09	111.48	0.67	97.82	27.79	7.16	68.85	0.96
PBDE-28	0.0015	21.94	0.16	0.11	1.69	1.45	17.47	0.31	0.24	2.11	2.13
PBDE-47	0.0025	20.65	0.26	0.19	5.62	2.53	34.06	0.56	0.41	5.46	4.04
PBDE-99	0.0023	59.35	1.63	0.19	11.28	1.47	28.82	0.50	0.37	9.45	3.00
PBDE-153	0.0027	86.45	2.52	0.15	9.55	1.81	14.41	0.30	0.2	3.35	3.31
PBDE-154	0.0012	93.55	2.60	0.25	11.14	1.06	44.54	0.57	0.42	3.92	2.58
PBDE-209	0.0091	37.42	1.02	0.72	14.53	1.58	13.54	1.87	1.40	16.18	1.97

HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; 4,4′-DDD, 1,1-bis(4-chlorophenyl)-2,2-dichloroethane; 4,4′-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene; 4,4′-DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; PCB, polychlorinated biphenyl; PBDE, polybrominated diphenyl ethers.
LOD, limit of detection; 5th, percentile 5; 95th, percentile 95; CV, coefficient of variation.

(n = 229). Summary statistics were calculated for continuous variables and categorical variables were presented as n (%).

We used the anogenital index (AGI; calculated as AGD divided by weight at age of examination [mm/kg]) as previously described (Swan et al., 2005). The AGI was normally distributed and treated as the dependent variable in the linear regression models. Statistical analyses were stratified by child's sex for both maternal serum and cord serum samples. POP levels were expressed in nanograms per milliliter (ng/ml) and adjusted by total serum lipid concentrations (nanograms per gram lipid, ng/g lipid), and then natural-log transformed to reduce skewness before being introduced in the linear regression models. Although POPs concentrations standardized by lipid content are widely used in POPs relevant analysis, we have repeated the analysis with POPs concentrations expressed on a volume basis (ng/ml), with total serum lipids as a covariate and as a sensitivity analysis. Spearman's coefficients were calculated for each POP pair. We calculated the sum of maternal and cord DDT (ΣDDTs; i.e., 4,4′-DDE, 4,4′-DDT, 4,4′-DDD), PCBs (ΣPCBs; i.e., PCB-28, -101, −118, −138, −153, −180), and PBDE (ΣPBDEs; i.e., PBDE-28, -47, −99, −153, −154, −209) as biomarkers of ΣPOPs exposure and they were used as global indicators of exposure to different groups of POPs.

Covariates used as potential confounders in our models were based on prior studies using direct acyclic graphs and selecting the minimally sufficient adjustment set (Figure S4) (Textor et al., 2016). In both males and females, models were adjusted by height at age 4 years, maternal height, maternal pre-pregnancy BMI, and maternal gravidity, as well as by the ΣDDTs, ΣPCBs, and ΣPBDEs (excluding the family of compounds –Σ– to which the dependent variable belonged). Interactions between the compounds PBDE-99, PCB-138, and 4,4′-DDE (in pairs) were introduced into the model if associations were statistically significant at $\alpha = 0.05$. To examine the associations between POPs and AGI, compounds with a detection rate > 50% were categorized according to tertiles, whereas those with a detection rate < 50% were divided depending on whether the value was below or above the LOD. *P*-trends across tertiles of POPs were obtained by including the median of each quartile as a continuous variable in the linear regression models. Values below the lower LOD were entered as the LOD divided by the square root of 2 and included in statistical analyses. For hypothesis testing, $p < 0.05$ (both main effects and interactions) was considered statistically significant. The Benjamini-Hochberg procedure was used to control for false discovery rate (Benjamini and Hochberg, 1995). We used the Variance Inflation Factor (VIF) to study the multicollinearity of the linear regression models (Fox and Weisberg, 2019). All statistical analyses and graphics were performed using R version 3.5.1 (R Core Team, 2014).

3. Results

Table 1 summarizes the physiological and sociodemographic variables of the mothers, fathers, and children in the INMA Asturias study. The median maternal age was 31.5 years (range: 19–42 years) and mean (standard deviation [SD]) maternal height and weight were 162.6 (5.60) cm and 63.14 (10.96) kg, respectively. Approximately 28% of mothers were overweight or obese, and 40% had a university education at delivery. Regarding tobacco use, 46.5% were passive smokers and 27.7% were smokers at the beginning of pregnancy, but only 13.5% were smokers at week 12 of pregnancy and 12.9% at week 32. Children's mean (SD) height, weight, and BMI at age 4 years were 106.3 (4.47) cm, 18.57 (2.94) kg, and 16.36 (1.77) kg/m^2, respectively. The mean (SD) birth weight was 3.35 (4.39) kg and mean (SD) birth length was 49.92 (1.90) cm. No genital malformations or disorders were observed in the evaluated children, and no parameter appeared grossly abnormal in any child. The AGD in males was 13–62 mm, with a mean (SD) distance of 33.95 (10.65) mm. In females, the mean (SD) AGD was 16.48 (9.25) mm (range: 10–30 mm). The mean (SD) AGI was 1.81 (0.58) mm/kg in males and 1.07 (0.72) mm/kg in females. The descriptive analyses results were similar among those with cord serum samples (Table 1).

Table 2 summarizes maternal serum and cord serum levels for two HCH isomers, HCB, DDT and its metabolites, six PCB congeners, and six PBDE congeners. In maternal serum, HCB, β-HCH, PCB-138, PCB-153, and PCB-180 were detected in all samples, and 4,4′-DDE, 4,4′-DDT, PCB-28, PCB-118, PBDE-153, and PBDE-154 were detected in the majority (> 75%) of samples. The detection frequency of the other analyzed compounds ranged from 10% to 60%. In general, POPs levels and detection rates were lower in cord serum samples. Only PCB-138 and PCB-153 were detected in all cord serum samples. The detection rates of HCB, β-HCH, 4,4′-DDE, 4,4′-DDT, PCB-118, and PCB-180 were ≥90%. The detection rate of the other compounds was 4%–63%. Spearman's correlations between maternal and cord serum paired samples ranged from weakly negative to strongly positive (Spearman rho [ρ] = −0.04 to 0.91, Figure S1). No correlation was observed for PBDE

Table 3
Association between prenatal exposure to persistent organic pollutants measured in maternal blood and anogenital index at 4 years of age.

Maternal serum	Male[1]					Female[1]				
POP (ng/g lipid)	Beta	95% CI		*p*-value	*p*-trend	Beta	95% CI		*p*-value	*p*-trend
HCB (ref: ≤ T1)										
T2: 51.72–91.36	0.003	−0.031	0.036	0.850		−0.011	−0.053	0.030	0.588	
T3: > 91.36	0.011	−0.032	0.054	0.612	0.608	−0.008	−0.050	0.033	0.683	0.671
β-HCH (ref: ≤ T1)										
T2: 20.60–38.50	−0.010	−0.045	0.024	0.548		−0.020	−0.061	0.022	0.351	
T3: > 38.50	−0.017	−0.052	0.018	0.336	0.333	−0.017	−0.066	0.032	0.491	0.493
44-DDE (ref: ≤ T1)										
T2: 200.61–355.84	−0.014	−0.047	0.019	0.392		0.034	−0.007	0.074	0.100	
T3: > 355.84	0.004	−0.030	0.039	0.803	0.757	0.029	−0.012	0.070	0.163	0.151
44-DDT (ref: ≤ T1)										
T2: 13.04–25.83	0.010	−0.021	0.044	0.489		0.032	−0.009	0.073	0.121	
T3: > 25.83	0.025	−0.008	0.059	0.128	0.122	0.024	−0.019	0.066	0.266	0.284
PCB-28 (ref: ≤ T1)										
T2: 1.65–5.52	0.010	−0.019	0.040	0.460		0.011	−0.029	0.051	0.588	
T3: > 5.52	0.010	−0.023	0.040	0.520	0.495	0.023	−0.015	0.060	0.231	0.228
PCB-101 (ref: ≤ T1)										
T2: 1.80–8.61	0.010	−0.023	0.045	0.518		0.039	0.002	0.076	0.041	
T3: > 8.61	−0.007	−0.038	0.025	0.664	0.634	0.008	−0.032	0.047	0.701	0.621
PCB-118 (ref: ≤ T1)										
T2: 7.59–13.90	−0.014	−0.050	0.022	0.444		−0.016	−0.058	0.026	0.451	
T3: > 13.90	0.009	−0.022	0.040	0.560	0.457	0.027	−0.014	0.068	0.193	0.127
PCB-138 (ref: ≤ T1)										
T2: 30.15–46.64	−0.041	−0.074	−0.008	0.017		−0.029	−0.046	0.034	0.149	
T3: > 46.64	−0.022	−0.061	0.009	0.136	0.218	−0.006	−0.057	0.021	0.745	0.727
PCB-153 (ref: ≤ T1)										
T2: 53.50–76.44	−0.052	−0.085	−0.020	0.002[a]		0.031	−0.008	0.070	0.126	
T3: > 76.44	−0.024	−0.057	0.008	0.134	0.204	−0.006	−0.045	0.034	0.775	0.695
PCB- 180 (ref: ≤ T1)										
T2: 36.50–58.50	−0.065	−0.096	−0.035	< 0.001[a]		−0.006	−0.046	0.034	0.782	
T3: > 58.40	−0.042	−0.073	−0.011	0.009[a]	0.046	−0.018	−0.057	0.021	0.368	0.360
PBDE-99 (ref: ≤ T1)										
T2: 0.26–3.06	0.019	−0.010	0.048	0.197		0.017	−0.025	0.060	0.426	
T3: > 3.06	0.000	−0.036	0.036	0.988	0.748	−0.015	−0.054	0.024	0.451	0.313
PBDE-153 (ref: ≤ T1)										
T2: 1.94–3.52	−0.002	−0.034	0.031	0.923		0.010	−0.033	0.053	0.634	
T3: > 3.52	0.004	−0.028	0.036	0.800	0.802	0.025	−0.016	0.066	0.227	0.218
PBDE-154 (ref: ≤ T1)										
T2: 1.68–3.92	−0.004	−0.033	0.025	0.766		0.022	−0.019	0.039	0.278	
T3: > 3.92	0.002	−0.031	0.036	0.896	0.938	−0.001	−0.041	0.049	0.945	0.883
γ-HCH (ref: ≤LOD)										
> LOD: 0.020	−0.008	−0.038	0.081	0.079	-	−0.026	−0.077	0.024	0.297	-
44-DDD (ref: ≤LOD)										
> LOD: 0.003	−0.008	−0.034	0.018	0.531	-	−0.006	−0.039	0.027	0.732	-
PBDE-28 (ref: ≤LOD)										
> LOD: 0.001	−0.010	−0.043	0.023	0.534	-	0.008	−0.029	0.045	0.675	-
PBDE-47 (ref: ≤LOD)										
> LOD: 0.003	0.003	−0.037	0.042	0.890	-	0.003	−0.033	0.040	0.849	-
PBDE-209 (ref: ≤LOD)										
> LOD: 0.010	−0.031	−0.058	−0.006	0.016	-	−0.014	−0.049	0.020	0.415	-

LOD, limit of detection; T1, 1st tertile; T2, 2nd tertile: T3, 3rd tertile; ***β***, regression coefficient; ***p*-trend**: P for trend from a linear regression model where the AGI was the outcome of interest and POPs variable was the predictor (continuous). **CI**: confidence interval. [1]adjusted by height at age 4 years, maternal height, pre-pregnancy body mass index, gravidity, and ΣDDTs (sum of 4,4′-DDE, 4,4′-DDT, and 4,4′-DDD), ΣPCBs (sum of PCB-28, -101, −118, −138, −153, and −180), and/or ΣPBDEs (sum of PBDE-28, -47, −99, −153, −154, and −209). [a] Applying Benjamini and Hochberg's procedure keeps the *p*-values significant.

concentrations between maternal serum and sum of PCBs or sum of DDTs. The concentrations of PCBs in maternal serum were not strongly correlated with ΣDDTs and ΣPBDEs (ρ = 0 to 0.36 and 0.12 to 0.20, respectively). In addition, 4,4′-DDT and its derivates did not exhibit statistically significant correlations with ΣPCBs and sum of ΣPBDEs (ρ = 0.05 to 0.36) (Figure S2B). No substantial correlation differences were observed when considering the concentrations in cord serum (Figure S3).

The AGI of male offspring was inversely associated with maternal serum concentrations of the second tertile of PCB-138 (β = −0.041, 95% confidence interval [CI]: −0.074, −0.008), second tertile of PCB-153 (β = −0.052, 95% CI: −0.085, −0.020), and the second and third tertiles of PCB-180 (β = −0.065, 95% CI: −0.096, −0.035 and β = −0.042, 95% CI: −0.073, −0.011, respectively), and PBDE-209 (β = −0.031, 95% CI: −0.058, −0.006) (Table 3). PCB-153 (β = −0.029, 95% CI: −0.59, −0.000 and β = −0.047, 95% CI: −0.085, −0.008, second and third tertiles, respectively) and PCB-180 (β = −0.041, 95% CI: −0.078, −0.005) in cord serum samples were also associated with a lower AGI value (Table 4). Furthermore, for PCB-153 and PCB-180, the interaction between PCB-138 and 4,4′-DDE was statistically significant ($p = 0.01$ and $p = 0.03$, respectively). However, a statistically significant positive association was found between PBDE-153 in cord serum samples and AGI (β = 0.023, 95% CI: 0.001, 0.045) (Table 4). In females, the AGI was positively related to maternal serum concentrations in the second tertile of PCB-101 (β = 0.039, 95% CI: 0.002, 0.076) (Table 3) and cord serum concentrations in the third

Table 4
Association between prenatal exposure to persistent organic pollutants measured in cord blood and anogenital index at 4 years of age.

Cord Serum	Male[1]					Female[1]				
POP (ng/g lipid)	Beta	95% CI		*p*-value	*p*-trend	Beta	95% CI		*p*-value	*p*-trend
HCB (ref: ≤ T1)										
T2: 37.81–70.69	−0.009	−0.036	0.018	0.499		−0.001	−0.031	0.029	0.952	
T3: > 70.69	0.011	−0.021	0.044	0.484	0.493	0.003	−0.029	0.035	0.845	0.841
B-HCH (ref: ≤ T1)										
T2: 11.62–25.19	0.029	0.001	0.057	0.402		0.000	−0.028	0.027	0.975	
T3: > 25.19	0.006	−0.022	0.035	0.661	0.659	0.007	−0.023	0.038	0.648	0.065
44-DDE (ref: ≤ T1)										
T2: 132.13–259.79	−0.016	−0.043	0.011	0.249		0.007	−0.021	0.034	0.633	
T3: > 259.79	0.005	−0.022	0.032	0.711	0.652	0.032	0.003	0.061	0.033	0.034
44-DDT (ref: ≤ T1)										
T2: 24.11–47.26	−0.010	−0.039	0.019	0.499		−0.002	−0.029	0.025	0.881	
T3: > 47.26	−0.004	−0.035	0.026	0.772	0.844	0.040	0.011	0.069	0.006[a]	0.010
PCB-101 (ref: ≤ T1)										
T2: 27.16–39.02	−0.006	−0.032	0.019	0.626		0.001	−0.027	0.030	0.917	
T3: > 39.02	0.000	−0.027	0.027	0.986	0.966	−0.004	−0.032	0.023	0.749	0.742
PCB-118 (ref: ≤ T1)										
T2: 3.27–8.77	−0.012	−0.038	0.014	0.361		−0.027	−0.054	0.000	0.053	
T3: > 8.77	−0.003	−0.029	0.023	0.821	0.819	−0.024	−0.051	0.003	0.084	0.085
PCB-138 (ref: ≤ T1)										
T2: 25.72–40.82	0.004	−0.023	0.031	0.785		−0.007	−0.034	0.020	0.605	
T3: > 40.82	−0.003	−0.031	0.025	0.833	0.822	−0.027	−0.056	0.003	0.081	0.084
PCB-153 (ref: ≤ T1)										
T2: 40.41–61.07	−0.029	−0.059	0.000	0.049[b]		−0.002	−0.030	0.025	0.859	
T3: > 61.07	−0.047	−0.085	−0.008	0.017[b]	0.016	−0.023	−0.052	0.006	0.122	0.118
PCB- 180 (ref: ≤ T1)										
T2: 19.82–36.42	−0.011	−0.040	0.018	0.463		0.025	−0.004	0.054	0.091	
T3: > 36.42	−0.041	−0.078	−0.005	0.027[b]	0.025	0.010	−0.032	0.052	0.639	0.464
γ-HCH (ref: ≤LOD)										
> LOD: 0.020	0.035	−0.033	0.104	0.311	-	−0.006	−0.056	0.045	0.818	-
44-DDD (ref: ≤LOD)										
> LOD: 0.003	0.001	−0.028	0.030	0.935	-	−0.001	−0.039	0.037	0.966	-
PCB-28 (ref: ≤LOD)										
> LOD: 0.006	−0.025	−0.055	0.004	0.085	-	−0.009	−0.042	0.024	0.596	-
PBDE-28 (ref: ≤LOD)										
> LOD: 0.001	−0.020	−0.051	0.011	0.197	-	−0.005	−0.032	0.023	0.727	-
PBDE-47 (ref: ≤LOD)										
> LOD: 0.003	0.009	−0.014	0.033	0.425	-	−0.008	−0.031	0.016	0.521	-
PBDE-99 (ref: ≤LOD)										
> LOD: 0.002	0.014	−0.009	0.037	0.222	-	−0.014	−0.038	0.011	0.268	-
PBDE-153 (ref: ≤LOD)										
> LOD: 0.003	0.023	0.001	0.045	0.037	-	−0.021	−0.043	0.000	0.051	-
PBDE-154 (ref: ≤LOD)										
> LOD: 0.001	0.005	−0.026	0.036	0.764	-	−0.009	−0.040	0.023	0.592	-
PBDE-209 (ref: ≤LOD)										
> LOD: 0.010	0.026	−0.003	0.056	0.083	-	−0.006	−0.042	0.029	0.71	-

LOD, limit of detection; T1, 1st tertile; T2, 2nd; tertile: T3, 3rd tertile; ***β***: regression coefficient; ***p*-trend**: P for trend from a linear regression model where the AGI was the outcome of interest and POPs variable was the predictor (continuous). **CI**: confidence interval. [1]Adjusted by height at age 4 years, maternal height, pre-pregnancy body mass index, gravidity, and ΣDDTs (sum of 4,4′-DDE, 4,4′-DDT, and 4,4′-DDD), ΣPCBs (sum of PCB-28, -101, −118, −138, −153, and −180), and/or ΣPBDEs (sum of PBDE-28, -47, −99, −153, −154, and −209. [a] Applying Benjamini and Hochberg's procedure keeps the *p*-values significant. [b] In males, associations with PCB-153 and PCB-180 were adjusted by the interaction between 4,4′-DDE and PCB-138.

tertile of 4,4′-DDE (β = 0.032, 95% CI: 0.003, 0.061) and third tertile of 4,4′-DDT (β = 0.040, 95% CI: 0.011, 0.069) (Table 4). When using POPs concentration expressed on a volume basis (ng/ml) with total serum lipids as a covariate, the results were similar to those using lipid adjusted concentrations.

4. Discussion

This study provided epidemiological evidence that prenatal exposure to PCB-138, PCB-153, PCB-180, and PBDE-209 in the first trimester was associated with a shorter AGI in 4-year-old boys. In girls, the AGI was positively associated with maternal serum concentrations of PCB-101, and 4,4′-DDT and 4,4′-DDE concentrations in cord blood serum at delivery. Pre/perinatal exposure to POPs could be associated with shortened AGI in male offspring and with lengthened in females.

Gestation is a stage of vulnerability to EDCs, as even low levels of these compounds may produce irreversible damage (Costa et al., 2016; Legler, 2008). In this respect, the first trimester of pregnancy is a critical period for the differentiation of external genitalia, which occurs in males when appropriate androgen levels are reached. The biological mechanism for this has been widely studied in the literature (Agramunt et al., 2011; Thankamony et al., 2016). If this process is incomplete, it may result in genital tract malformation and be related to shortened AGD in males, because it reflects diminished androgenization during development (Freire et al., 2018). AGD is a sexually dimorphic trait and is therefore a marker of prenatal estrogenic or androgenic influence that can be used as an index of the anti-androgenic or anti-estrogenic effects produced by EDCs in humans (Dean and Sharpe, 2013; Mendiola et al., 2016; Salazar-Martinez et al., 2004; Yang et al., 2010). In male offspring, androgenicity is typically measured by testicular volume and penile dimensions rather than AGD (Longnecker et al., 2007). However, human data suggest AGD may be responsive to androgenic exposures in

utero, and that AGD is more reliable than penile dimensions (Salazar-Martinez et al., 2004; Swan et al., 2005).

In the present study, we used a previously published protocol for measuring AGD (Salazar-Martinez et al., 2004) because the anatomical landmarks are easily identifiable. The mean AGD in 4-year-old males was 33.95 mm, which was comparable with data reported in the UK (Thankamony et al., 2009), Nigeria (Avidime et al., 2011), the US (Wenzel et al., 2018), South Africa (Bornman et al., 2016), and China (Luan et al., 2019). However, the AGIs in the present study were 3–4 times lower than those reported in other studies conducted among newborns and 2-year-old children (Huang et al., 2009; Swan et al., 2015, 2005; Torres-Sanchez et al., 2008). Therefore, available data show variations between AGD measured in geographically distinct populations, most likely because of ethnic factors or different measuring devices or measurement techniques (i.e., anoscrotal, anopenile). Differences may also be influenced by the ages at which the measurements were collected, and could also be explained by the influence of weight on children's growth. In particular, the high prevalence of obesity in our study population (Riaño-Galán et al., 2017) could be a factor influencing the AGI.

Our results for males showed a negative association between AGI and exposure to PCB-138, PCB-153, PCB-180, and PBDE-209. These findings were supported by a human study conducted in Mexico that evaluated the associations between AGD/height during the first year of life and PCBs and DDT concentrations in maternal venous serum collected in the third trimester of pregnancy (Loreto-Gómez et al., 2018). That study found noteworthy negative associations between PCB-28, PCB-74, and PCB-170 and AGD/height in males. Similarly, notable inverse associations between anoscrotal distance and fourth-quartile PBDE-47 cord plasma concentrations in male children aged 12 and 48 months were reported in China (Luan et al., 2019). Other studies focused on persistent compounds found high dioxin-like activity in maternal blood were associated with shortened AGD in newborn boys, indicating that dioxins may generate endocrine-disrupting effects in male infants (Vafeiadi et al., 2013). Maternal serum DDT concentrations at delivery were also associated with larger penile measurements in male offspring at 12 months (Bornman et al., 2016). Another study reported an association between maternal venous concentrations of DDE and a reduction in the anal position index, which is another metric for measuring the effects of chemicals on reproductive organs in males (Torres-Sanchez et al., 2008). However, Longnecker and colleagues did not find a clear association between AGD and maternal blood DDE levels (Longnecker et al., 2007). The differences in time of AGD measurements made the comparison between other studies and our study difficult. In addition, the relatively low exposure level in our study may also make it difficult to explore the potential association between POPs and AGI.

Recent work in the INMA-Asturias cohort found inverse associations between AGI at 18 months and exposure levels of PBDE-153 and PBDE-99 in maternal venous blood (García-Villarino et al., 2018). Although it is possible that the shorter AGD associated with higher exposures of POPs may be related to a feminizing effect, the mechanism of action and the effects of these compounds have not yet been identified. However, the results from human studies were consistent with those from animal studies, showing that exposure to phthalates, PCBs, dioxins, and PBDEs may affect fetal development (Jin et al., 2008; Rosenberg et al., 2008; Saillenfait et al., 2009). POPs (i.e., DDE, PCBs, and PBDEs) have been reported to be anti-androgenic in in-vitro and in-vivo assays (Hoffmann and Kloas, 2016; Stoker et al., 2005). In rats, prenatal exposure to PCBs led to dose-dependent decreases in steroid hormone levels (Kaya et al., 2002), supporting the hypothesis that PCBs interfere with androgen-induced transcriptional activation and hormone binding (Portigal et al., 2002). The anti-androgenic activity of DDT and its derivates has also been studied in animals, with results showing that prenatal exposure led to shortenings of AGD in male rats (Hoffmann and Kloas, 2016). Prenatal PBDE-99 exposure has also been shown to be related to shorter AGD in male rats (Lilienthal et al., 2006). Evidence suggests that PBDEs exposure could downregulate steroidogenic proteins, decrease androgen levels, and inhibit Leydig cells testosterone synthesis and androgen receptor (AR) binding. Prenatal androgen levels are associated with a shortened AGD (Moore et al., 2001); a possible mechanism underlying the adverse effects of PBDEs on AGD is the antiandrogenic effect caused by the decrease in androgen levels. Another potential mechanism of PBDEs may be explained by estrogenic pathways, which also has been suggested in previous studies exploring the effects of estrogenic (Dang et al., 2007; Meerts et al., 2001), anti-estrogenic (Meerts et al., 2001), and even anti-androgenic (Stoker et al., 2005) activity.

Among females, very low levels of androgens, an absent or an inactive AR are necessary for external genitalia differentiation (Speroff et al., 1999). In addition, according to the rodent model of inactivated estrogen alpha receptor gene (ERα), estrogen action seems to be necessary for the final female morphology (Yang et al., 2010). In our study, the mean measured AGD for females was 16.48 mm, which was similar than the AGD reported in other studies carried out in Mexico (Loreto-Gómez et al., 2018; Salazar-Martinez et al., 2004), in the US (Barrett et al., 2017; Callegari et al., 1987; Wenzel et al., 2018), and in Israel (Phillip et al., 1996). The mean AGI for girls in the present study was lower than that reported in Taiwan (Huang et al., 2009). As for results pertaining to males, differences in AGD in these studies may be explained by different measuring devices or measurement techniques (i.e., anofourchette or anoclitoral distance), age at examination, body size, and ethnicity.

To our knowledge, no human studies have previously linked PCB or PBDE exposure to AGD in females. Our data showing a meaningfully increased AGD in girls exposed to 4,4′-DDE, 4,4′-DDT, and PCB-101 may be the first findings suggesting a role of estrogen action during human female external genitalia development. In particular, 4,4′-DDE is best known for its estrogenic activity (Chen et al., 1997; Kuiper et al., 1998), and the mechanisms underlying the relationship between prenatal 4,4′-DDE and AGD are uncertain. A possible explanation is that the balance between the estrogenic and anti-androgenic effect of DDE is marked by increased estrogenic activity. Evidence suggests that exposure to DDT/DDE is associated with younger age at menarche and increased incidence of shortened menstrual cycles (Ouyang et al., 2005). A South African study found maternal serum DDT concentrations at delivery were associated with shorter AGD in female offspring (Bornman et al., 2016), which contrasts with our findings, perhaps because of higher environmental DDT levels in South Africa. Maternal exposure to bisphenol A (BPA) has been associated with shorter distance from the center of the anus to the clitoris in newborn females, but no association was found between maternal BPA and infant distance from the center of the anus to the fourchette (Barrett et al., 2017). This study hypothesized that prenatal exposure to EDCs may affect AGD through estrogenic pathways, which may explain our results. Androgenic environments during gestation can develop masculinization, generating females with higher AGD (Hotchkiss et al., 2007; Wolf et al., 2002).

Studies in female animals support the hypothesis that prenatal exposure to compounds with androgenic activity results in longer AGD (Dean et al., 2012; Wolf et al., 2002; Wu et al., 2010), perhaps because exposure to androgens or estrogens can regulate their own expression of the receptor; that is, estrogens/androgens can act in a differentiated way and the expression of the receptor of one of them can be repressed. This idea is supported by studies on ERα knockout in mouse females that in the absence of estrogen action, showed masculinized external genitalia that was explained by a balance between sex hormones during pregnancy; for a long time, it was thought that female genitalia represent the default state (Yang et al., 2010). In humans, no such model of ERα absence with slightly virilized external genitalia at birth has been described. However, this is an extremely rare genetic condition in which virilization signs have been described in adults in the context of

increased testosterone production from polycystic ovaries (Quaynor et al., 2013). As genital development models in rodents reproduce human ones, it may be that external genitalia in human females with ERα at birth or prepuberty have not yet been explored.

We previously observed that OC compounds (Lopez-Espinosa et al., 2016) and PBDEs (Lopez-Espinosa et al., 2015) can interfere in fetal growth and be transported across the placenta (Vizcaino et al., 2014a). We also observed that PBDE-99 and PBDE-153 were associated with reduced AGD in males at age 18 months (García-Villarino et al., 2018). Differences in the results from this study and our previous work may be explained by the difference in the sample size and by the inclusion of interactions with other compounds in the present study. The interactions studied in our models evaluated the synergistic effect of the concentration of compounds such as 4,4-DDE, PCB-138, or PBDE-99. All of these are considered potent antiandrogenic agents (Lilienthal et al., 2006; Portigal et al., 2002; Torres-Sanchez et al., 2008). One potential explanation for this interaction is that higher levels of each compound (PCB-138 and 4,4′-DDE) might induce the decrease of AGI in males and the interactions effects appear to be synergistic, which may be of significance given the common coexposures to multiple contaminants. It is not clear, however, which pathways participate in this mechanism, so further investigation is needed. Nevertheless, in this study and our previous study, we showed that prenatal exposure to POPs was inversely associated with AGI in boys, which was consistent with most scientific literature published to date. In addition, there is a strong maternal-fetal correlation for the concentration of these pollutants measured in both matrices of the present study. This is consistent with the transfer of pollutants from mother to fetus (Vizcaino et al., 2014a), which may explain why AGI in males was associated with PCB-153 and PCB-180 in both maternal and cord serum samples. However, this finding has not been confirmed in females, which may be attributable to the small sample size for maternal serum compared with cord serum and the relatively low exposure level in our study. This may make the exploration of potential association difficult. Furthermore, the effects of POPs on AGD may be time-delayed in females, because at age 4 years we found some associations that had not been observed in our previous study at age 18 months. Individual susceptibility may also play a role in the effects of these hormonally active agents.

Several limitations of this study should be noted. It is possible that our sample size lacked the power to detect associations because stratified our study population by the sex of the children, which decreased the sample size. Interactions with compounds such as phthalates, PCB-like dioxins, BPA, or perfluoroalkyl substances and residual confounders could also have affected the association between the pollutants we studied and AGD (Luan et al., 2019; Sun et al., 2018). AGD measurements were performed by two pediatricians, leading to possible variability in the results, which could also be affected by the child's movements during the physical examination. However, only one measurement for each AGD was recorded, as assessment of intra-rater reliability (10% of the sample) showed no substantial bias. Another limitation was the possibility of false positive results; it should be noted that if there was actually no association between AGI and any POP, we would obtain around 3.6 out of 72 *p*-values lower than 0.05. Nevertheless, we obtained four statistically significant results after applying the Benjamini-Hochberg procedure, that is a powerful tool that decreases the false discovery rate. A major strength of this study was the longitudinal cohort design with a long follow-up period and good participation rates (94% participation rate). No clear pattern of dose-response relationship was observed in our study. But the stronger and more accurate effects of some congeners for the second and third tertiles might be explained by the fact that hormones have complicated concentration-response patterns, and these lay the foundation for the dose-response characteristics exhibited by EDCs (Gore et al., 2015). These endocrine characteristics demonstrate why it is difficult to define the power of an EDC, why action thresholds are not possible to identify, and why non-linear dose responses cannot be ignored, despite the continuing controversy on this point. Measurements of AGD were taken at 18 months and 4 years, with maternal samples collected in the first trimester of pregnancy, which is possibly the most important period for fetal reproductive system development. This study also contributed data on AGD at an age that has not previously been investigated and included data for female children, which to date have been scarce. We measured a large number of OC and brominated compounds in both maternal blood serum and cord blood serum, and analyzed summed congeners and the interaction between compounds (i.e., 4,4′-DDE, PCB-138, and PBDE-99) because they are compounds that have a high anti-androgen capacity and that have been reported to have an effect on the AGD. VIF values greater than 10 would indicate that the linear regression model presents a degree of worrying multicollinearity, in the case of our models the VIF values vary in the range between 1.12 and 7.34 and therefore do not present a multicollinearity. Lastly, to capture the complex nature of environmental exposure as a whole, it might be necessary to move from a single-pollutant to a multipollutant approach.

In summary, our findings provide evidence of inverse associations between specific POPs and AGI in boys and positive association in girls at age 4 years, perhaps through a feminizing effect in males and a masculinizing effect in females. These findings are consistent with previous rodent and human studies. Whether the changes we observed in AGI persist throughout life and contribute to clinically relevant results (e.g., endometriosis or testicular cancer) should be further investigated and confirmed in subsequent studies.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113563.

References

Agramunt, S., Kogevinas, M., Carreras, R., 2011. [Anogenital distance in newborns: a sensitive marker of prenatal hormonal disruption]. Med. Clin. 137, 459–463. https://doi.org/10.1016/j.medcli.2010.03.024.

Avidime, O.M., Olorunshola, K.V., Dikko, A.A.U., 2011. Anogenital distance of newborns and causes of variation in Zaria , northern Nigeria. Asian J. Med. Sci. 3, 74–78.

Barrett, E.S., Parlett, L.E., Redmon, J.B., Swan, S.H., 2014. Evidence for sexually dimorphic associations between maternal characteristics and Anogenital distance, a marker of reproductive development. Am. J. Epidemiol. https://doi.org/10.1093/aje/kwt220.

Barrett, E.S., Sathyanarayana, S., Mbowe, O., Thurston, S.W., Redmon, J.B., Nguyen, R.H.N., Swan, S.H., 2017. First-trimester urinary bisphenol a concentration in relation to anogenital distance, an androgen-sensitive measure of reproductive development, in infant girls. Environ. Health Perspect. https://doi.org/10.1289/EHP875.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bornman, M.S., Chevrier, J., Rauch, S., Crause, M., Obida, M., Sathyanarayana, S., Barr, D.B., Eskenazi, B., 2016. Dichlorodiphenyltrichloroethane exposure and anogenital distance in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) birth cohort study, South Africa. Andrology 4, 608–615. https://doi.org/10.1111/andr.12235.

Callegari, C., Everett, S., Ross, M., Brasel, J.A., 1987. Anogenital ratio: measure of fetal virilization in premature and full-term newborn infants. J. Pediatr. 111, 240–243.

https://doi.org/10.1016/S0022-3476(87)80075-6.
Carrizo, D., Grimalt, J.O., Ribas-Fito, N., Sunyer, J., Torrent, M., 2006. Physical-chemical and maternal determinants of the accumulation of organochlorine compounds in four-year-old children. Environ. Sci. Technol. 40, 1420–1426.
Casas, M., Nieuwenhuijsen, M., Martinez, D., Ballester, F., Basagana, X., Basterrechea, M., Chatzi, L., Chevrier, C., Eggesbo, M., Fernandez, M.F., Govarts, E., Guxens, M., Grimalt, J.O., Hertz-Picciotto, I., Iszatt, N., Kasper-Sonnenberg, M., Kiviranta, H., Kogevinas, M., Palkovicova, L., Ranft, U., Schoeters, G., Patelarou, E., Petersen, M.S., Torrent, M., Trnovec, T., Valvi, D., Toft, G.V., Weihe, P., Weisglas-Kuperus, N., Wilhelm, M., Wittsiepe, J., Vrijheid, M., Bonde, J.P., 2015. Prenatal exposure to PCB-153, p,p'-DDE and birth outcomes in 9000 mother-child pairs: exposure-response relationship and effect modifiers. Environ. Int. 74, 23–31. https://doi.org/10.1016/j.envint.2014.09.013.
Chen, C.W., Hurd, C., Vorojeikina, D.P., Arnold, S.F., Notides, A.C., 1997. Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. Biochem. Pharmacol. 53, 1161–1172. https://doi.org/10.1016/S0006-2952(97)00097-X.
Costa, O., Lopez-Espinosa, M.J., Vizcaino, E., Murcia, M., Iniguez, C., Navarrete-Munoz, E.M., Grimalt, J.O., Tardon, A., Ballester, F., Fernandez-Somoano, A., 2016. Dietary and household sources of prenatal exposure to polybrominated diphenyl ethers (PBDEs) in the INMA birth cohort (Spain). Environ. Sci. Technol. 50, 5935–5944. https://doi.org/10.1021/acs.est.5b06263.
Dalsager, L., Christensen, L.E., Kongsholm, M.G., Kyhl, H.B., Nielsen, F., Schoeters, G., Jensen, T.K., Andersen, H.R., 2018. Associations of maternal exposure to organophosphate and pyrethroid insecticides and the herbicide 2,4-D with birth outcomes and anogenital distance at 3 months in the Odense Child Cohort. Reprod. Toxicol. https://doi.org/10.1016/j.reprotox.2017.12.008.
Dang, V.H., Choi, K.C., Jeung, E.B., 2007. Tetrabromodiphenyl ether (BDE 47) evokes estrogenicity and calbindin-D9k expression through an estrogen receptor-mediated pathway in the uterus of immature rats. Toxicol. Sci. 97, 504–511. https://doi.org/10.1093/toxsci/kfm051.
De Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere. https://doi.org/10.1016/S0045-6535(01)00225-9.
Dean, A., Sharpe, R.M., 2013. Anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. J. Clin. Endocrinol. Metab. 98, 2230–2238. https://doi.org/10.1210/jc.2012-4057.
Dean, A., Smith, L.B., Macpherson, S., Sharpe, R.M., 2012. The effect of dihydrotestosterone exposure during or prior to the masculinization programming window on reproductive development in male and female rats. Int. J. Androl. https://doi.org/10.1111/j.1365-2605.2011.01236.x.
Drake, A.J., Van Den Driesche, S., Scott, H.M., Hutchison, G.R., Seckl, J.R., Sharpe, R.M., 2009. Glucocorticoids amplify dibutyl phthalate-induced disruption of testosterone production and male reproductive development. Endocrinology. https://doi.org/10.1210/en.2009-0700.
Fernández-Somoano, A., Estarlich, M., Ballester, F., Fernández-Patier, R., Aguirre-Alfaro, A., Herce-Garraleta, M.D., Tardón, A., 2011. Outdoor NO2and benzene exposure in the INMA (environment and childhood) Asturias cohort (Spain). Atmos. Environ. https://doi.org/10.1016/j.atmosenv.2011.02.010.
Fernández-Somoano, A., Tardon, A., 2014. Socioeconomic status and exposure to outdoor NO2 and benzene in the Asturias INMA birth cohort, Spain. J. Epidemiol. Community Health 68, 29–36. https://doi.org/10.1136/jech-2013-202722.
Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third ed. R topics documented.
Freire, C., Ocón-Hernández, O., Dávila-Arias, C., Pérez-Lobato, R., Calvente, I., Ramos, R., Olea, N., Fernández, M.F., 2018. Anogenital distance and reproductive outcomes in 9- to 11-year-old boys: the INMA-Granada cohort study. Andrology 1–8. https://doi.org/10.1111/andr.12544.
García-Villarino, M., Riaño-Galán, I., Rodriguez-Dehli, A.C., Vizcaíno, E., Grimalt, J.O., Tardón, A., Fernández-Somoano, A., 2018. Prenatal exposure to persistent organic pollutants and anogenital distance in children at 18 months. Horm. Res. Paediatr. 90, 1–7. https://doi.org/10.1159/000492236.
Gari, M., Grimalt, J., 2010. Use of proficiency testing materials for the calculation of detection and quantification limits in the analysis of organochlorine compounds in human serum. Anal. Bioanal. Chem. 397, 1383–1387. https://doi.org/10.1007/s00216-010-3638-3.
Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, E1–E150. https://doi.org/10.1210/er.2015-1010.
Gray, L.E., 2001. Effects of environmental antiandrogens on reproductive development in experimental animals. Hum. Reprod. Update 7, 248–264. https://doi.org/10.1093/humupd/7.3.248.
Gray, L.E., Ostby, J.S., 1995. In utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. Toxicol. Appl. Pharmacol. https://doi.org/10.1006/taap.1995.1153.
Grimalt, J.O., Carrizo, D., Garí, M., Font-Ribera, L., Ribas-Fito, N., Torrent, M., Sunyer, J., 2010a. An evaluation of the sexual differences in the accumulation of organochlorine compounds in children at birth and at the age of 4 years. Environ. Res. 110, 244–250. https://doi.org/10.1016/j.envres.2010.01.005.
Grimalt, J.O., Howsam, M., Carrizo, D., Otero, R., De Marchi, M.R.R., Vizcaino, E., 2010b. Integrated analysis of halogenated organic pollutants in sub-millilitre volumes of venous and umbilical cord blood sera. Anal. Bioanal. Chem. 396, 2265–2272. https://doi.org/10.1007/s00216-010-3460-y.
Hass, U., Scholze, M., Christiansen, S., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Metzdorff, S.B., Kortenkamp, A., 2007. Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. Environ. Health Perspect. https://doi.org/10.1289/ehp.9360.
Hoffmann, F., Kloas, W., 2016. p,p′-Dichlordiphenyldichloroethylene (p,p′-DDE) can elicit antiandrogenic and estrogenic modes of action in the amphibian Xenopus laevis. Physiol. Behav. https://doi.org/10.1016/j.physbeh.2016.09.012.
Hotchkiss, A.K., Lambright, C.S., Ostby, J.S., Parks-Saldutti, L., Vandenbergh, J.G., Gray, L.E., 2007. Prenatal testosterone exposure permanently masculinizes anogenital distance, nipple development, and reproductive tract morphology in female sprague-dawley rats. Toxicol. Sci. https://doi.org/10.1093/toxsci/kfm002.
Huang, P.C., Kuo, P.L., Chou, Y.Y., Lin, S.J., Lee, C.C., 2009. Association between prenatal exposure to phthalates and the health of newborns. Environ. Int. 35, 14–20. https://doi.org/10.1016/j.envint.2008.05.012.
Jin, M.H., Ko, H.K., Hong, C.H., Han, S.W., 2008. In utero exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin affects the development of reproductive system in mouse. Yonsei Med. J. 49, 843–850. https://doi.org/10.3349/ymj.2008.49.5.843.
Kaya, H., Hany, J., Fastabend, A., Roth-Härer, A., Winneke, G., Lilienthal, H., 2002. Effects of maternal exposure to a reconstituted mixture of polychlorinated biphenyls on sex-dependent behaviors and steroid hormone concentrations in rats: dose-response relationship. Toxicol. Appl. Pharmacol. https://doi.org/10.1006/taap.2001.9318.
Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., Van Der Saag, P.T., Van Der Burg, B., Gustafsson, J.Å., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139, 4252–4263. https://doi.org/10.1210/endo.139.10.6216.
Legler, J., 2008. New insights into the endocrine disrupting effects of brominated flame retardants. Chemosphere 73, 216–222. https://doi.org/10.1016/j.chemosphere.2008.04.081.
Lilienthal, H., Hack, A., Roth-Härer, A., Grande, S.W., Talsness, C.E., 2006. Effects of developmental exposure to 2,2′,4,4′, 5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. Environ. Health Perspect. https://doi.org/10.1289/ehp.8391.
Lind, D.V., Priskorn, L., Lassen, T.H., Nielsen, F., Kyhl, H.B., Kristensen, D.M., Christesen, H.T., Jørgensen, J.S., Grandjean, P., Jensen, T.K., 2017. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod. Toxicol. 68, 200–206. https://doi.org/10.1016/j.reprotox.2016.08.019.
Longnecker, M.P., Gladen, B.C., Cupul-Uicab, L.A., Romano-Riquer, S.P., Weber, J.P., Chapin, R.E., Hernandez-Avila, M., 2007. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, Mexico. Am. J. Epidemiol. 165, 1015–1022. https://doi.org/10.1093/aje/kwk109.
Lopez-Espinosa, M.J., Costa, O., Vizcaino, E., Murcia, M., Fernandez-Somoano, A., Iñiguez, C., Llop, S., Grimalt, J.O., Ballester, F., Tardon, A., 2015. Prenatal exposure to polybrominated flame retardants and fetal growth in the INMA cohort (Spain). Environ. Sci. Technol. 49, 10108–10116. https://doi.org/10.1021/acs.est.5b01793.
Lopez-Espinosa, M.J., Murcia, M., Iñiguez, C., Vizcaino, E., Costa, O., Fernández-Somoano, A., Basterrechea, M., Lertxundi, A., Guxens, M., Gascon, M., Goñi-Irigoyen, F., Grimalt, J.O., Tardón, A., Ballester, F., 2016. Organochlorine compounds and ultrasound measurements of fetal growth in the INMA cohort (Spain). Environ. Health Perspect. https://doi.org/10.1289/ehp.1408907.
Loreto-Gómez, C., Farías, P., Moreno-Macías, H., Guzmán, C., Riojas-Rodríguez, H., 2018. Prenatal exposure to persistent organic compounds and their association with anogenital distance in infants. Reprod. Biomed. Online 37, 732–740. https://doi.org/10.1016/j.rbmo.2018.09.008.
Luan, M., Liang, H., Yang, F., Yuan, W., Chen, A., Liu, X., Ji, H., 2019. Prenatal polybrominated diphenyl ethers exposure and anogenital distance in boys from a Shanghai birth cohort. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2019.01.008. 0–1.
McIntyre, B.S., Barlow, N.J., Foster, P.M., 2001. Androgen-mediated development in male rat offspring exposed to flutamide in utero: permanence and correlation of early postnatal changes in anogenital distance and nipple retention with malformations in androgen-dependent tissues. Toxicol. Sci. 62, 236–249.
Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, Å., Lemmen, J.G., Van Der Burg, B., Brouwer, A., 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. Environ. Health Perspect. 109, 399–407. https://doi.org/10.1289/ehp.01109399.
Mendiola, J., Oñate-Celdrán, J., Samper-Mateo, P., Arense-Gonzalo, J.J., Torres-Roca, M., Sánchez-Rodríguez, C., García-Escudero, D., Fontana-Compiano, L.O., Eisenberg, M.L., Swan, S.H., Torres-Cantero, A.M., 2016. Comparability and reproducibility of adult male anogenital distance measurements for two different methods. Andrology. https://doi.org/10.1111/andr.12202.
Mendiola, J., Roca, M., Mínguez-Alarcón, L., Mira-Escolano, M.P., López-Espín, J.J., Barrett, E.S., Swan, S.H., Torres-Cantero, A.M., 2012. Anogenital distance is related to ovarian follicular number in young Spanish women: a cross-sectional study. Environ. Health. https://doi.org/10.1186/1476-069X-11-90.
Miao, M., Yuan, W., He, Y., Zhou, Z., Wang, J., Gao, E., Li, G., Li, D.K., 2011. In utero exposure to bisphenol-A and anogenital distance of male offspring. Birth Defects Res. Part A Clin. Mol. Teratol. 91, 867–872. https://doi.org/10.1002/bdra.22845.
Mira-Escolano, M.P., Mendiola, J., Mínguez-Alarcõn, L., Melgarejo, M., Cutillas-Tolín, A., Roca, M., Lõpez-Espín, J.J., Noguera-Velasco, J.A., Torres-Cantero, A.M., 2014. Longer anogenital distance is associated with higher testosterone levels in women: a cross-sectional study. BJOG An Int. J. Obstet. Gynaecol. https://doi.org/10.1111/1471-0528.12627.
Moore, R.W., Rudy, T.A., Lin, T.M., Ko, K., Peterson, R.E., 2001. Abnormalities of sexual development in male rats with in utero and lactational exposure to the anti-androgenic plasticizer di(2-ethylhexyl) phthalate. Environ. Health Perspect. 109,

229–237. https://doi.org/10.1289/ehp.01109229.
Ouyang, F., Perry, M., Venners, S., Chen, C., Wang, B., Yang, F., Fang, Z., Zang, T., Wang, L., Xu, X., Wang, X., 2005. Serum DDT, age at menarche, and abnormal menstrual cycle length. Occup. Environ. Med. 62, 878–884. https://doi.org/10.1136/oem.2005.020248.
Phillip, M., De Boer, C., Pilpel, D., Karplus, M., Sofer, S., 1996. Clitoral and penile sizes of full term newborns in two different ethnic groups. J. Pediatr. Endocrinol. Metab. 9, 175–179.
Phillips, D.L., Pirkle, J.L., Burse, V.W., Bernert, J.T., Henderson, L.O., Needham, L.L., 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch. Environ. Contam. Toxicol. 18, 495–500.
Portigal, C.L., Cowell, S.P., Fedoruk, M.N., Butler, C.M., Rennie, P.S., Nelson, C.C., 2002. Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. Toxicol. Appl. Pharmacol. https://doi.org/10.1006/taap.2002.9371.
Quaynor, S.D., Stradtman, E.W., Kim, H.G., Shen, Y., Chorich, L.P., Schreihofer, D.A., Layman, L.C., 2013. Delayed puberty and estrogen resistance in a woman with estrogen receptor α variant. N. Engl. J. Med. https://doi.org/10.1056/NEJMoa1303611.
R Core Team, 2014. R Core Team (2014). R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria. ISBN 3–900051-07–0, URL. http://www.R-project.org/.
Riaño-Galán, I., Fernández-Somoano, A., Rodríguez-Dehli, C., Valvi, D., Vrijheid, M., Tardón, A., 2017. Proatherogenic lipid profile in early childhood: association with weight status at 4 Years and parental obesity. J. Pediatr. 187, 153–157. https://doi.org/10.1016/j.jpeds.2017.04.042. e2.
Rosenberg, B.G., Chen, H., Folmer, J., Liu, J., Papadopoulos, V., Zirkin, B.R., 2008. Gestational exposure to atrazine: effects on the postnatal development of male offspring. J. Androl. 29, 304–311. https://doi.org/10.2164/jandrol.107.003020.
Saillenfait, A.M., Sabate, J.P., Gallissot, F., 2009. Effects of in utero exposure to di-n-hexyl phthalate on the reproductive development of the male rat. Reprod. Toxicol. 28, 468–476. https://doi.org/10.1016/j.reprotox.2009.06.013.
Salazar-Martinez, E., Romano-Riquer, P., Yanez-Marquez, E., Longnecker, M.P., Hernandez-Avila, M., 2004. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. Environ. Health Perspect. 3, 8. https://doi.org/10.1186/1476-069X-3-8.
Sánchez-Ferrer, M.L., Mendiola, J., Jiménez-Velázquez, R., Cánovas-López, L., Corbalán-Biyang, S., Hernández-Peñalver, A.I., Carmona-Barnosi, A., Maldonado-Cárceles, A.B., Prieto-Sánchez, M.T., Machado-Linde, F., Nieto, A., Torres-Cantero, A.M., 2017. Investigation of anogenital distance as a diagnostic tool in endometriosis. Reprod. Biomed. Online 34, 375–382. https://doi.org/10.1016/j.rbmo.2017.01.002.
SCOPS, 2009. Stockholm convention on persistent organic pollutants (POPs). [WWW Document]. URL. http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx.
Singal, A.K., Jain, V.G., Gazali, Z., Shekhawat, P., 2016. Shorter anogenital distance correlates with the severity of hypospadias in pre-pubertal boys. Hum. Reprod. https://doi.org/10.1093/humrep/dew115.
Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum. Reprod. 16, 972–978.
Speroff, L., Glass, R.H., Kase, N.G., 1999. Clinical Gynecologic Endocrinology and Infertility. Lippincott Williams & Wilkins.
Stoker, T.E., Cooper, R.L., Lambright, C.S., Wilson, V.S., Furr, J., Gray, L.E., 2005. In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. Toxicol. Appl. Pharmacol. 207, 78–88. https://doi.org/10.1016/j.taap.2005.05.010.
Sun, X., Li, D., Liang, H., Miao, M., Song, X., Wang, Z., Zhou, Z., Yuan, W., 2018. Maternal exposure to bisphenol A and anogenital distance throughout infancy: a longitudinal study from Shanghai, China. Environ. Int. 121, 269–275. https://doi.org/10.1016/j.envint.2018.08.055.
Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113, 1056–1061.
Swan, S.H., Sathyanarayana, S., Barrett, E.S., Janssen, S., Liu, F., Nguyen, R.H.N., Redmon, J.B., 2015. First trimester phthalate exposure and anogenital distance in newborns. Hum. Reprod. 30, 963–972. https://doi.org/10.1093/humrep/deu363.
Textor, J., van der Zander, B., Gilthorpe, M.S., Liśkiewicz, M., Ellison, G.T., 2016. Robust causal inference using directed acyclic graphs: the R package "dagitty.". Int. J. Epidemiol. 45, 1887–1894. https://doi.org/10.1093/ije/dyw341.
Thankamony, A., Lek, N., Carroll, D., Williams, M., Dunger, D.B., Acerini, C.L., Ong, K.K., Hughes, I.A., 2014. Anogenital distance and penile length in infants with hypospadias or cryptorchidism: comparison with normative data. Environ. Health Perspect. https://doi.org/10.1289/ehp.1307178.
Thankamony, A., Ong, K.K., Dunger, D.B., Acerini, C.L., Hughes, I.A., 2009. Anogenital distance from birth to 2 years: a population study. Environ. Health Perspect. 117, 1786–1790. https://doi.org/10.1289/ehp.0900881.
Thankamony, A., Pasterski, V., Ong, K.K., Acerini, C.L., Hughes, I.A., 2016. Anogenital distance as a marker of androgen exposure in humans. Andrology 4, 616–625. https://doi.org/10.1111/andr.12156.
Torres-Sanchez, L., Zepeda, M., Cebrián, M.E., Belkind-Gerson, J., Garcia-Hernandez, R.M., Belkind-Valdovinos, U., López-Carrillo, L., 2008. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Ann. N. Y. Acad. Sci. 1140, 155–162. https://doi.org/10.1196/annals.1454.004.
Vafeiadi, M., Agramunt, S., Papadopoulou, E., Besselink, H., Mathianaki, K., Karakosta, P., Spanaki, A., Koutis, A., Chatzi, L., Vrijheid, M., Kogevinas, M., 2013. In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. Environ. Health Perspect. https://doi.org/10.1289/ehp.1205221.
Vizcaino, E., Arellano, L., Fernandez, P., Grimalt, J.O., 2009. Analysis of whole congener mixtures of polybromodiphenyl ethers by gas chromatography-mass spectrometry in both environmental and biological samples at femtogram levels. J. Chromatogr. A 1216, 5045–5051. https://doi.org/10.1016/j.chroma.2009.04.049.
Vizcaino, E., Grimalt, J.O., Fernández-Somoano, A., Tardon, A., 2014a. Transport of persistent organic pollutants across the human placenta. Environ. Int. 65, 107–115. https://doi.org/10.1016/j.envint.2014.01.004.
Vizcaino, E., Grimalt, J.O., Glomstad, B., Fernández-Somoano, A., Tardón, A., 2014b. Gestational weight gain and exposure of newborns to persistent organic pollutants. Environ. Health Perspect. 122, 873–879. https://doi.org/10.1289/ehp.1306758.
Vizcaino, E., Grimalt, J.O., Lopez-Espinosa, M.J., Llop, S., Rebagliato, M., Ballester, F., 2010. Maternal origin and other determinants of cord serum organochlorine compound concentrations in infants from the general population. Environ. Sci. Technol. 44, 6488–6495. https://doi.org/10.1021/es101397e.
Wenzel, A.G., Bloom, M.S., Butts, C.D., Wineland, R.J., Brock, J.W., Cruze, L., Unal, E.R., Kucklick, J.R., Somerville, S.E., Newman, R.B., 2018. Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls. Environ. Int. https://doi.org/10.1016/j.envint.2017.10.007.
Wolf, C.J., Hotchkiss, A., Ostby, J.S., LeBlanc, G.A., Gray, L.E., 2002. Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. Toxicol. Sci. https://doi.org/10.1093/toxsci/65.1.71.
Wolf, C.J., LeBlanc, G.A., Gray Jr., L.E., 2004. Interactive effects of vinclozolin and testosterone propionate on pregnancy and sexual differentiation of the male and female SD rat. Toxicol. Sci. 78, 135–143. https://doi.org/10.1093/toxsci/kfh018.
Wu, X.-Y., Li, Z.-L., Wu, C.-Y., Liu, Y.-M., Lin, H., Wang, S.-H., Xiao, W.-F., 2010. Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague-Dawley rats. Endocr. J.
Yang, J.H., Menshenina, J., Cunha, G.R., Place, N., Baskin, L.S., 2010. Morphology of mouse external genitalia: implications for a role of erstrogen in sexual dimorphism of the mouse genital tubercle. J. Urol. https://doi.org/10.1016/j.juro.2010.03.079.