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Published in:
Biomonitoring

DOI:
[10.2478/bimo-2014-0002](https://doi.org/10.2478/bimo-2014-0002)

[Link to publication](#)

Citation for published version (APA):

Leijts, M. M., van der Linden, L. M., Koppe, J. G., de Voogt, P., Olie, K., van Aalderen, W. M. C., & ten Tusscher, G. W. (2014). The influence of perinatal and current dioxin and PCB exposure on puberty: a review. *Biomonitoring*, 1(1), 16-24. <https://doi.org/10.2478/bimo-2014-0002>

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Review Article

Open Access

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The influence of perinatal and current dioxin and PCB exposure on puberty: a review

Abstract: Over the last two decades much has been written about the consequences of perinatal dioxin and PCB exposure in humans. In this paper we strive to elucidate the data on puberty in relation to these endocrine disruptive compounds in human populations. Effects in PCB/dioxin-exposed human populations on puberty are seen, not only in highly exposed cohorts, but also in average populations with background exposures. Study showed effects like increased weight, a delay in pubic hair growth and male genital development in boys, sex-hormone homeostasis, reduced penile length, and delayed age at first ejaculation after PCB exposure. Effects seen after dioxin exposure include retarded initiation and stage of breast development in girls, earlier menarche, disruption of sex hormone homeostasis, reduced testicular volume and reduced penile length in boys. The data published by different studies were inconclusive as a result of different methodological setup as well as because of multiple exposure settings. Populations were exposed to different mixtures of dioxin/PCB congeners or mixtures with other endocrine disrupters, and therefore synergistic and antagonistic effects with PCBs and dioxins are possible. Dioxin-like compounds disturb the hormonal balance mainly

through interaction with the Ah receptor, which may influence the synthesis of hormones or their transport proteins. However, we have to keep in mind that hormonal balance during puberty could also be altered by disruption of the thyroid homeostasis. Another important possible mechanism is the induction of epigenetic changes or effects on genetic polymorphism. The fact that exposure to background concentrations of dioxin-like compounds and PCBs also has effects on the reproductive development is disconcerting and warrants further research and long term follow-up studies.

Keywords: dioxin, furan, polychlorinated biphenyls (PCBs), puberty, perinatal exposure, follow-up

Doi: 10.2478/bimo-2014-0002

Received February 23 2014; accepted April 15 2014.

1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are among the most dangerous environmental toxicants. PCDDs, PCDFs and planar PCBs are often grouped together as ‘dioxins’ or ‘dioxin-like compounds’, because of their common mode of (toxic) action. Once ingested, dioxins and PCBs are primarily stored in adipose tissue and liver, as a result of their hydrophobic nature. The half-life of each PCB and dioxin congener varies. The mean half-life of PCBs and dioxins with chlorine at the 2, 3, 7, and 8 position in the human body is assumed to be 7 to 9 years [1].

Dioxins and PCBs interact with hormonal pathways and are therefore known as hormone and growth disruptors. They possess the potential to interfere with a multitude of biological functions. These compounds may alter the (developing) reproductive system by estrogenic or anti-estrogenic properties or

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by interfering with thyroid homeostasis. Dioxin-like compounds (PCDDs/Fs and planar PCBs) are able to bind to the AhR-receptor and are thereby considered to behave like anti-estrogens. However, individual PCB congeners can differ in their chemical and toxicological properties, mainly depending on the position of the chlorine atoms on the PCB molecule in the ortho, para or meta position. Congeners which may possess a planar structure are referred to as dioxin-like (dl). Certain classes of PCB congeners have common mechanisms of action with regard to their toxicity. Some (non-planar) PCB congeners are considered to be estrogenic; however, the *in vitro* estrogen receptor (ER) binding of these congeners appears weak in some studies [2,3]. PCBs and their hydroxylated metabolites are also known for their effects on the thyroid regulatory pathway, both reducing and increasing serum thyroid hormone (T4) levels [4,5]. Thyroid hormone is essential for normal body metabolism, growth, and development including reproduction, maturation and ageing. Fluctuations in thyroid hormone levels are able to alter reproductive outcomes in children [6].

Dioxins and PCBs are able to cross the placenta. In addition, they are excreted in breast milk and thereby cause significant exposure to nursing offspring [7-9]. The foetal and nursing periods of a child are considered to be the most sensitive exposure windows in terms of reproductive effects [10-13]. Adolescents undergoing hormonal changes during puberty are probably also more susceptible, and are therefore at higher risk with regards to environmental exposure health effects [14].

Mice studies showed earlier sexual maturation in females and an increased number of genital abnormalities in males after exposure to estrogenic endocrine-disrupting chemicals [15-17]. Delayed breast development in rats was found following TCDD exposure [18].

Various studies are dedicated to the effects on human development following perinatal exposure. Some of these studied cohorts were exposed to high concentrations as a result of chemical accidents. Yet background concentrations – concentrations that average individuals are daily exposed to – have also been related to various negative health effects. Studies have shown negative effects on lung function [19,20] and haematological and immunological disturbances [21-24], as well as neurodevelopmental changes and behavioural problems [25].

In this review we summarise the most important results of studies performed on the relationship between exposure to dioxin-like compounds and PCBs and pubertal end-points in humans.

2 Materials and methods

A literature search was performed in Pubmed for references to PCB, dioxin and furan exposure and puberty development. MeSH terms and keywords used included, 'menarche', 'TannerScale', 'pubertal development', 'testis' and 'penis', all in combination with the MESH terms 'PCB' and 'dioxin'. We focused exclusively on human studies available to the broad public via medical literature. All the articles found by this search and linked 'related articles' were considered. Publications related to abnormalities caused by disturbances other than PCBs or dioxins were excluded. Articles related to exposure to PCBs or dioxins considering endpoints other than the reproductive system were also excluded. All articles written in English were included. The search encompassed articles written from all years found in Pubmed.

The results from the 16 found research articles were compared. To determine the effects of PCBs and dioxins on puberty, the Tanner Scale, height, weight, age at menarche, testicular volume and genital abnormalities were used as end-points.

3 Results and discussion

3.1 Girls; Tanner scale, height, weight and age at menarche

In a North Carolina study [26], 594 children (316 girls) were studied to determine the effects of background exposure (prenatal and lactational) to PCBs and p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) on pubertal growth and development, by self-reporting through annual mailed questionnaires. Caucasian girls with the highest trans-placental PCB exposures (≥ 3 ppm = $\mu\text{g/g}$ lipids) were heavier for their heights than girls with PCB exposure of 0-1 ppm = $\mu\text{g/g}$ lipids, by an average of 5.4 kg. There was a non-significant association where girls with the highest transplacental PCB exposures reached the early stages of puberty sooner. No relation was seen between lactational or transplacental PCB exposure and age at menarche. Also, no relation was found between transplacental and/or lactational DDE exposure and age at menarche. Possible correlations with individual PCB congeners were not reported. A weak point of the study, which might influence the outcomes, is that the puberty stages were not determined by physicians, but determined through annual mailed questionnaires.

A Belgian study [27] with a cross-sectional design investigated the effect of polychlorinated aromatic

hydrocarbons on sexual maturation. Concentrations of PCB 138, 153 and 180 and dioxin-like compounds were measured in the serum of 200 adolescents (120 females and 80 males) from two polluted suburbs of Antwerp (Hoboken and Wilrijk) and from 100 adolescents (60 girls and 40 boys) in a rural control area (Peer). The dioxin levels in the serum of girls (measured using the CALUX method) were higher in one of the polluted areas (Hoboken 0.21 ng TEQ/L, Wilrijk 0.17 ng TEQ/L) vs the rural area Peer (0.11 ng TEQ/L). Pubertal development was measured by school physicians. In girls, a doubling of serum dioxin concentration increased the odds of not having reached the adult stage (breast stage B5) by 2.3. Furthermore, there was a delay of breast development in relation with higher serum dioxins. No correlation between weight and height and the measured compounds was found.

In a prospective longitudinal Dutch study [28] on 33 adolescents (19 girls), pubertal development was compared with perinatal PCDD/F exposure and current serum PCDD/Fs, dl-PCBs and PBDEs (polybrominated diphenyl-ethers) in a dose-response manner. A delay in initiation of breast development was found in girls (n=18) with higher prenatal (p=0.023) and lactational PCDD/F exposure (p=0.048). No effects were seen in relation to pubic hair development, BMI (body mass index), axillar hair stage or age at menarche. In this study, physical examinations and Tanner stages were evaluated by the same physician. Questionnaires were completed in face-to-face interviews with the adolescents and their parents.

In a Michigan cohort, people were exposed to fire retardant polybrominated biphenyls (PBB) during a distributional mistake in 1973, whereby bags with PBB were mixed up with cattle feed (magnesium oxide called Nutrimaster) and were sent to farms to be fed to dairy cattle [29]. While the incident was only discovered later, PBBs had entered the food-chain. In the Michigan cohort, pubertal development (using the Tanner stages) was compared to serum polybrominated biphenyls (PBB), PCB (Aroclor 1254) and maternal PCB in 201 daughters, aged 5-17 years. Maternal initial serum PCB levels were used as the maternal serum PCB levels during pregnancy, regardless of the time since the daughter's birth. The mean *in utero* exposure level was estimated as 5.6 ng/g lipids. For estimation of this exposure, hazard models were used. No significant association was seen between serum PCB concentrations and the Tanner scale. Perinatal PBB exposure was associated with earlier pubic hair development in breastfed girls. No association was found between the PCB exposure levels and age at menarche. However, the Tanner stage was reported through questionnaires and not examined by trained physicians.

Only girls exposed to high levels of PBB *in utero* had an earlier age at menarche (mean age 11.6 years) compared to those with lower exposure (mean age 12.2-12.6 years) [30].

In another study of the Michigan cohort, female participants and their offspring were studied. DDE and PCB levels had been determined previously in the serum of the mothers. The age at menarche was retrospectively assessed in the 151 female offspring, aged 20–50 years at the time of the study. Based on repeated maternal serum measurements between 1973 and 1991, the PCB and DDE serum levels at the time of pregnancy were derived. No association with age at menarche and prenatal PCB exposure was found. An increase in the *in utero* DDE exposure of 15 µg/L reduced age at menarche by 1 year [31].

Warner et al. reported age at menarche among the 282 Seveso Women's Health Study participants who were pre-menarcheal at the time of the explosion. The age at menarche was compared with serum levels of TCDD in the women. The mean age of menarche in this cohort was 12.8 years. No relationship was seen between the age at menarche and the serum 2-3-7-8-TCDD in the cohort [32]. In a correspondence re-evaluation of the study however, Wolff and Britton suggested that children younger than 5 years at the time of the explosion were more susceptible to hormonal effects of environmental contaminants. When Warner and Eskenazi re-evaluated the study using age stratification, they found that the children exposed before 5 years of age may have been at increased risk for earlier menarche; however, this relation was not significant (p=0.07) [33]. A negative point of this study might be the retrospective evaluation of the age at menarche.

3.2 Boys; Tanner scale, height, weight, testicular volume and penis length

The North Carolina (study details mentioned above) cohort showed no significant associations with perinatal PCB exposure and height or weight. The boys revealed no significant pattern of early maturation in association to higher trans-placental exposure to PCBs. However, higher trans-placental DDE exposure was significantly associated with increased height and weight [26].

In a puberty study on the sons of Yucheng victims (of an accidental leakage of PCBs and PCDFs into rice oil destined for human consumption in Taiwan, 1979), 60 Yucheng boys and 60 controls were examined. Tanner status, testicular size and serum hormone (LH=luteinising hormone, PRL=prolactin, T4= thyroxin, thyroid hormone, T3=thyroid hormone, TSH=thyroid stimulating hormone) levels were not statistically different between Yucheng and control boys in the subgroups prior to and at the

age of puberty. However, the serum estradiol levels were significantly higher in Yucheng boys at the age of puberty, and there was a decrease in serum testosterone levels and increase in serum follicle-stimulating hormone (FSH) levels in Yucheng boys at the age of puberty, as compared with the controls. The testicular volume of boys prenatally exposed to PCBs and PCDFs was unaffected [34].

In the aforementioned Dutch study, the males (n=14) revealed a negative trend with age at first ejaculation. However, the amount of studied participants of this long-term follow up study was too low. For other endpoints on puberty and growth (pubic hair, axillary hair, genital stage, length, BMI, testicular volume), no significant relation was found with any of the measured compounds [28].

As mentioned above, the Antwerp study measured serum PCBs (congeners 138, 153 and 180) and dioxin concentrations in a total of 120 boys in 2 polluted cities and 1 (control) rural area. Height, weight and Tanner scale were measured. The PCB congeners, when individually evaluated, showed congener 138 to be inversely correlated to male genital development ($p=0.04$). Similarly, concentrations of congeners 153 and 180 were inversely correlated to pubic hair growth [27]. In other words, a higher exposure resulted in inhibited sexual development. For measuring the testicular volume, a Prader's orchidometer was used. Although left and right testicular volume was lower in both polluted areas than in the control area (42.4 ml vs. 47.3 ml), no significant relation was seen with the current PCB and dioxin-like compounds. Serum hormone concentrations (testosterone, SHBG (sex-hormone binding globulin), inhibin B, LH and FSH) were all within the normal ranges and did not differ between the areas [27].

Similarly, in a study of 305 young average Swedish men aged 18-21 years, lipid-adjusted serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) were measured and correlated to markers of male reproductive function. No association between PCB-153 and testicular volume was seen. A significant negative relationship with PCB 153 and free testosterone and sperm motility was seen [35].

Similarly, in Henriksen's study on Agent Orange veterans, no association was found between serum TCDD levels and testicular volume or testicular abnormalities. Serum dioxin levels were measured 5 years before the examination [36,37].

In a study of 499 boys (aged between 8-12 years) in Chapaevsk, a town in Russia contaminated with PCBs and dioxins resulting from industrial activity in the past, higher dioxin levels was associated with later pubertal onset (lower testicular volume). However, no effect was seen on genital stage. The sum-PCBs showed a non-

significant association with delayed puberty [38]. In another evaluation of the study on 444 mother-son pairs, higher maternal serum concentrations of Σ PCBs were associated with earlier onset of puberty (reaching >G2 Stage). However, no relationship with testicular volume or pubic hair staging was seen [39].

In a study on 438 boys of the Faroe Island birth cohort (mean age 14 years), prenatal PCB (138, 153, 180) and DDE exposure showed a non-significant inverse relation with testicular volume ($p=0.14$ for prenatal PCB exposure) and a significant inverse relation with Tanner stage ($p=0.014$ for prenatal PCB exposure). Nevertheless higher prenatal PCB exposure was associated with lower serum concentrations of both luteinizing hormone (LH) and testosterone. Sex hormone-binding globulin (SHBG) was positively associated with both prenatal and concurrent PCB exposures [40].

Fifty-five pairs of Yucheng boys and their controls were measured for penile length. Boys aged 11 to 14, who were born in the earlier years after the mothers' intoxication, had normal progression through the Tanner stages but reduced penile length compared to that of their controls [41,42]. It was hypothesised that the effects of PCBs (and PCDFs) on the length of penis might be due to hormonal effects of the toxicants, since animals exposed to these chemicals exhibit hormone dysfunction and altered sexual maturation [41].

While often cited, this study remains the only such study of penile length performed to date.

3.3 Discussion of the studies and mechanism of action

Results of the abovementioned studies were not consistent. The puberty assessment relied on self-reporting in two of the mentioned studies [26,30]. It is arguable that a physical examination by a singular physician might reveal a more accurate assessment of the Tanner stage of a subject. A good point of most studies was that they were able to include a large cohort. A limitation of the Michigan study is that the *in utero* and postnatal chemical exposures were not directly measured, but estimated by modelling [30]. The North Carolina study performed no evaluation of correlations between puberty development and individual congeners [26].

Age at menarche as an indicator of endocrine disruption was evaluated in six papers. Three of the four abovementioned papers showed no difference in the age at menarche in relation to PCB or dioxin exposure. In the two Michigan studies, DDE and PBB exposure was associated with earlier age at menarche [30, 1], and a re-evaluation of

the Seveso study [32,33] found that the children exposed before 5 years of age may have been at increased risk for earlier menarche. A limitation of all the studies is the retrospective recall of age of menarche.

From the seven studies mentioned above considering testicular volume, six did not show a relationship between concentrations of PCBs, dioxin-like compounds and testicular volume. The study on boys in Chapaevsk showed an inverse relationship between testicular volume and dioxin levels [38].

Effects on breast development were seen in two studies [27,28]. This is consistent to what is seen in animal studies. A study in mice showed an important role for the Arylhydrocarbon-receptor (AhR) in normal mammary development. The AhR is expressed and immunohistochemically localised in the epithelial portions of the mouse mammary gland during periods of ductal proliferation. Treatment of mammary gland explants with TCDD resulted in suppressed lobule development of the glands [18,43].

The onset of puberty, including the physical growth spurt and development of secondary sexual characteristics, is controlled by complex neuro-endocrine mechanisms. Puberty changes occur as a consequence of the activation of the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) axis [44]. The HPG axis is under control of both inhibitory and stimulatory mechanisms. Puberty is marked by the reactivation of the HPG axis after a low-activity state during childhood, leading to a rise in pulsatile secretion of LH and FSH from the anterior pituitary, which leads to stimulation of the gonads [45]. The pubertal period is also characterised by the adrenarche, leading to pubarche (forming of pubic hair), acne and body odour – an independent physiologic effect during puberty [46].

In each step of the mechanism leading to activation of puberty, dioxin-like compounds and PCBs may interfere. It is not yet clear how and during which step(s) these compounds interfere with the normal initiation of pubertal development.

On the one hand, xeno-estrogens, like some of the PCB congeners, may impair sexual maturation in men, not only by decreasing the testosterone secretion by the testis, but also through direct interference with the androgen receptor. On the other hand, dioxins and planar PCBs disturb the hormonal balance, mainly through interaction with the Ah receptor, which may influence the synthesis of hormones or their transport proteins [3].

Interference of dioxins or PCBs at gene level is most probable. Effects of environmental changes at the gene level have been described [47,48]. Possible effects include

(heritable) epigenetic changes and effects on genetic polymorphisms. Epigenetic changes (mediated by the histone demethylase Jarid1b) on the androgen receptor have been reported in relation to PCB exposure [49]. Effects on genetic polymorphism in the glucocorticoid receptor and the estrogen receptor α genes are reported to modify the association between peripubertal serum dioxin concentration and male pubertal onset (defined by genitalia staging), as seen in another follow up of the study of the dioxin-exposed Russian boys [39].

In the above mentioned studies, varying results are seen. It must be borne in mind that PCB exposure in humans represents exposure to a mixture of different congeners, with different mechanisms of action: some congeners display estrogenic effects whilst others have anti-estrogenic effects. In short, different PCB congeners show different effects through different mechanisms of action, whether through inhibition, stimulation or synergism. An explanation for the varying results seen in different studies may also be sought in the study limitations. The varying results probably arise from the different mechanisms of influence by different endocrine disrupters, as well as differences in age of exposure and the age of the participants at physical examination. Finally, concomitant exposure to other pollutants such as furans, polybrominated aromatic hydrocarbons, phthalates and alkyl phenols – which may exert estrogenic, anti-estrogenic or androgenic effects – may also play a role.

Summarising, it can be concluded that effects on puberty in PCB/dioxin-exposed human populations are seen, not only in highly exposed cohorts, but also in average populations with background exposures. Effects seen in relation to dl- and ndl-PCB exposure include increased weight, a delay in pubic hair growth and male genital development in boys, sex-hormone homeostasis, reduced penis length, and delayed age at first ejaculation. Effects seen after dioxin exposure include retarded initiation and stage of breast development in girls, earlier menarche, disruption of sex hormone homeostasis, reduced testicular volume and reduced penis length in boys. The fact that exposure to background concentrations of dioxin-like compounds and PCBs have effects on the reproductive development is disconcerting and warrants further research and long term follow-up studies.

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Table 1: Summary of the mentioned studies.

End-point	Author	Cohort	Relevant results
Pubertal development and age at menarche	Gladen et al 2000	North Carolina; 594 children	(Caucasian) Girls with higher (transplacental) PCB exposure were heavier for their heights by \5.4 kg on average. There were (non-significant) signs that girls and boys with higher PCB exposure reach puberty earlier. No relation to transplacental PCB concentration and age at menarche was seen.
	Blanck et al 2000	Michigan; 201 daughters (5-17 yrs) of exposed mothers	No association with transplacental PCB concentration and Tanner scale was seen. (PBB showed effects on pubic hair/breast development.) No relation between transplacental PCB concentration and age at menarche was seen (only girls with higher <i>in utero</i> PBB exposure had menarche at an earlier age).
	Den Hond et al 2002	Antwerp; 200 adolescents and 100 controls	The serum PCBs were inversely correlated with pubic hair growth and breast stage. In girls, doubling serum dioxin increased the odds of not having reached adult stage by 2.3. PCB congener 138 in serum was inversely correlated with male genital development; PCB 153 and 180 were inversely correlated with pubic hair growth.
	Vasiliiu et al 2004	Michigan 151 female offspring	No association with age at menarche and maternal PCB exposure was seen. A reduced age at menarche after DDE exposure (<i>in utero</i>) was seen.
	Warner et al 2004	Seveso; 282 women premenarcheal at time of explosion	No relation with TCDD exposure and age at menarche was seen.
	Warner, Eskenazi 2005 Wolff, Britton 2005	Re-evaluation of the Seveso cohort 84 women < 5 years of age at time of explosion	A non-significant increased risk for earlier menarche in relation to TCDD exposure was seen.
	Hsu et al 2005	Yucheng; 60 Yucheng boys and 61 controls	A relation between PCB/PCDF and sex hormone homeostasis at puberty was seen.
	Leijds et al 2008	Netherlands: 33 adolescents	A retarded initiation in breast development was seen in girls with higher perinatal PCDD/F exposure. A retarded first ejaculation was seen in boys with higher serum dl-PCBs.
	Korrick et al 2011, Humblet et al 2011	499 Boys, 444 Mother-Son pair	Higher dioxin levels were associated with lower testicular volume; no effect on genital stage was seen. The sum-PCBs showed a non-significant association with delayed puberty. Higher maternal serum concentrations of ΣPCBs were associated with earlier onset of puberty (reaching >G2 Stage). No relation with pubic hair staging was seen.
	Grandjean et al 2012	Faroe Island birth cohort. 438 boys	Higher prenatal PCB exposure was associated with lower LH and testosterone concentration. SHBG was associated with both prenatal and concurrent PCB exposures. Prenatal exposure to PCB and DDE showed weak, non-significant inverse associations with testicular size and Tanner stage.
Testicular volume	Henriksen et al 1996	US: agent orange veterans (474 and 532 comparisons)	No relation between TCDD serum levels and testicular volume/abnormalities was seen.
	Den Hond et al 2002	Belgium: 80 boys (17 yrs)	Testicular volume was lower in 2 polluted areas than the control area. However, there was no significant relation seen with PCB (138, 153 and 180) or dioxin-like compounds and testicular volume.
	Richthoff et al 2003	Sweden: 305 men (18-21 yrs)	No relation between PCB-153 and testicular volume was seen.
	Hsu et al 2005	Yucheng; 60 Yucheng boys and 61 controls	No relation between PCBs/PCDFs and testicular volume was seen.

End-point	Author	Cohort	Relevant results
Penile length	Leijs et al 2008	Netherlands: 33 adolescents, 14 boys	No effects of PCDDs/Fs or dl-PCBs on testicular volume were seen. A retarded first ejaculation was seen in boys with higher serum dl-PCBs.
	Korrick et al 2011, Humblet et al 2011	499 Boys, 444 Mother-Son pair	Higher dioxin levels were associated with lower testicular volume; no effect on genital stage was seen. The sum-PCBs showed a non-significant association with a delayed puberty. Higher maternal serum concentrations of Σ PCBs were associated with earlier onset of puberty (reaching >G2 Stage). No relation with pubic hair staging was seen.
	Grandjean et al 2012	Faroe Island birth cohort. 438 boys	Prenatal exposure to PCB and DDE showed weak, non-significant inverse associations with testicular size and Tanner stage.
	Guo et al 2004, Rogan and Ragan 2003	Yucheng: 55 boys (11-14 yrs)	A reduced penile length was seen in PCB/PCDF-exposed boys in comparison to controls.