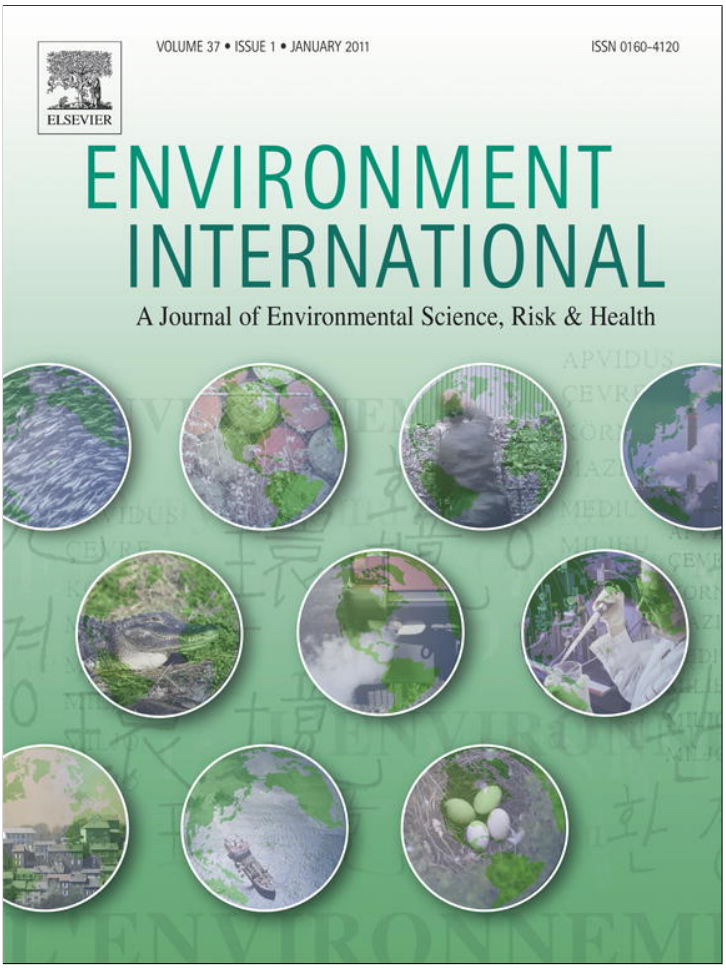

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The effect of in utero exposure to dioxins and polychlorinated biphenyls on reproductive development in eight year-old children ☆,☆☆

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ABSTRACT

We have previously reported on the effects of in utero exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) on thyroid function and growth hormone concentrations at birth and in two and five year-old children. Herein, we present our most recent follow-up examination findings for the same cohort of children at eight-years of age. A total of 56 children (23 boys, 33 girls) were examined. Bone age (BA), hormone concentrations, and indicators of reproductive development including Tanner, breast, genital, and armpit stages were assessed. Estradiol concentrations were significantly lower in children exposed to higher levels than median of PCDD/Fs + PCBs TEQ compared to the children exposed to levels lesser than median ($P = 0.003$). Girls exposed to higher levels than median of indicator PCBs had a significantly greater proportion in genital stage 1 and shorter fundi and uteri lengths, as compared to those exposed to low levels ($P = 0.025$ and $P < 0.05$, respectively). There was a significant negative relationship between estradiol concentrations and PCDD/Fs + PCB exposure level ($P = 0.005$). After adjusting for BA, there was a significant association between fundus length and indicator PCB exposure level ($P = 0.034$). Exposure to both high levels of ΣPCDD/Fs + PCBs TEQ and high levels of total PCBs was associated with decreased fundus length ($P = 0.016$) and uterus length ($P = 0.016$). In utero exposure to high levels of PCDD/Fs and PCBs may result in lower estradiol concentrations in eight year-old children and impaired reproductive development in girls.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are byproducts of industrial processes that can become persistent organic pollutants. Accumulation of, or acute exposure to, PCDD/Fs may cause a variety of deleterious effects, including altered hormone levels, growth, immune responsiveness, metabolism, cardiovascular disease, and cancer (White and Birnbaum, 2009).

PCDD/Fs are readily transferred from mother to fetus via the placenta and to infant via breast milk (Chao et al., 2004; Koopman-Esseboom et al., 1994; Ma et al., 2002; Wang et al., 2004). Being much smaller than adults, fetuses and infants would therefore appear to be at a much greater risk of being adversely affected by exposure to PCDD/Fs. Indeed, there is evidence that in utero exposure to PCDD/Fs and other environmental contaminants (such as polychlorinated biphenyls (PCBs)) can result in altered thyroid hormone levels (Baccarelli et al., 2008; Langer, 2008), neurobehavioral deficits (Lundqvist et al., 2006), and altered timing of puberty onset (Leijs et al., 2008). However, as these were observational studies, further evidence with continuing follow-up is required before we can confirm the effects of in utero exposure to PCDD/Fs on subsequent development.

We have been following a cohort of children exposed to PCDD/Fs and PCBs in utero and have previously reported on the effects of this exposure on thyroid function and growth hormone concentrations at birth (Wang et al., 2005) and at two and five years of age (Su et al., 2010). In our most recently published report we described how two year-old girls exposed to high levels of PCDD/Fs and PCBs in utero were significantly taller and heavier and had higher serum concentrations of several thyroid hormones than girls exposed to low levels of PCDD/Fs and PCBs (Su et al., 2010). In this report, we present our

Abbreviations: BA, bone age; CA, chronological age; E2, estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; PCBs, polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans.

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most recent findings from the same group of children who were prepubertal (aged eight-years) at the time of follow-up. As well as examining indicators of growth and hormone levels, we focused on assessing indicators of reproductive development for girls.

2. Methods

2.1. Participants

A total of 56 children were included in this phase of follow-up which took place between November 2001 and August 2009. All children were eight years-old at the time of follow-up. The characteristics of these children and their mothers have been fully described previously (Wang et al., 2005).

As previously described (Su et al., 2010; Wang et al., 2005), children were stratified into low median placental PCDD/Fs + PCBs exposure and high median placental PCDD/Fs + PCBs exposure groups according their mother's overall median exposure level. The overall median ΣPCDD/Fs + ΣPCBs toxic equivalent (TEQ) level was 14.83 pg WHO_{98}-TEQ/g (cut-off point), while the overall median Σco-planer PCBs level was 22.56 ng/g lipid (cut-off point), and the median Σortho-PCBs level was 4870.2 ng/g lipid (cut-off point). The TEQ assesses the toxicity of dioxins and dioxin-like compounds relative to that of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). The TEQ is calculated by multiplying the amount of each toxic compound by the designated 1998 WHO toxic equivalency factor (TEF) and then adding the values (the notation WHO_{98}-TEQ may be used as above) (Van den Berg et al., 1998).

This study was approved by the ethics review committee of National Health Research Institutes, Taiwan and each participating child's parent provided written informed consent at eight years-old.

2.2. Measurements and blood sampling

Demographic information included gender, chronological age (CA), bone age (BA), and the ratio of BA to CA. Bone age was determined by examining left hand radiographs using the method of Greulich and Pyle (Tanner et al., 2001).

Eight-hour fasting blood samples were obtained at follow-up and immediately centrifuged and the serum separated and frozen at −70 °C for later analysis. Serum concentrations of testosterone (TT), estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), triglyceride (TG), cholesterol, and insulin were measured and compared between gender and PCDD/Fs and PCB median levels.

Serum TT concentrations were measured by solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000 Advanced Immunoassay System, Siemens Medical Solutions Diagnostics, Deerfield, IL). The sensitivity of this assay was 1.5 ng/mL. Intra- and interassay coefficients of variation (CV) were 9.7% and 12.0%.

Serum E2 levels were measured using a radioimmunoassay kit (RIA, Diagnostic Systems Laboratories, Santa Monica, CA). The sensitivity of this assay was 0.22 ng/dL. Intra- and interassay CVs were 7.5% and 9.3%.

Serum FSH, LH, and insulin concentrations were measured by enzyme immunoassay (FSH: Abbott Laboratories, Rome, Italy; LH: Dade Behring, Milan, Italy; Insulin: Siemens, Washington, DC). The sensitivity for FSH, LH, insulin assays were 0.2 mIU/mL, 0.2 mIU/mL and 2 μIU/mL, respectively. Intra- and interassay CVs were 4.7% and 8.9% for FSH, 3.1% and 4.0% for LH, and 5.5% and 7.3% for insulin, respectively.

Serum concentrations of cholesterol and TG were measured by enzymatic color test using an OLYMPUS analyser (AU600, Clare, Ireland). The sensitivity for cholesterol was 3 mg/dL (0.07 mmol/L), while the intra- and interassay CVs were 0.91% and 1.06%. The sensitivity for TG was 1.77 mg/dL (0.02 mmol/L). The intra- and interassay coefficients of variation (CVs) were 1.37% and 1.81%.

2.3. Evaluation of reproductive development

All children underwent detailed physical examinations and reproductive development was scored by the same investigator according to Tanner staging (Marshall and Tanner, 1969). Genital and armpit development were defined according to the patterns of pubertal change defined by Marshall and Tanner (Marshall and Tanner, 1969, 1970).

All girls also underwent full-bladder, transabdominal ultrasound scans for complete pelvic organ examination (Badouraki et al., 2008). Scans were performed using a SIEMENS ACUSON Antares ultrasound (Siemens Medical. Solutions, Inc., Malvern, PA) with a transabdominal probe set at 3.64 MHz. Uterine measurements included longitudinal diameter, transverse diameter, uterine body length, and cervix length. Bilateral ovarian measurements included longitudinal and transverse diameters.

2.4. Statistical analysis

Continuous, non-normally distributed data are expressed as median and range (minimum to maximum), while categorical data are expressed as frequency and percentage (%). Median values were compared between groups by Wilcoxon rank sum test, while frequencies were compared by chi-square test or Fisher's exact test if there were fewer than five data points for a given stratified cell. The relationships between fundus length, uterus length, and E2 concentrations and PCDD/Fs and PCBs exposure levels were determined by performing Spearman's correlation analysis. Binary logistic regression analysis was performed to determine the factors associated with hormone levels and reproductive development with or without adjusting for BA. All comparisons were considered statistically significant when $P<0.05$. All data were analyzed using SAS version 9.0 statistical software (SAS Institute Inc., Cary, NC).

3. Results

Twenty-three boys and 33 girls who were exposed to PCDD/Fs and PCBs in utero were included in this study. Age and hormone measurements were generally similar between boys and girls (Table 1); however, several between sex differences were found. Both BA and the BA to CA ratio were significantly higher in girls compared with boys ($P<0.001$). Median insulin concentrations were also significantly higher in girls compared with boys ($P<0.001$) (Table 1).

There were no significant differences in PCDD/Fs or PCBs exposure levels between the boys and girls included in our study (Table 2). Children exposed to PCDD/Fs and PCBs in utero were stratified by high and low median exposure levels to PCDD/Fs + PCBs, indicator PCBs, and ortho-PCBs, and compared. Age and hormone levels were generally unaffected by PCDD/Fs, indicator PCBs, and total ortho-PCBs exposure levels (Table 3). However, E2 concentrations were significantly lower in children exposed to higher levels of PCDD/Fs + PCBs compared with children exposed to lower levels of PCDD/Fs + PCBs ($P=0.003$) (Table 3).

No sex characteristics of boys were affected by the level of exposure to PCDD/Fs and PCBs in utero (data not shown), while several sex characteristics of girls were affected by the level of exposure to PCDD/Fs and PCBs in utero (Table 3, Supplementary Table 1). All 23 boys were of genital stage 1, armpit stage 1, Tanner stage 1, and had no voice changes (data not shown). In contrast, several girls were of genital stage 2, Tanner stages 2 and 3, armpit stage 2, and breast stages 2 and 3 (Supplementary Table 2). None of the girls had experienced menses. All of the girls (100%) exposed to higher levels of total PCBs were of genital stage 1, which was a significantly higher proportion than the girls exposed to lower levels of total PCBs in utero ($P=0.025$). Girls exposed to high levels of total PCBs also had significantly shorter fundi and uteri lengths ($P=0.008$ and 0.013, respectively) than girls exposed to low levels of total PCBs (Table 3). Girls exposed to high levels of total ortho-PCBs also had significantly shorter fundi than girls exposed to low levels of total ortho-PCBs ($P=0.028$) (Table 3).

Correlations between PCDD/Fs + PCBs exposure level and serum E2 concentrations (Fig. 1A), fundus length (Fig. 1B) and uterus length (Fig. 1C) were borderline significant ($P=0.079$, $P=0.074$, $P=0.090$, respectively). There was no significant correlation between serum E2 concentrations and total PCBs exposure level ($P=0.333$, Fig. 2A), while both fundus length (Fig. 2B) and uterus length (Fig. 2C) were significantly correlated with total PCBs exposure level ($P=0.003$, $P=0.004$, respectively).

Binary logistic regression analysis revealed that there was a significant relationship between serum E2 concentrations and PCDD/Fs + PCBs exposure level ($P=0.005$, Table 4). Neither gender, age, nor any hormone variables were related to PCDD/Fs or PCBs exposure levels (Table 4, Supplementary Table 3).

Table 1
Summary of age variables and hormone levels in children exposed to PCDD/Fs and PCBs in utero.

Variable	Reference range[a]	Total (n = 56)	Boys (n = 23)	Girls (n = 33)	*P* value
BA (years)	–	6.5 (4, 11)	6 (4, 9)	9 (5, 11)	<0.001[b]
CA (years)	–	8 (7, 9)	8 (7, 8)	8 (8, 9)	0.168
BA/CA ratio	–	0.8 (0.5, 1.4)	0.8 (0.5, 1.1)	1.1 (0.6, 1.4)	<0.001[b]
Estradiol (ng/dL)	(M: 0.5–2.0; F: 0.5–1.1)	2.3 (ND, 7)	2 (ND, 7)	2.5 (ND, 6.9)	0.218
FSH (mIU/mL)	(M: 1.0–4.2; F: 0.26–3.0)	1.6 (0.4, 11.4)	1.4 (0.4, 2.4)	1.8 (0.5, 11.4)	0.069
LH (mIU/mL)	(M: 0.02–0.18; F: 0.02–0.3)	0.2 (0.1, 47.8)	0.2 (0.1, 35.5)	0.2 (0.1, 47.8)	0.556
Testosterone[c] ($\times 10^{-3}$ ng/mL)	(<3–10)	ND (ND, 0.1)	ND (ND, 0.1)	ND (ND, 0.1)	0.154
Progesterone[c] (ng/dL)	(<10–33)	0.1 (ND, 1.1)	0.1 (ND, 1.1)	0.1 (ND, 0.8)	0.868
Triglyceride (mg/dL)	(15–190)	83 (39, 267)	85 (40, 267)	83 (39, 260)	0.979
Cholesterol (mg/dL)	(M: 122–209; F: 126–191)	162 (118, 289)	155 (126, 250)	163 (118, 289)	0.465
Insulin (μIU/mL)	(<29.1)	14.7 (1.4, 121)	10 (1.4, 25.1)	20.8 (1.4, 121)	<0.001[b]

Data are presented as median (minimum, maximum), and were compared between gender by Wilcoxon rank-sum test.
Abbreviations: BA, bone age; CA, chronological age; FSH, follicle stimulating hormone; F, female ; M, male; LH, luteinizing hormone ; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls.
[a] Reference range from Nelson Textbook of Pediatrics, 17th edition (Behrman et al., 2004).
[b] $P<0.001$, indicates a statistically significant difference between gender for a given variable.
[c] Some children had undetectable testosterone and progesterone levels (ND). ND was calculated as lower limit/√2. Hormone assay sensitivities were: 15 ng/dL for testosterone; 0.22 ng/dL for estradiol; 0.2 mIU/mL for FSH; 0.2 mIU/mL for LH; 2 μIU/mL for insulin; and 3 mg/dL for cholesterol.

Binary logistic regression analysis adjusting for BA revealed that there were significant relationships between fundus length and total PCBs exposure level ($P=0.034$, Table 4), and ovary follicle and PCDD/Fs or PCBs exposure level ($P=0.023$, Supplementary Table 4). No other female sex characteristics were related to ortho-PCBs exposure level (Table 4, Supplementary Table 4).

Table 5 summarizes the combined effects of ΣPCDD/Fs + PCBs TEQ and total PCBs on fundus length, uterus length, and E2 after adjusting for BA. Children were stratified into high and low level subgroups according to the cut-off values for E2 (median = 1.99 ng/dL for boys, 2.49 ng/dL for girls), fundus length (median = 2.08 cm), and uterus length (median = 3.5 cm). Exposure to both high levels of ΣPCDD/Fs + PCBs TEQ and high levels of indicator PCBs was significantly associated with decreased fundus length (OR = 0.05, 95% CI = 0–0.27, $P=0.016$) and uterus length (OR = 0.06, 95% CI = 0.01–0.59, $P=0.016$) (Table 5).

4. Discussion

In this study we examined BA, hormone levels, and indicators of reproductive development in eight-year-old children exposed in utero to PCDD/Fs and PCBs. We found that there were no differences between gender with regards to exposure to PCDD/Fs, PCBs and other compounds; hence there was no need to categorize PCDD/Fs and PCB exposure by sex. We generally found that there were few differences between eight-year-old children exposed to high levels of PCDD/Fs and PCBs in utero and children exposed to low levels of PCDD/Fs and PCBs in utero. Of note, BA appeared to be unaffected by exposure level, indicating normal growth. However, we found that E2 concentrations were significantly related to PCDD/Fs + PCBs exposure level. Interestingly, we also found a negative and significant relationship between fundus length and PCBs exposure level in girls.

We found that E2 concentrations were significantly lower in eight year-old children exposed to high levels of PCDD/Fs and PCBs and further, that there was a significant negative relationship between E2 concentrations and PCDD/Fs and PCBs exposure levels. Interestingly, we found no such difference or relationship in the same children when they were two and five-years old (Su et al., 2010), probably because the 8-year old children are more close to the puberty developing stage. To our knowledge, no previous report has described E2 concentrations in eight year-old children exposed in utero to PCDD/Fs and PCBs. Our findings, however, are consistent with those reported by Mocarelli et al. (2008), who found that E2 concentrations were significantly decreased in men exposed to TCDD during infancy compared with normal age-matched health boys. Findings from animal experiments (Kaya et al., 2002) and in vitro studies of human MCF-7 hepatoma cell lines (Pang et al., 1999) also suggest that PCBs can reduce E2 concentrations/production. The consequences of the decreased E2 concentrations observed in our study are unclear at this point; however altered reproductive maturation and/or functioning might be a possibility.

We found evidence that in utero exposure to PCDD/Fs and PCBs affects reproductive development in girls. Specifically, we found that girls exposed to higher levels of indicator PCBs had shorter fundi and uteri lengths compared with girls exposed to lower levels of indicator PCBs. Further, a significantly higher proportion of girls exposed

Table 2
Comparison of PCDD/Fs and PCB exposure levels between boys and girls.

PCDD/Fs and PCBs levels	Total (n = 56)	Boys (n = 23)	Girls (n = 33)	*P* value
2,3,7,8 TCDD (pg/g lipid)	2.1 (0.8, 4.0)	2.2 (0.9, 4.0)	2.0 (0.8, 3.6)	0.188
ΣPCDD/Fs (pg/g lipid)	168 (0, 574.7)	155 (17.1, 324.3)	174 (0, 574.7)	0.637
ΣPCDD/Fs TEQ (pg WHO_{98}-TEQ_{DF}/g lipid)	12 (5.3, 25)	12 (6.6, 25)	11.9 (5.3, 22.3)	0.529
ΣPCBs TEQ (pg WHO_{98}-TEQ/g lipid)	2.8 (0.5, 6.3)	2.8 (0.5, 4.1)	2.8 (1, 6.3)	0.974
ΣPCDD/Fs TEQ + ΣPCBs TEQ (pg WHO_{98}-TEQ/g lipid)	14.8 (6.8, 29.1)	15.2 (7, 29.1)	14.7 (6.8, 25.1)	0.492
Total non-ortho-PCBs ($\times 10^{-3}$ ng/g lipid)	29.2 (6.1, 130.6)	28.3 (6.1, 52.8)	31.8 (12.9, 130.6)	0.262
Total-mono-ortho-PCBs (ng/g lipid)	4870 (1080, 13,322)	4405 (2183, 8164)	5003 (1080, 13,322)	0.927
Total ortho-PCBs (ng/g lipid)	4870 (1080, 13,322)	4405 (2183, 8164)	5003 (1080, 13,322)	0.419
Indicator PCB138 (ng/g lipid)	7.4 (2, 27)	6.5 (2, 16)	8.4 (2, 27)	0.196
Indicator PCB153 (ng/g lipid)	8.8 (2.4, 32)	8.2 (3.8, 19.1)	9.2 (2, 32)	0.546
Indicator PCB180 (ng/g lipid)	6.2 (1.5, 17)	6.0 (2.4, 16.9)	6.7 (1.5, 17)	0.503
Indicator PCBs (138 + 153 + 180) (ng/g lipid)	22.6 (5.9, 76)	20.3 (10.5, 52)	25.2 (5.9, 76)	0.395

Data are presented as median (range: minimum, maximum) and were compared between gender by Wilcoxon rank-sum test.
Abbreviations: PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent; WHO, World Health Organization.

Table 3
Summary of age, hormone levels, fundus length, and uterus length in children exposed to PCDD/Fs and PCBs in utero as stratified by PCDD/Fs + PCBs, indicator PCBs, and total ortho-PCBs exposure level.

	Total TEQ (PCDD/Fs + PCBs) level			Indicator PCBs level			Total ortho-PCBs level		
	Low (<14.83 pg WHO_{98} TEQ/g lipid)	High (≥14.83 pg- WHO_{98} TEQ/g lipid)	*P* value	Low (<22.56 ng/g lipid)	High (≥22.56 ng/g lipid)	*P* value	Low (<4870 ng/g lipid)	High (≥4870 ng/g lipid)	*P* value
Variable	(n = 28)	(n = 28)		(n = 28)	(n = 28)		(n = 28)	(n = 28)	
Gender, girls (%)	17 (51.5%)	16 (48.5%)	0.786	14 (42.4%)	19 (57.6%)	0.174	15 (45.5%)	18 (54.6%)	0.415
BA (years)	6.0 (4.0, 11.0)	7.0 (4.0, 11.0)	0.423	6.0 (4.0, 11.0)	7.0 (4.0, 11.0)	0.941	6.5 (4.0, 11.0)	6.5 (4.0, 11.0)	1.000
BA/CA	0.8 (0.5, 1.4)	0.9 (0.5, 1.4)	0.453	0.8 (0.5, 1.4)	0.9 (0.5, 1.4)	0.914	0.8 (0.5, 1.4)	0.8 (0.5, 1.4)	0.569
Estradiol (ng/dL)	3.0 (ND, 7.0)	1.8 (ND, 4.2)	0.003[a]	2.3 (ND, 7.0)	2.3 (ND, 4.8)	0.706	2.7 (ND, 7.0)	2.3 (ND, 4.5)	0.221
FSH (mIU/mL)	1.5 (0.4, 3.9)	1.8 (0.5, 11.4)	0.437	1.6 (0.4, 11.4)	1.6 (0.7, 5.9)	0.841	1.4 (0.4, 11.4)	1.8 (0.7, 3.9)	0.514
LH (mIU/mL)	0.2 (ND, 47.8)	0.2 (ND, 41.5)	0.893	0.2 (ND, 41.5)	0.2 (ND, 47.8)	0.834	0.2 (ND, 47.8)	0.2 (ND, 35.5)	0.536
Testosterone[b] ($\times 10^3$ ng/mL)	ND (ND, 0.05)	ND (ND, 0.1)	0.616	ND (ND, 0.1)	ND (ND, 0.1)	0.735	ND (ND, 0.02)	ND (ND, 0.1)	0.224
Fundus length[c] (cm)	2.3 (1.3, 2.8)	2.0 (1.2, 2.8)	0.123	2.4 (1.2, 2.8)	2.0 (1.3, 2.6)	0.008[a]	2.4 (1.2, 2.8)	2.0 (1.5, 2.6)	0.028[a]
Uterus length[c] (cm)	3.6 (2.1, 5.1)	3.2 (2.0, 4.6)	0.140	3.9 (2.0, 5.1)	3.2 (2.1, 4.6)	0.013[a]	3.8 (2.0, 5.1)	3.2 (2.2, 4.6)	0.069

All data except those pertaining to sex are presented as median (range: minimum, maximum) and were compared between groups by Wilcoxon rank-sum test. Sex data are presented as number (percent) and were compared between groups by Pearson Chi-square test.

Abbreviations: BA, bone age; CA, chronological age; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent; WHO, World Health Organization.

[a] Indicates a statistically significant difference between the low and high exposure groups for a given variable ($P<0.05$).

[b] Some children had undetectable testosterone, estradiol and LH levels (ND). ND was calculated as lower limit/√2. The sensitivity of hormones was 15 ng/dL in testosterone, 2.2 pg/mL in estradiol, 0.2 mIU/mL in FSH, and 0.2 mIU/mL in LH.

[c] n = 33, fundus length and uterus length were observed for girls only.

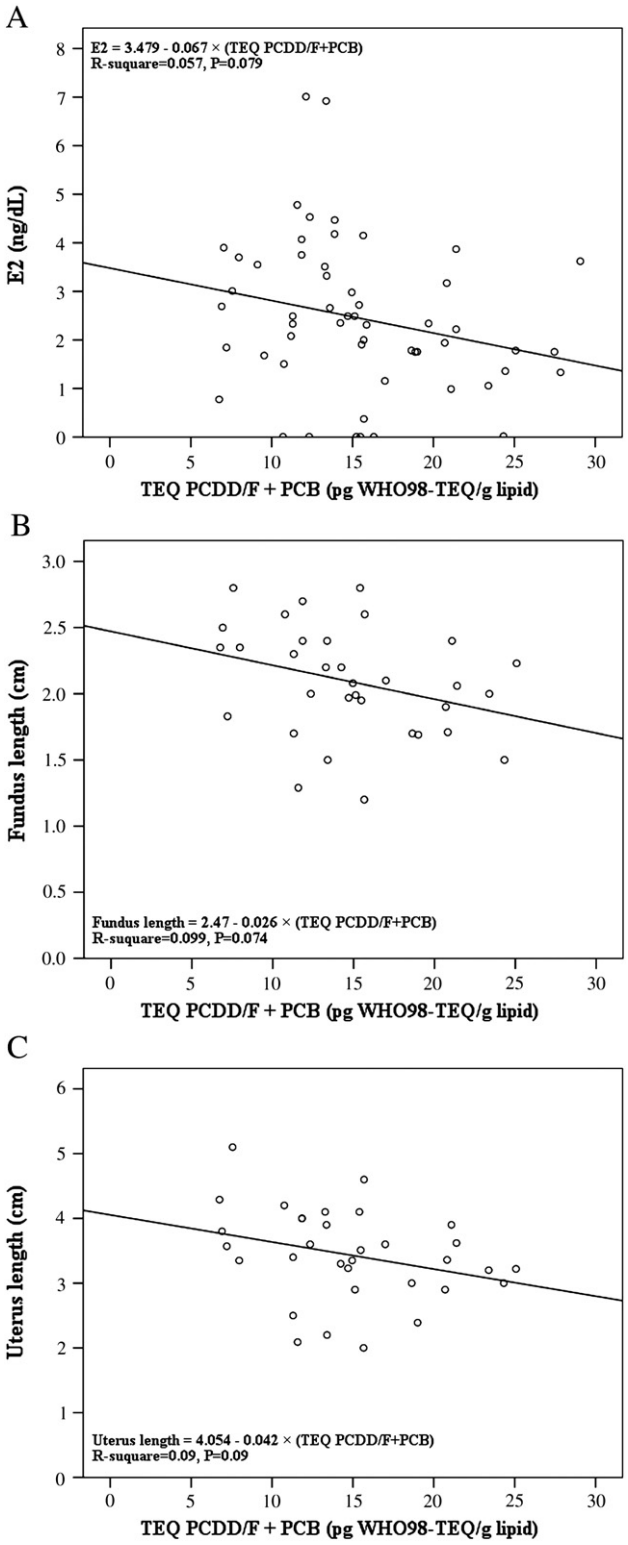

Fig. 1. Scatter plots illustrating the correlations between in utero polychlorinated dibenzo-*p*-dioxins and dibenzofurans + co-planar polychlorinated biphenyl (PCDD/Fs + PCBs) exposure level and estradiol concentrations (E2: A), fundus length (B), and uterus length (C) in eight year-old girls. Simple linear models are shown.

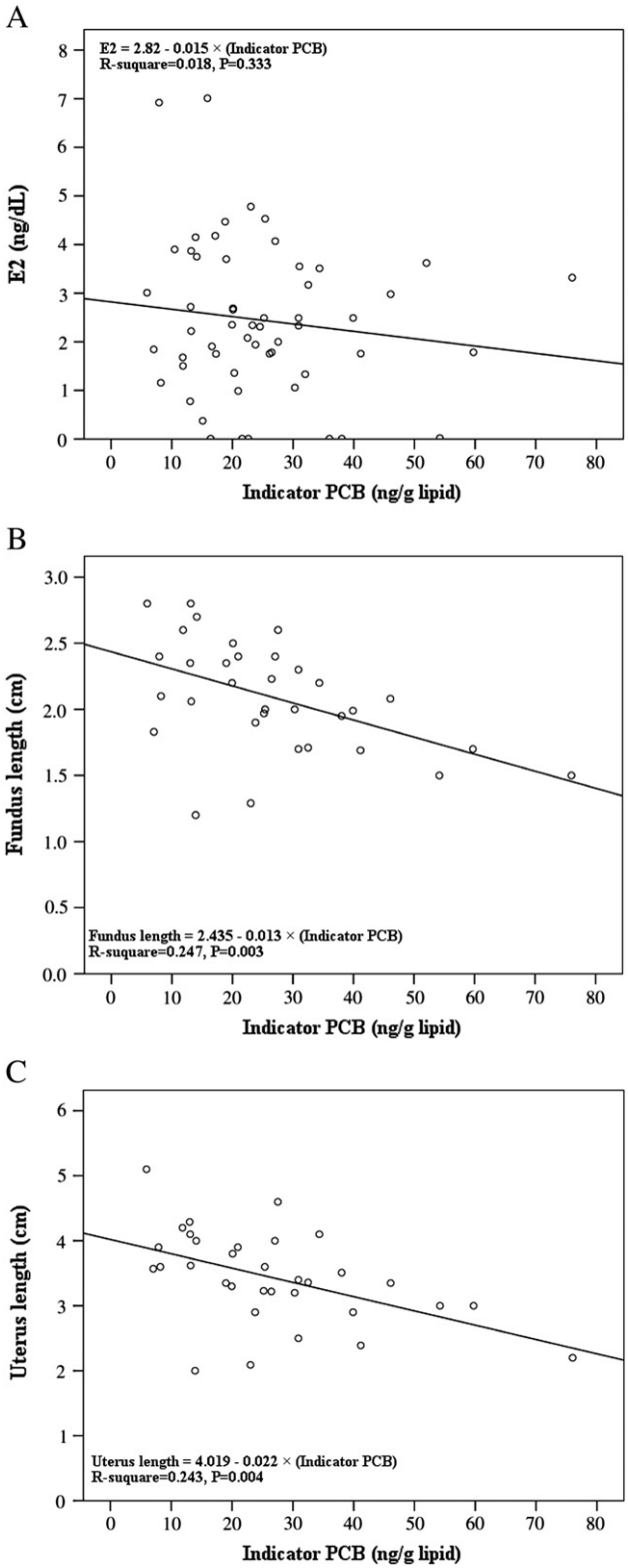

Fig. 2. Scatter plots illustrating the correlations between in utero total polychlorinated biphenyl (total PCBs) exposure level and estradiol concentrations (E2: A), fundus length (B), and uterus length (C) in eight year-old girls. Simple linear models are shown.

to higher levels of indicator PCBs were of genital stage 1 than the proportion of girls exposed to lower levels of indicator PCBs. We also observed a borderline significant effect of exposure level on breast and Tanner stages and found that there was a significant relationship between PCBs median exposure level and fundus length after adjusting for BA. Previous studies have found that in utero exposure to organic pollutants alters reproductive development in girls. For instance, Leijs et al. (2008) reported that breast development was delayed with higher prenatal levels of exposure to PCDD/Fs. To our knowledge, no previous studies have reported on the effects of in

Table 4
Binary logistic regression analysis findings showing the relationship between childrens' characteristics and total PCDD/Fs + PCBs TEQ, indicator PCBs, and total ortho-PCBs exposure level.

	Total (PCDD/Fs + PCBs) TEQ level		Indicator PCBs level		Total ortho-PCBs level	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Gender (girl vs. boy)	0.86 (0.30–2.5)	0.786	2.13 (0.71–6.25)	0.177	1.56 (0.53–4.55)	0.416
BA (years)	1.10 (0.87–1.40)	0.435	0.98 (0.77–1.24)	0.857	0.92 (0.73–1.17)	0.509
BA/CA ratio	2.12 (0.31–14.66)	0.448	0.86 (0.13–5.87)	0.881	0.50 (0.07–3.45)	0.482
Estradiol (ng/dL)	0.51 (0.32–0.82)	0.005[a]	0.99 (0.95–1.02)	0.430	0.97 (0.94–1.01)	0.123
FSH (mIU/mL)	1.36 (0.82–2.26)	0.240	0.96 (0.69–1.35)	0.819	0.91 (0.65–1.30)	0.615
LH (mIU/mL)	0.98 (0.92–1.02)	0.335	1.02 (0.98–1.07)	0.332	0.99 (0.95–1.04)	0.859
Fundus length[b] (cm)	0.18 (0.02–1.30)	0.088	0.08 (0.01–0.83)	0.034[a]	0.19 (0.02–1.48)	0.114
Uterus length[b] (cm)	0.41 (0.13–1.28)	0.125	0.26 (0.07–1.07)	0.062	0.48 (0.15–1.54)	0.216

Data are presented as OR (95% CI).
Abbreviations: BA, bone age; CA, chronological age; FSH, follicle-stimulating hormone; LH, luteinizing hormone; OR(95% CI), odds ratio (95% confidence interval); PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent.
[a] Indicates statistical significance ($P<0.05$).
[b] Analysis was performed for the 33 girls after adjusting for bone age.

utero exposure to PCDD/Fs and PCBs on uterine and fundal measures. Our findings suggest that in utero exposure to PCBs may delay the reproductive development of eight year-old girls. Ongoing continuous follow-up is required to determine whether the observed changes have long-term consequence.

We found no evidence that in utero exposure to PCDD/Fs and PCBs affects reproductive development in eight year-old boys. Given the lack of any reproductive development our findings suggest that in utero exposure to PCDD/Fs and PCBs does not hasten reproductive development in boys. Indeed, all of the boys studied were of genital, armpit, and Tanner stages 1, and exhibited no voice changes. Hence these boys were clearly too young at the time of examination to allow us to detect any detrimental effect of in utero PCDD/Fs and PCBs exposure on reproductive development. Thus, future follow-up observation is warranted.

Several mechanisms may explain the observed effects of in utero dioxin and PCBs exposure on serum estrogen concentrations and reproductive development in 8 year-old girls. Indeed, dioxins and PCBs are considered to be endocrine disruptors, and as such may interfere with the normal action or regulation of endocrine hormones, which play a crucial role in reproductive development (Mouritsen et al., 2010; Rogan and Ragan, 2003; Roy et al., 2009). Findings from previous studies suggest that exposure to dioxins and PCBs may alter estrogen catabolism, reducing estrogen concentrations (Wang et al., 2006). This effect may be mediated by altered aryl hydrocarbon receptor–estrogen receptor signaling (Safe et al., 1998).

Our study has several limitations that warrant acknowledgment. Although we focused on assessing indicators of reproductive development, many of the children (including all of the boys) lacked any signs of reproductive maturation. Given this, follow-up examinations with sufficient sample size at a later point are needed, especially in the cohort of boys, to determine whether reproductive development is affected by in utero exposure to PCDD/Fs and PCBs.

5. Conclusions

In conclusion, our findings indicate that in utero exposure PCDD/Fs and PCBs leads to decreased serum E2 concentrations in eight year-old children. We also found evidence that reproductive development may be delayed in girls exposed to higher levels than median of PCDD/Fs and PCB levels in utero. Continued monitoring of this cohort of children will allow us to determine the consequences of these alterations and whether other manifestations of in utero PCDD/Fs and PCB exposure become apparent with age.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2011.09.009.

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Table 5
Effect of PCDD/Fs + PCBs TEQ and indicator PCB exposure level on estradiol concentration, fundus length, and uterus length after adjusting for bone age.

			Estradiol (ng/dL) [a]		Fundus length (cm)[a]		Uterus length (cm)[a]	
PCDD/Fs + PCBs TEQ	Indicator PCBs	Number of children[b]	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Low	Low	18	Reference		Reference		Reference	
Low	High	10	4.84 (0.49–48.3)	0.179	0.11 (0.01–1.49)	0.097	0.27 (0.03–2.57)	0.252
High	Low	10	0.44 (0.09–2.18)	0.313	0.17 (0.01–2.79)	0.214	1.09 (0.07–17.2)	0.950
High	High	18	0.32 (0.08–1.28)	0.108	0.05 (0–0.27)	0.016[c]	0.06 (0.01–0.59)	0.016[c]

Data are presented as OR (95% CI).
Abbreviations: OR (95% CI), odds ratio (95% confidence interval); PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent.
[a] Children were stratified according to the following cut-off values: estradiol, median = 1.99 ng/dL for boys, median = 2.49 ng/dL for girls; fundus length, median = 2.1 cm; uterus length, median = 3.5 cm.
[b] The number of children are summarized according to PCDD/Fs + PCBs TEQ and indicator PCBs exposure levels (low or high).
[c] Indicates statistical significance ($P<0.05$).

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