Sexual Maturation in Relation to Polychlorinated Aromatic Hydrocarbons: Sharpe and Skakkebaek's Hypothesis Revisited

Elly Den Hond,[1] Harry A. Roels,[2] Karel Hoppenbrouwers,[3] Tim Nawrot,[1] Lutgarde Thijs,[1] Corinne Vandermeulen,[3] Gerhard Winneke,[4] Dirk Vanderschueren,[5] and Jan A. Staessen[1]

[1]Studiecoördinatiecentrum, Hypertensie en Cardiovasculaire Revalidatie Eenheid, Departement Moleculair en Cardiovasculair Onderzoek, Katholieke Universiteit Leuven, Leuven; [2]Unité de Toxicologie Industrielle et de Médecine du Travail, Université catholique de Louvain, Bruxelles; [3]Dienst Jeugdgezondheidszorg, Departement Maatschappelijke Gezondheidszorg, Katholieke Universiteit Leuven, Leuven, Belgium; [4]Medizinische Institut für Umwelthygiene, Heinrich-Heine-Universität, Düsseldorf, Germany; [5]Afdeling Experimentele Geneeskunde, Departement Ontwikkelingsbiologie, Katholieke Universiteit Leuven, Leuven, Belgium

Polychlorinated aromatic hydrocarbons (PCAHs) have been described as endocrine disruptors in animals and in accidentally or occupationally exposed humans. In the present study we examined the effect of moderate exposure to PCAHs on sexual maturation. Two hundred adolescents (mean age, 17.4 years) who resided in two polluted suburbs and a rural control area in Flanders (Belgium) participated. We measured the serum concentration of polychlorinated biphenyl (PCB) congeners 138, 153, and 180 and dioxin-like compounds [chemically activated luciferase expression (CALUX) assay] as biomarkers of exposure. School physicians assessed the pubertal development of boys and girls and measured testicular volume. In one suburb near two waste incinerators, compared with the other suburb and the control area, fewer boys ($p < 0.001$) had reached the adult stages of genital development (62% vs. 92% and 100%, respectively) and pubic hair growth (48% vs. 77% and 100%). Also, in the same suburb, fewer girls ($p = 0.04$) had reached the adult stage of breast development (67% vs. 90% and 79%). In individual boys, a doubling of the serum concentration of PCB congener 138 increased the odds of not having matured into the adult stage of genital development by 3.5 ($p = 0.04$); similarly for PCB congener 153 in relation to male pubic hair growth, the odds ratio was 3.5 ($p = 0.04$). In girls, a doubling of the serum dioxin concentration increased the odds of not having reached the adult stage of breast development by 2.3 ($p = 0.02$). Left plus right testicular volume was lower in both polluted areas than in the control area (42.4 mL vs. 47.3 mL, $p = 0.005$) but was not related to the current exposure of the adolescents to PCAHs. Through endocrine disruption, environmental exposure to PCAHs may interfere with sexual maturation and in the long-run adversely affect human reproduction. *Key words:* dioxins, endocrine disruption, PCAHs, PCBs, puberty, testicular volume, xenoestrogens. *Environ Health Perspect* 110:771–776 (2002). [Online 14 June 2002]
http://ehpnet1.niehs.nih.gov/docs/2002/110p771-776den_hond/abstract.html

Since the 1970s, the public in the Western world has become increasingly concerned about the toxicity of environmental pollutants in relation to fetal development and human reproduction (*1–3*). The discovery in 1977 that the nematocide 1,2-dibromo-3-chloropropane decreased fertility in male pesticide workers (*4*) and the more recent epidemiologic evidence of the decreasing quality of human sperm (*5,6*) has drawn attention to effects on the male reproductive system. Some polychlorinated biphenyls (PCBs) and dioxins behave as endocrine disruptors (*7*), have the potential to interfere with multiple biologic functions, and may exert estrogenic, androgenic, and antiestrogenic effects, which in turn may adversely influence human reproduction. Sharpe and Skakkebaek (*8*) hypothesized that reproductive abnormalities in males may be related to increased estrogen exposure *in utero*.

Regarding health effects caused by environmental exposure to chemical toxicants, children and adolescents have a greater susceptibility and are at higher risk than adults (*9*). Biomonitoring may be a sensitive method to track exposure to common pollutants and their biologic effects long before overt disease develops (*10*). We recently found in 200 Flemish adolescents that sexual maturation was slower in relation to biomarkers of exposure to polychlorinated aromatic hydrocarbons (PCAHs) (*10*). In an attempt to further identify the toxicants and the hormonal pathways involved, we investigated the influence of individual PCB congeners and dioxin-like compounds on pubertal stages, testicular volume, and sexual hormones in the adolescents enrolled in our study. In addition, we reviewed our results in the light of Sharpe and Skakkebaek's hypothesis.

Methods

The geographic areas where we performed the study, enrollment of the subjects, questionnaires, and biochemical methods have been described in detail elsewhere (*10*). In short, we recruited 100 adolescents living in two polluted suburbs of Antwerp (Wilrijk and Hoboken) and enrolled 100 adolescents in a rural control area (Peer). Lifelong residence in the area was an inclusion criterion. The participation rate was 58.3% (*10*).

Compared with the participants, nonparticipants had a similar age, sex distribution, parental social class, and regional distribution, and in the suburbs they resided at similar distances from the main sources of pollution (*10*). The suburbs are the seat of a primary nonferrous smelter (Hoboken), two waste incinerators (Wilrijk), and a crematory (Wilrijk). The waste incinerators have been in operation since 1971 and 1980. In 1997, they had annual turnovers of 23,000 and 110,000 metric tons, respectively. At the time of the study (1999), the major incinerator in Wilrijk was inoperative because the installations had to be brought up to the current Belgian emission standards (*11*).

The Ethics Committee of the University of Leuven approved the study. We obtained informed written consent from the adolescents and their parents. Four trained school physicians recorded medical history, staged sexual maturation according to Marshall and Tanner (*12,13*), and in boys also measured testicular volume using Prader's orchidometer (*14*). In girls, they recorded menstrual history and use of contraceptives. Two physicians examined the teenagers recruited in Peer, and two others staged the pupils in

Wilrijk and Hoboken. Three school physicians participated in a separate validation study. With the physician who worked in Wilrijk as a reference, the κ coefficients for the staging of sexual maturity were 0.72 [95% confidence interval (CI), 0.56–0.88; p = 0.001] and 0.68 (95% CI, 0.52–0.85; p = 0.001), respectively. κ Coefficients between 0.61 and 0.80 represent good agreement beyond chance (15). The mean differences in estimated testicular volume were –3.0 (± 4.8) mL (p = 0.08) and 0 (± 3.3) mL (p > 0.99), respectively.

The study nurses administered questionnaires to assess lifestyle, use of tobacco and alcohol, food frequencies, special dietary habits, intake of medications, and social class of the parents. Using the Dutch food composition table (16), we computed the dietary intake of animal fat from the reported food frequencies of meat, fish, and dairy products during the year before the study. We defined regular alcohol intake as a positive answer to the question "Do you regularly consume alcohol" with the specification of at least one type of an alcohol-containing beverage in a subsequent question.

The dioxin congener 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the reference compound of the chemical class of PCAHs, which in addition to dioxins and PCBs also include polychlorinated dibenzofurans (17). Concentrations are usually expressed in toxic equivalents (TEQs), relative to the toxicity of TCDD itself. In keeping with current recommendations (17), we determined congeners 138, 153, and 180 in serum as biomarkers of exposure to PCBs. We estimated exposure to biologically active PCAHs via the *in vitro* activation of the aryl hydrocarbon (Ah) receptor of cultured H4IIE cells by the dioxin-like compounds present in 2.5 mL of serum [chemically activated luciferase expression (CALUX) assay] (18). We measured blood fat gravimetrically. In boys, we determined the serum concentration of testosterone, estradiol, steroid hormone binding globulin (SHBG), inhibin B (Serotec, Oxford, UK), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), using commercially available immunoassay kits. We calculated free testosterone and free estradiol in serum from the total serum hormone concentrations, serum SHBG, and serum albumin (19).

We performed statistical analysis with SAS software, version 6.12 (SAS Institute, Cary, NC, USA). We log-transformed data that were not normally distributed and described them by their geometric mean and its 95% CI or the median and interquartile range. Before any adjustment, we compared normally and non-normally distributed variables and proportions by analysis of variance,

Wilcoxon's rank test, and Fisher's exact test, respectively. We compared correlation coefficients using Fisher's transformation and the z statistic. In further analyses, we traced confounders by linear regression for continuous variables or by logistic regression for categorical outcomes. We used stepwise regression procedures in which we set the p-values for the independent variables to enter and to stay in the model at 0.05. Age is a major confounder of sexual development and was forced in the regression models regardless of significance. Allowing for the covariables, we looked for differences between the three areas, using analysis of covariance for continuous outcomes and logistic regression for odds ratios. If we found significant geographical differences across the three areas, we performed multiple comparisons between individual areas with Bonferroni's correction of the significance levels. Across individual subjects, we computed dose–effect and dose–response relationships between biomarkers of exposure and effect, using multiple linear regression and multiple

logistic regression, respectively. We calculated effect sizes and odds ratios with 95% CIs from the linear and logistic regression coefficients for a 2-fold increase in the biomarker of exposure.

Results

Characteristics of the study population. The 200 adolescents included 120 girls (60.0%), none of whom was pregnant. Mean age was 17.4 years (SD, 0.8; range, 15.8–19.6 years). Age, body mass index, the proportion of smokers, animal fat intake, the child's history of breast feeding, and parental social class were similar in boys and girls. In boys compared with girls, body height, body weight, and the daily amount of tobacco and alcohol consumption were higher (Table 1).

Between-area comparison of exposure biomarkers. In boys, the serum concentrations of PCB congeners 138 and 153 and the sum of congeners 138, 153, and 180 were significantly higher in Wilrijk than in Peer and Hoboken (Table 2). In girls, the dioxin-like compounds in serum (CALUX

Table 1. Characteristics of the adolescents.

Characteristics	Boys (n = 80)	Girls (n = 120)	p-Value
Anthropometric data			
Age (years)[a]	17.3 ± 0.8	17.4 ± 0.8	0.70
Body height (cm)[a]	179 ± 6	165 ± 7	< 0.001
Body weight (kg)[a]	67.7 ± 11.9	58.0 ± 9.3	< 0.001
Body mass index (kg/m²)[a]	21.1 ± 2.9	21.2 ± 2.9	0.65
Questionnaire data			
No. (%) of current smokers	19 (24)	31 (26)	0.74
Grams tobacco/day[b]	11.4 (5.7–16.4)	5.9 (4.2–9.3)	0.03
No. (%) consuming alcohol	52 (65)	35 (29)	0.001
Grams ethanol/day[b]	11.4 (4.3–24.7)	4.3 (2.3–7.1)	< 0.001
Animal fat intake (g/day)[b]	66 (55–75)	61 (46–75)	0.14
No. (%) breast-fed	48 (61)	65 (55)	0.39
Weeks of breast-feeding[b]	11 (6–14)	8 (5–13)	0.10
No. (%) according to parental social class			
Workers	13 (16)	34 (28)	
Middle class	54 (68)	75 (63)	
Learned professionals	13 (16)	11 (9)	0.08
No. (%) using oral contraceptives	—	49 (41)	—

[a]Mean ± SD. [b]Medians (interquartile range).

Table 2. Biomarkers of exposure in boys and girls by area of residence.

Measurements in serum	Peer (rural)	Wilrijk (urban)	Hoboken (urban)	p-Value[a]
No. boys	40	21	19	
Marker PCB 138 (nmol/L)	0.43 (0.36–0.50)	0.63 (0.49–0.81)*	0.46 (0.36–0.60)[b]	0.04
Marker PCB 153 (nmol/L)	0.70 (0.63–0.77)	0.92 (0.78–1.08)**#	0.71 (0.60–0.83)[b]	0.03
Marker PCB 180 (nmol/L)	0.41 (0.37–0.46)	0.51 (0.43–0.60)	0.42 (0.36–0.50)[b]	0.13
Sum of marker PCBs (nmol/L)	1.55 (1.38–1.74)	2.08 (1.75–2.49)**	1.60 (1.34–1.91)[b]	0.03
CALUX assay (ng TEQ/L)	0.15 (0.12–0.20)	0.15 (0.10–0.22)	0.20 (0.13–0.29)	0.51
No. girls	60	21	39	
Marker PCB 138 (nmol/L)	0.26 (0.22–0.30)	0.35 (0.27–0.46)	0.26 (0.22–0.33)	0.12
Marker PCB 153 (nmol/L)	0.46 (0.42–0.51)	0.56 (0.47–0.65)	0.46 (0.41–0.52)	0.12
Marker PCB 180 (nmol/L)	0.26 (0.23–0.28)	0.28 (0.24–0.33)	0.24 (0.22–0.27)	0.36
Sum of marker PCBs (nmol/L)	0.99 (0.89–1.10)	1.20 (1.01–1.43)	0.97 (0.86–1.11)	0.13
CALUX assay (ng TEQ/L)	0.11 (0.09–0.13)	0.17 (0.13–0.22)**	0.21 (0.17–0.26)***	< 0.001

Values are geometric means (95% CI). To convert to molar units: PCB congeners 138 and 153, 1 µg = 2.771 nmol; PCB congener 180, 1 µg = 2.530 nmol. [a]Significance of the differences between three areas; significant differences between control and polluted areas (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001) and between both polluted areas (#p ≤ 0.05). Means and p-values were adjusted for body mass index, smoking, number of weeks breast-feeding, parental social class, and dietary fat intake. [b]n = 18.

assay) were significantly higher in both suburbs than in the control area.

Between-area comparison of effect biomarkers. In boys (Table 3) and girls (Table 4) alike, mean age was slightly but significantly higher in Wilrijk, because these adolescents had been examined after the end of the school year. To allow for possible between-area differences due to age, we adjusted all analyses for age.

In boys, both genital development and pubic hair growth were at a lower stage in Wilrijk than in Peer and Hoboken (Table 3). A history of undescended testis was reported in one boy living in Hoboken and in none of the boys living in the other areas. Testicular volume was significantly lower in both polluted suburbs compared with the control area (Table 3). Testicular volume was associated with genital stage (40.9 ± 8.3 mL in 11 boys in stages G3–G4 compared with 45.4 ± SD 6.3 mL in 67 boys in stage G5, $p = 0.04$). In contrast, testicular volume was not associated with pubic hair growth

(44.2 ± 7.1 mL in 20 boys in stages PH3–PH4 compared with 45.0 ± 6.6 mL in 59 boys in stage PH5; $p = 0.67$]. After adjustment for genital stage, the between-area differences in testicular volume remained significant (46.8 ± 6.8 mL in Peer, 44.1 ± 7.3 mL in Wilrijk, 41.4 ± 6.5 mL in Hoboken; $p = 0.01$), demonstrating regional variation in testicular volume independent of the Tanner stage.

The serum hormone concentrations in boys were within the expected ranges and did not differ between the areas (Table 3). We found a strong negative correlation between the serum concentrations of inhibin B and FSH ($r = –0.57$, $p < 0.001$). Testicular volume correlated significantly with FSH ($r = –0.28$, $p < 0.01$) but not with inhibin B ($r = 0.11$, $p = 0.31$). The correlation coefficients between testicular volume and inhibin B were 0.30 ($p = 0.05$) in Peer, 0.26 ($p = 0.25$) in Wilrijk, and –0.05 ($p = 0.83$) in Hoboken; the correlation coefficients did not differ significantly between the areas.

Among girls, fewer participants had reached the adult stage of breast development in Wilrijk than in Peer. Pubic hair growth was more developed in girls in Hoboken than in those in Wilrijk and Peer (Table 4). Mean age at menarche was 13.1 ± 1.2 years and was similar in all areas (Table 4). It was significantly associated with breast development (13.7 ± 1.5 years in 21 girls in stages B3–B4 compared with 12.9 ± 1.1 years in 99 girls in stage B5; $p = 0.03$] but not with pubic hair growth (13.2 ± 1.4 years in 37 girls in stages PH3–PH4 compared with 13.0 ± 1.1 years in 83 girls in stage PH5; $p = 0.62$). Exclusion of six adolescents (one boy and five girls) of non-European origin who resided in Hoboken did not change the findings presented in Tables 3 and 4.

Dose–effect and dose–response relationships. In boys, a 2-fold increase in the summated serum concentrations of marker PCBs was weakly ($p = 0.06$) associated with higher odds ratios for the presence of genital stages G3–G4 and pubic hair stages PH3–PH4

Table 3. Anthropometric characteristics, sexual maturation, and serum hormones in boys by area of residence.

Characteristics	Peer (rural; $n = 40$)	Wilrijk (urban; $n = 21$)	Hoboken (urban; $n = 19$)	p-Value[a]
Anthropometric data				
Age (years)	17.1 (0.7)	17.9 (0.8)*#	17.3 (0.7)	0.002
Height (cm)	179 (7)	180 (7)	177 (6)	0.38
Weight (kg)	66.8 (12.0)	70.1 (12.5)	67.0 (11.7)	0.61
Body mass index (kg/m²)	20.7 (2.8)	21.6 (3.0)	21.3 (2.8)	0.49
Sexual maturation				
No. (%) of boys in genital stage G5[b]	35 (92)[c]	13 (62)**##	19 (100)	0.001
No. (%) of boys in pubic hair stage PH5[b]	30 (77)[d]	10 (48)**##	19 (100)	< 0.001
Left plus right testicular volume (mL)	47.3 (6.5)[d]	42.8 (6.7)*	42.1 (6.3)**	0.005
Serum hormones				
Total testosterone (nmol/L)	18.3 (5.5)	17.8 (5.7)	17.0 (5.4)	0.66
Free testosterone (nmol/L)	0.44 (0.14)	0.43 (0.15)	0.38 (0.14)	0.37
SHBG (nmol/L)	26.5 (9.0)	25.0 (9.4)	28.5 (8.8)	0.49
Total estradiol (pmol/L)	74.2 (19.3)	74.9 (20.1)	71.0 (18.9)	0.78
Free estradiol (pmol/L)	1.44 (0.42)	1.48 (0.44)	1.35 (0.41)	0.61
Inhibin B (ng/dL)	189 (70)	199 (73)	218 (69)	0.32
Luteinizing hormone (mIU/L)	5.02 (1.88)	4.48 (1.96)	4.07 (1.85)	0.17
FSH (mIU/L)	4.37 (3.68–5.17)	4.27 (3.34–5.44)	4.26 (3.34–5.42)	0.98

Anthropometric data, testicular volume, and serum hormone levels are means ± SD, except FSH, which is presented as geometric mean (95% CI).
[a]Significance of the differences between three areas; significant differences between control and polluted areas (*$p \le 0.05$; **$p \le 0.01$) and between both polluted areas (#$p \le 0.05$; ##$p \le$ 0.01). All values were adjusted for age. p-Values for genital development and pubic hair growth were adjusted for age, body mass index, and parental social class. [b]Defined according to Marshall and Tanner (13). [c]$n = 38$. [d]$n = 39$.

Table 4. Anthropometric characteristics, oral contraceptive use, and sexual maturation in girls by area of residence.

Characteristics	Peer (rural; $n = 60$)	Wilrijk (urban; $n = 21$)	Hoboken (urban; $n = 39$)	p-Value[a]
Anthropometric data				
Age (years)	17.4 (0.8)	17.8 (0.5)*#	17.3 (0.9)	0.03
Height (cm)	166 (7)	165 (7)	165 (7)	0.78
Weight (kg)	57.6 (9.4)	58.5 (9.6)	58.4 (9.4)	0.89
Body mass index (kg/m²)	21.0 (2.9)	21.5 (3.0)	21.5 (2.9)	0.52
Questionnaire data				
No. (%) of girls using oral contraceptives	21 (35)	11 (52)	17 (44)	0.35
Duration of oral contraceptive use (months)	12.7 (2.2–30.7)	13.3 (10.7–25.4)	16.7 (7.7–20.7)	0.49
Sexual maturation				
No. (%) of girls in breast stage B5[b]	54 (90)	14 (67)*	31 (79)	0.04
No. (%) of girls in pubic hair stage PH5[b]	37 (62)	10 (48)	36 (92)***###	< 0.001
Age of menarche (years)	13.2 (1.2)	12.8 (1.2)	13.0 (1.2)	0.58

Age, height, weight, body mass index, and age of menarche are means (SD); duration of oral contraceptive use are medians (interquartile range).
[a]Significance of the differences between three areas; significant differences between control and polluted areas (*$p \le 0.05$; ***$p \le 0.001$) and between both polluted areas (#$p \le 0.05$; ###$p \le 0.001$). All values were adjusted for age. p-Values for breast development and pubic hair growth were adjusted for age, body mass index, use of oral contraceptives, and parental social class. [b]Defined according to Marshall and Tanner (12).

(Figure 1). These odds ratios were 3.8 and 2.7, respectively. If we considered the PCB congeners separately, the serum concentration of PCB congener 138 was inversely (p = 0.04) correlated with male genital development (Figure 1). Similarly, the serum concentrations of PCB congeners 153 and 180 were inversely correlated with pubic hair growth (Figure 1). Testicular volume and serum hormone concentrations did not correlate with any of the markers of exposure.

In girls, the odds of belonging to breast stages B3–B4 were 2.3 (p = 0.02) times higher for a 2-fold increase in the serum level of dioxin-like compounds (Figure 2). Pubic hair growth and age of menarche were not significantly correlated with biomarkers of exposure to PCAHs.

Discussion

To the best of our knowledge, our study provides the first epidemiologic evidence that environmental exposure to PCAHs may influence sexual maturation of adolescents without occupational or accidental exposure. Indeed, in boys, genital development and pubic hair growth were inversely associated with the serum concentrations of marker PCBs. Furthermore, in girls, a lower stage of breast development was associated with higher serum concentration of dioxin-like compounds. Testicular volume was significantly lower in both polluted areas but, in contrast with pubertal development, was not significantly correlated with any biomarker reflecting internal exposure to PCAHs.

PCAHs are toxins that accumulate in the human body. The exposure levels in our adolescents were relatively low, but higher in boys than in girls. Possible explanations for the apparent differences in biomarkers of exposure between boys and girls in our study group could be differences in lifestyle or differences in body composition, particularly fat stores. The serum concentration of summated PCB congeners 138, 153, and 180 averaged 107 ng per gram of blood fat. This is substantially lower than the internal exposure levels observed in adults (> 250 ng/g lipids) (20–22) but comparable with the serum concentrations found in 286 German children 9–12 years old (181 ng/g lipids) (22). The geometric mean for dioxin-like compounds in the serum of the adolescents in our study was 29 pg TEQ/g blood fat. This is considerably lower than the mean value of 47 pg TEQ/g lipids reported in 106 Belgian women or 104 pg TEQ/g lipids in 12 Dutch women (21). Simultaneously with the present study, we assessed the exposure to chlorinated pesticides in women 50–65 years old. The serum levels of pentachlorophenol (5.10 vs. 3.76 ng/mL), lindane (0.051 vs. 0.033 ng/mL), active p,p'-DDT [1,1,1-

trichloro-2,2-bis(p-chlorophenyl)ethane; 0.026 vs. 0.014 ng/mL] and its inactive metabolite p,p'-DDE [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethylene; 7.13 vs. 6.22 ng/mL] were higher in the rural area than in the suburbs (100 women per area), but we observed the opposite for hexachlorobenzene (0.68 vs. 0.85 ng/mL).

PCAHs behave as endocrine disruptors, but their precise mechanism of action remains under investigation. PCBs interact with the receptors of sex steroid hormones, by which they may exert estrogenic, androgenic, or antiestrogenic effects (7). Dioxins disturb the hormonal balance mainly through interaction with the Ah receptor, which in turn may influence the synthesis of hormones or their transport proteins (7). In 1993, Sharpe and Skakkebaek (8) hypothesized that xenoestrogens such as PCAHs may interfere with the development of Sertoli and Leydig cells during fetal development (Figure 3A). Indeed, these pollutants may inhibit the pituitary FSH secretion, a hormone that stimulates the Sertoli cells. The PCAH-induced inhibition of the FSH secretion may reduce the multiplication of Sertoli cells and, ultimately, lower testicular volume. Furthermore, during fetal development, xenoestrogens may also injure the Leydig precursor cells, leading to a reduced number of Leydig cells, the main source of testosterone (Figure 3A).

In our study, we found decreased testicular volume in both polluted suburbs (Wilrijk

and Hoboken) that was not accompanied by a slower pubertal maturation around the nonferrous smelter (Hoboken). In addition, testicular volume did not correlate with any of the biomarkers of exposure to PCAHs. Testicular volume, however, may be more closely associated with maternal exposure during pregnancy (8). In keeping with Sharpe and Skakkebaek's hypothesis (8), we therefore speculate that during fetal development xenoestrogens may inhibit the proliferation of Sertoli cells. Our hypothesis of an excessive maternal exposure to PCAHs during pregnancy is plausible. Indeed, industrial processes involving combustion are known sources of PCAHs. The two waste incinerators and the nonferrous smelter were fully operational at the time of the conception and fetal and neonatal development of our male adolescents (1980–1983). Unfortunately, measurements of PCBs or dioxins in air, soil, or blood in the early 1980s are not available. In 1997, however, the dioxin concentration was measured in the topsoil layer in a radius of 0.5–3.0 km around the main waste incinerator and ranged from 3.9 to 27.2 ng TEQ/kg dry weight (mean, 9.8) (11). In 2000, the deposition of dioxins in Hoboken was higher than acceptable (27 pg TEQ/m^2/day vs. a norm of 6.8 pg TEQ/m^2/day) (23).

Inhibin B is a testicular hormone (Figure 3) that regulates pituitary FSH secretion via a negative feedback loop. Its serum concentration probably reflects testicular function:
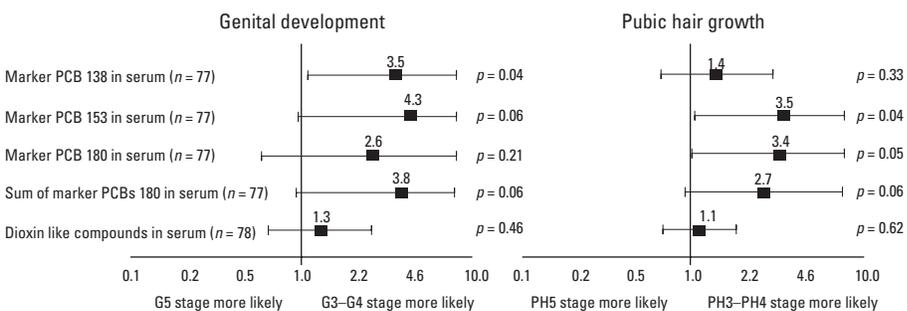

Figure 1. Odds of having reached male sexual maturity associated with a 2-fold increase in the serum levels of PCAHs. We adjusted all odds ratios for age, body mass index, and parental social class. Sum of marker PCBs indicates the sum of PCB congeners 138, 153, and 180.
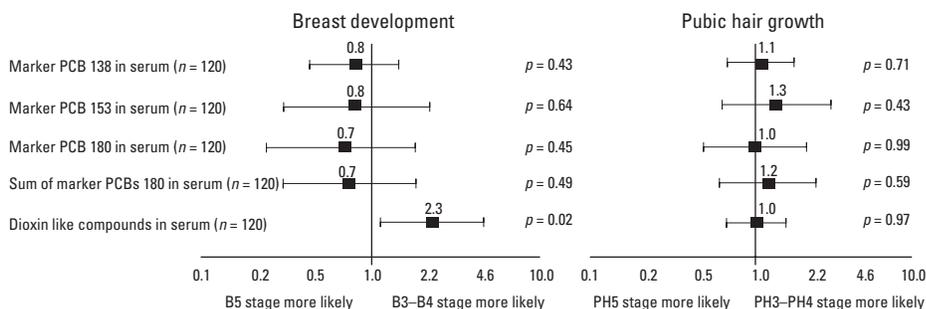

Figure 2. Odds of having reached female sexual maturity associated with a 2-fold increase in the serum levels of PCAHs. We adjusted all odds ratios for age, body mass index, use of oral contraceptives, and parental social class. Sum of marker PCBs indicates the sum of PCB congeners 138, 153, and 180.

In 703 healthy young men (median age, 19.0 years), serum inhibin B was positively associated with testicular volume ($r = 0.33$, $p < 0.001$) (*24*). This value was not significantly different from the correlation coefficients that we observed in Peer ($r = 0.30$), Wilrijk ($r = 0.26$), and Hoboken ($r = -0.05$). Indeed, using Fisher's *z* transformation, the *p*-values for the latter comparisons were 0.84, 0.75, and 0.12, respectively. The low correlation coefficient in Hoboken may have been due to the relatively small sample size, diurnal variation in the inhibin B concentration, and the semicategorical measurement of testicular volume. Moreover, we cannot exclude the possibility that an unknown toxic compound emitted along with PCAHs (e.g., lead or cadmium) adversely influenced the function of Sertoli cells and the secretion of inhibin B.

We found that in boys genital development and pubic hair growth were inversely correlated with the serum concentration of PCBs. Similarities between these two stages are expected because they are both androgenic effects. The findings suggest that, in agreement with the concept of endocrine disruption, xenoestrogens may impair male pubertal maturation. Indeed, PCB congener 153 has a strong estrogenic activity (*17*), and in our study its serum concentration was inversely correlated both with the boys' genital development ($p = 0.06$) and pubic hair growth ($p = 0.02$). We did not find an inverse correlation between the serum testosterone concentration and the biomarkers of exposure to PCAHs. This may be explained by the strong diurnal variation and/or the large inter- and intraindividual variability in the serum testosterone concentrations. Moreover, xenoestrogens may impair sexual maturation not only by decreasing the testosterone secretion by the testis but also through direct interference with the androgen receptors (Figure 3B).

The possible effects of PCAHs on sexual differentiation and maturation are less documented in females than in males. In female rats, both dioxin (*25*) and PCBs (*26*) delay puberty. In areas with accidental PCB poisoning, women reported a shorter menstrual cycle (*27*) and abnormal menstrual bleeding (*28*). In girls, we found an inverse relationship between breast development and the serum concentration of dioxin-like compounds. Breast development depends strongly on stimulation by estrogens, in contrast to pubic hair development, which is stimulated by androgens. The CALUX assay used in our study is sensitive to a range of compounds activating the Ah receptor, including dioxins and coplanar and mono-*ortho*-PCBs (*7*), which all may have anti-estrogenic activity (*29*). It is therefore tempting to speculate that the biologically active PCAHs detected by the CALUX assay in girls interacted with estrogen receptors and via this mechanism may have retarded pubertal breast development in girls. Menarche is also initiated by estrogenic stimulation, but the sensitivity and distribution of estrogen receptors may vary according to the target tissue and age.

It would be interesting to measure, both in boys and in girls, the global estrogenic (*30*) and androgenic (*31*) activity in serum and to study the association with sexual maturation on the one hand and with serum concentrations of PCBs or dioxin-like compounds on the other hand. However, other pollutants such as furans, polybrominated aromatic hydrocarbons, phthalates, alkyl phenols, and so forth also may exert estrogenic, antiestrogenic, or androgenic effects.

If confirmed, our present findings in boys agree with the concept of endocrine disruption and support Sharpe and Skakkebaek's hypothesis (*8*). They may have important implications for human reproduction. Because each Sertoli cell can produce only a fixed number of spermatozoa, smaller testes will reduce sperm output in adult life (*8*). The question of whether this may also lead to reduced fertility remains unsettled. However, in this context, in 1997 the Flemish government reported a higher percentage of medically assisted conceptions in the district around the waste incinerators compared with the rest of Flanders, regardless of whether singleton (5.6 vs. 3.4%) or multiple (59.0 vs. 33.4%) births were considered (*32*). Furthermore, epidemiologic studies produced evidence suggesting that environmental exposure to endocrine disruptors may explain the decreasing quality and quantity of human sperm (*5,6*), the increasing incidence of testicular cancer (*33*), and cryptorchidism (*34*) and the lower male-to-female sex ratio in offspring of fathers exposed to TCDD in puberty (*35*).

In conclusion, our findings suggest that, in line with the concept of endocrine disruption and Sharpe and Skakkebaek's hypothesis (*8*), environmental exposure to PCAHs may adversely interfere with the sexual maturation during the fetal and pubertal stages of development. In view of the potential implications for human reproduction, further studies should be undertaken to confirm or to refute our interpretation of the present findings.

Figure 3. Mechanisms by which xenoestrogens may interfere with the sexual development in boys during fetal and neonatal development (*A*) and during puberty (*B*).

REFERENCES AND NOTES

1. Stein ZA, Hatch MC. Reproductive problems in the workplace. Occup Med 1:361–539 (1986).
2. Safe S. Endocrine disruptors and human health—is there a problem? An update. Environ Health Perspect 108:487–493 (2000).
3. Tas S, Lauwerys R, Lison D. Occupational hazards for the male reproductive system. Crit Rev Toxicol 26:261–307 (1996).
4. Worthon D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. Lancet 2(8051):1259–1261 (1977).
5. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. Br Med J 305:609–613 (1992).
6. Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. Environ Health Perspect 105:1228–1232 (1997).
7. Brouwer A, Ahlborg UG, Vandenberg M, Birnbaum LS, Boersma ER, Bosveld B, Denison MS, Gray LE, Hagmar L, Holene E, et al. Functional aspects of development toxicity of polyhalogenated aromatic-hydrocarbons in experimental animals and human infants. Eur J Pharmacol Environ Toxicol Pharmacol 293: 1–40 (1995).
8. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1392–1395 (1993).
9. Goldman LR. Chemicals and children's environment: what we don't know about risks. Environ Health Perspect 106(suppl 3):875–880 (1998).
10. Staessen JA, Nawrot T, Den Hond E, Thijs L, Fagard R, Hoppenbrouwers K, Koppen G, Nelen V, Schoeters G, Vanderschueren D, et al. Renal function, cytogenetic
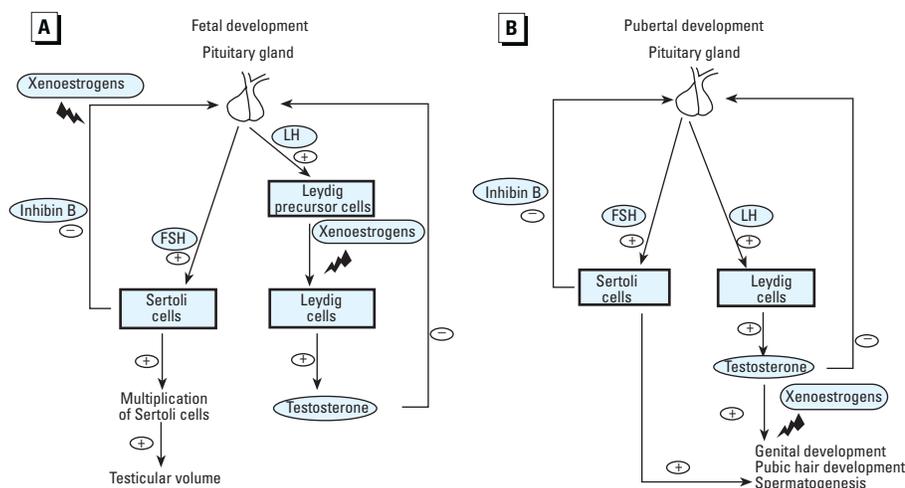
measurements and sexual development in adolescents in relation to common environmental pollutants. Lancet 357:1660–1669 (2001).

11. Schoeters G, Cornelis C, De Fré R, Hooghe R, Collier M, Maes A, Mensink A, Nouwen J, Palyn J, Verschaeve L, et al. Studie van de gezondheidsaspecten en gezondheidsrisico's ten gevolge van de milieuverontreiniging in de Neerlandwijk te Wilrijk. Studie uitgevoerd in opdracht van het Ministerie van de Vlaamse Gemeenschap (1998/TOX/R/0097). Departement Gezondheidsbeleid, Mol, Belgium:VITO, 1998.

12. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. Arch Dis Child 44:291–303 (1969).

13. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23 (1970).

14. Dörnberger V, Dörnberger G. Vergleichende Volumetrie des menschlichen Hodens unter besonderer Berücksichtigung der Hodensonogaphie, Praderorchidometer, Schirrenzirkel und Schublehre [Comparative volumetry of human testes using special types of testicular sonography, Prader's orchidometer, Schirren's circle and sliding caliber]. Andrologia 19:487–496 (1987).

15. Svanholm H, Starklint H, Gundersen HJG, Fabricius J, Barlebo H, Olsen S. Reproducibility of histomorphologic diagnoses with special reference to the kappa statistic. APMIS 97:689–698 (1989).

16. Breedveld BC, Hammink J, Van Oosten HM. Nederlands Voedingsstoffenbestand. Den Haag, the Netherlands: Voorlichtingsbureau voor de Voeding, 1989.

17. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. Environ Health Perspect 106(suppl 1):171–189 (1998).

18. Aerts JMMJG, Cenijn PH, Blankvoort BMG, Murk AJ, Brouwer A, Bovee TFH, Traag WA, Hoogeboom LAP, Patndin S, Weisglas-Kuperus N, et al. Application of the chemical activated luciferase expression (CALUX) bioassay for quantification of dioxin-like compounds in small samples of human milk and blood plasma. Organohalogen Compounds 27:285–290 (1996).

19. Vermeulen A, Verdonck L, Kaufman JD. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672 (1999).

20. Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Strömberg U, Hagmar L, Ostman C. Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption. Arch Environ Contam Toxicol 32:329–336 (1997).

21. Pauwels A, Cenijn PH, Schepens PJC, Brouwer A. Comparison of chemical-activated luciferase gene expression bioassay and gas chromatography for PCB determination in human serum and follicular fluid. Environ Health Perspect 108:553–557 (2000).

22. Wuthe J, Piechotowski I, Papke O, Zier B, Gabrio T, Krämer D, Kouros B, Schwenk M, Pfaff GI. First data on background levels of non-ortho and mono-ortho PCBs in blood of residents from southern Germany. Chemosphere 32:567–574 (1996).

23. Vlaamse Milieumaatschappij. Statistisch Nieuws. Meetresultaten Dioxinedeposities. Vlaamse Milieumaatschappij, 2000. Available: http://fred.vlaanderen.be/statistieken/nieuwsarch/2000oktober/dioxine.htm [cited 7 August 2001]

24. Andersen AG, Jensen TK, Carlsen E, Jorgensen N, Andersson A, Krarup T, Keiding N, Skakkebaek NEl. High frequency of sub-optimal semen quality in an unselected population of young men. Hum Reprod 15:366–372 (2000).

25. Gray LE, Ostby JS. In utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. Toxicol Appl Pharmacol 133:285–294 (1995).

26. Sager DB, Girard DM. Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. Environ Res 66:52–76 (1994).

27. Mendola P, Buck GM, Sever LE, Zielezny M, Vena JE. Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. Am J Epidemiol 146:955–960 (1997).

28. Yu ML, Guo YL, Hsu CC, Rogan WJ. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up of the women from the Taiwan Yucheng cohort. Int J Epidemiol 29:672–677 (2000).

29. Safe S, Astroff B, Harris M, Zacharewski T, Romkes M, Biegel L. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds as antioestrogens: characterization and mechanism of action. Pharmacol Toxicol 69:400–409 (1991).

30. Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB Jr. Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J Clin Invest 94:2475–2480 (1994).

31. Raivio T, Palvimo JJ, Dunkel L, Wickman S, Jänne OA. Novel assay for determination of androgen bioactivity in human serum. J Clin Endocrinol Metab 86:1539–1544 (2001).

32. Aelvoet W, Bekaert Y, Dejonghe M. Gezondheidsindicatoren 1997. Brussels, Belgium: Ministerie van de Vlaamse Gemeenschap, Departement Welzijn, Volksgezondheid en Cultuur, Administratie Gezondheidszorg, Afdeling Preventieve en Sociale Gezondheidszorg, Team Gezondheidsindicatoren met medewerking van de Vlaamse Vereniging voor Respiratoire Gezondheidszorg en Tuberculosebestrijding, 1997;130–141.

33. Fleming LE, Bean JA, Rudolph M, Hamilton K. Cancer incidence in a cohort of licensed pesticide applicators in Florida. J Occup Environ Med 41:279–288 (1999).

34. Weidner TK, Moller H, Jensen TK, Skakkebaek NE. Cryptorchidism and hypospadias in sons of gardeners and farmers. Environ Health Perspect 106:793–796 (1998).

35. Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG, Kieszak SM, Brambilla P, Vincoli N, Signorini S, Tramacere PL, Carreri V, et al. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858–1863 (2000).