Increased Concentrations of Polychlorinated Biphenyls, Hexachlorobenzene, and Chlordanes in Mothers of Men with Testicular Cancer

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An increasing incidence of testicular cancer has been reported from several countries in the Western world during the last decades. According to current hypothesis, testicular cancer is initiated during the fetal period, and exposure to endocrine disruptors, i.e., xenoestrogens, has been of concern. In this investigation we studied the concentrations of the sum of 38 polychlorinated biphenyls (PCBs), *p,p′*-dichlorodiphenyl-dichloroethylene, hexachlorobenzene (HCB), and chlordanes, in 61 cases with testicular cancer and 58 age-matched controls. Furthermore, case and control mothers were also asked to participate, and 44 case mothers and 45 control mothers agreed. They were of similar age. In cases only the concentration on lipid basis of *cis*-nonachlordane was significantly increased, whereas case mothers showed significantly increased concentrations of the sum of PCBs, HCB, *trans*- and *cis*-nonachlordane, and the sum of chlordanes. Among case mothers the sum of PCBs yielded an odds ratio (OR) of 3.8; 95% confidence interval (CI), 1.4–10 was calculated using the median concentration for the control mothers as cutoff value. For HCB, OR = 4.4 (95% CI, 1.7–12); for *trans*-nonachlordane, OR = 4.1 (95% CI, 1.5–11); for *cis*-nonachlordane, OR = 3.1 (95% CI, 1.2–7.8); and for sum of chlordanes, OR = 1.9 (95% CI, 0.7–5.0). No consistent different risk pattern was found for seminoma or nonseminoma testicular cancer. *Key words:* chlordanes, fetal period, hexachlorobenzene, persistent organic pollutants, polychlorinated biphenyls, testicular cancer. *Environ Health Perspect* 111:930–934 (2003). doi:10.1289/ehp.5816 available via *http://dx.doi.org/* [Online 19 December 2002]

An increasing incidence of testicular cancer has been reported from several western countries during the last decades (Toppari et al. 1995, 1996). In Sweden the annual age-adjusted incidence of testicular cancer increased significantly by 2.2% from 1980 to 1999 (National Board of Health and Welfare 2001). It is the most common cancer among young males. Testicular cancer has usually not been regarded as an occupational disease (Hardell et al. 1998), but cryptorchidism is an established risk factor. An increased risk has also been reported for the contralateral descendent testis (Henderson et al. 1979), suggesting common risk factors. Some prenatal risk factors seem to be common for both cryptorchidism and testicular cancer, such as high levels of estrogen in the first trimester (Bernstein et al. 1988; Cosgrove et al. 1977).

Prenatal exposures have been suggested to be of etiologic significance, such as environmental pollutants with estrogen potency, i.e., xenoestrogens (Toppari et al. 1995, 1996). In this context the so-called estrogen hypothesis has been expanded to include environmental antiandrogens as endocrine disruptors with potential adverse effects on male reproductive health (Skakkebæk et al. 2001; Toppari et al. 1996). Impacts of increasing levels of xenoestrogens have been observed in aquatic systems (Colborn et al. 1993). In humans, concern has been focused on endocrine-disrupting chemicals with either estrogenic or antiestrogenic effects, which may be related to an increasing incidence of hypospadia in newborn boys (Dolk 1998; Paulozzi 1999). Indications of a decrease in sperm counts have been observed in recent years, but the hypothesis of an association with exposure to xenoestrogens is still a controversial question (Jensen et al. 1995).

Our aim in this study was to investigate concentrations of certain persistent organic pollutants (POPs) in blood from men with testicular cancer compared with controls, and in mothers to cases compared with mothers to controls. Informed consent was obtained from all study persons. The study was approved by all involved ethical committees.

Materials and Methods

Incident cases with testicular cancer were recruited from 1997 to 2000 from the Department of Urology at Huddinge (n = 17) and Karolinska Hospital in Stockholm (n = 5), and the Departments of Oncology at the University Hospitals in Örebro (n = 13), Linköping (n = 13), and Lund (n = 10), Sweden. Patients were asked to participate by their respective physician. No case refused to participate. Of course, these patients did not represent all cases with testicular cancer admitted to these hospitals during this time. However, no selection bias occurred because the physicians treated patients regardless of tumor type. The patient, as well as his mother, was asked to give blood for chemical analysis of POPs.

For each case a control subject free from testicular cancer was drawn from the Swedish population registry (Stockholm, Sweden). This national registry covers the whole population and is updated continuously with emigration and death data, for example. Because of unique Swedish personal identification numbers, all inhabitants can be traced. The controls were drawn within 5-year age strata to the cases, for example, 20–24 years, 25–29 years, and randomly selected from all who qualified. Thereafter, the mother of the control subject was identified using the population registry. As one criterion, the mother was to be within the same 5-year age stratum as the mother to the respective case. If this was not the situation, a new set of controls, male control and his mother, was drawn. Both the male cases and controls and the mothers were within the same 5-year groups, respectively. Of the primarily included controls, 15 refused to participate, and each was replaced by the next randomly selected subject.

Blood was drawn from all study subjects during the same period (1997–2000). Study subjects were instructed to have only a light meal before this was done. Blood samples from cases were obtained before start of treatment with chemotherapy or radiotherapy. The sample was frozen for later analysis. Furthermore, all study participants were asked to answer a questionnaire, for example, on occupations, weight (present and 1 year previously) and length before blood was drawn. The mothers also answered questions on reproductive history, such as number of children and year of birth of each child. Duration of breast-feeding was assessed for the investigated child and all other children, if any.

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Valuable comments by A. Giwercman are appreciated.

This research was supported by grants from Cancer- och Allergifonden, Lions Research Foundation, and Örebro County Council Research Committee.

The authors declare they have no conflict of interest.

Received 6 June 2002; accepted 19 December 2002.

Chemical analysis. All blood samples were coded with an identification number that did not reveal sex or whether it was a case or a control. Approximately 20 mL of blood was used for analyses of 38 congeners of polychlorinated biphenyls (PCBs) most abundant in human samples. The sum of these 38 congeners is presented here in addition to *p,p′*-dichlorodiphenyl-dichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), and six chlordane congeners (*cis*-heptachlordane, *cis*-chlordane, oxychlordane, MC6, *trans*-nonachlordane, *cis*-nonachlordane). The plasma samples were fortified with ^{13}C-labeled internal standards. The lipid fraction, containing the organochlorines, was first removed from blood by use of a Hydromatrix column (Varian, Palo Alto, CA, USA). The lipid content was then determined gravimetrically, and lipids and interferences were removed by multilayer silica chromatography. Congener-specific analyses and quantification of the organochlorines was done by high-resolution gas chromatography and coupled mass spectrometry running in electro impact (EI) and selected ion monitoring (SIM) mode. The two most abundant ions of the chlorine cluster of the molecular ion for each compound were measured in addition to the one ion for the 12 ^{13}C-labeled internal standards (IS) and the three recovery standards (RS). We used a quantification mix including all compounds in addition to the IS and RS to calculate relative response factors (RRFs), and then used these RRFs to calculate the levels in the samples. In addition, the recovery of the IS was calculated. All recoveries of the 12 different IS were between 50 and 120%. In addition, one laboratory blank sample was processed with each set of nine samples. All blank levels were < 10% of the levels reported for all compounds. The methods detection level defined with a signal-to-noise (S/N) ratio > 3 was in the range of 0.3–1 ng/g, depending on the compound and the amount of sample. All results are expressed in nanograms per gram lipid. External quality assurance/quality control was assured by successfully participating in international round robin or intercalibration studies organized by both Arctic Monitoring and Assessment Program (AMAP) and International Union for Pure and Applied Chemicals (IUPAC). If some of the PCB congeners were under the detection limit (n.d.), 50% of the detection limit was used. However, for the sum of PCBs, chlordanes, HCB, and *p,p′*-DDE, all analytes were above detection limit.

Statistical methods. We performed unconditional logistic regression analysis using the SAS program (SAS Institute, Cary, NC, USA) for calculation of odds ratio (OR) and 95% confidence interval (CI). In the analyses, we adjusted for age and body mass index (BMI) at the time of sampling. We also adjusted for BMI 1 year before that time, but observed similar results without any significant differences, so the results are presented with adjustment for BMI at the time of blood sampling. We also adjusted for lactation of the respective child in one analysis and for the whole lactation time for all children in another analysis, without any significant change of results (data not shown). Of course, it would have been most appropriate to have an estimate of *in utero* exposure, but such data are lacking. To our knowledge, no other common risk factors for testicular cancer exist that should be adjusted for, e.g., smoking. Nevertheless, including smoking as a covariant in the analysis did not change the results significantly (data not shown). The median concentration in the controls was used as cutoff value in the calculations of ORs and CIs, because no biological relevant cutoff exists. The SAS program was also used for descriptive statistics and Wilcoxon rank sum tests for calculation of *p*-values.

Table 1. Age distribution among cases, controls, and mothers of cases and controls.

	No.	Mean	Median	Minimum	Maximum
Cases	58	31	30	18	45
Seminoma	22	34	33	23	45
Nonseminoma	36	29	29	18	42
Controls	61	32	31	19	47
Mothers of cases	44	57	54	41	75
Seminoma	14	60	61	48	75
Nonseminoma	30	55	54	41	69
Mothers of controls	45	57	55	43	75

Table 2. Concentrations of organochlorine compounds (nanograms per gram lipid) in cases with testicular cancer and controls.

	No.	Mean	Median	Minimum	Maximum	*p*-Value[a]
Sum of PCBs						
Cases	58	395	357	96	1,099	0.91
Controls	61	394	364	110	1,083	
HCB						
Cases	58	26	24	5.3	58	0.33
Controls	61	24	22	8.8	47	
p,p′-DDE						
Cases	58	152	117	35	529	0.27
Controls	61	140	98	29	601	
cis-Heptachlordane						
Cases	58	2.1	1.2	0.2	13	0.30
Controls	61	1.3	1.0	0.3	9.3	
cis-Chlordane						
Cases	58	1.1	0.8	0.1	4.6	0.71
Controls	61	1.0	0.9	0.2	2.6	
Oxychlordane						
Cases	58	8.3	6.9	0.9	33	0.61
Controls	61	7.5	6.5	2.0	32	
MC6						
Cases	58	2.5	2.0	0.7	7.5	0.79
Controls	61	2.3	1.9	0.5	7.0	
trans-Nonachlordane						
Cases	58	8.6	7.5	1.6	28	0.87
Controls	61	8.5	7.9	0.9	26	
cis-Nonachlordane						
Cases	58	1.8	1.5	0.4	7.6	0.04
Controls	61	1.4	1.1	0.3	7.8	
Sum of chlordanes						
Cases	58	24	21	8.0	72	0.41
Controls	61	22	21	8.2	70	

[a]Wilcoxon *p*-value.

Results

A total of 61 case and control pairs were recruited. However, for technical reasons, we

Table 3. OR (95% CI) for cases with testicular cancer, all types combined.[a]

	Cases/controls	OR (95% CI)
Sum of PCBs	28/30	1.1 (0.5–2.6)
HCB	35/30	1.7 (0.8–3.6)
p,p′-DDE	34/30	1.7 (0.8–3.7)
cis-Heptachlordane	34/29	1.6 (0.8–3.4)
cis-Chlordane	27/26	1.2 (0.6–2.6)
Oxychlordane	31/29	1.4 (0.7–2.9)
MC6	30/30	1.3 (0.6–2.9)
trans-Nonachlordane	27/30	1.0 (0.4–2.1)
cis-Nonachlordane	40/29	2.6 (1.2–5.7)
Sum of chlordanes	31/30	1.3 (0.6–2.8)

[a]The median concentration of the chemicals in the controls was used as cutoff value; numbers greater than the median (expressed in nanograms per gram lipid) are shown for cases and controls, and adjustment was made for age and BMI.

analyzed blood from only 58 cases. Of the case mothers, 44 agreed to participate compared with 45 of the control mothers.

Cases and controls. The 58 cases with testicular cancer were of mean age 31 years (median 30, range 18–45), and the 61 controls were of mean age 32 years (median 31, range 19–47) (Table 1). Cases with seminoma were somewhat older than cases with nonseminoma cancer, as was expected according to the age distribution for these tumor types.

The median number of birth order was two for both the cases and controls ($p = 0.27$). Median breast-feeding for the cases was 4 months (range 0–13.5) and for the controls 3 months (range 0–12; $p = 0.49$).

Of the cases, 22 had seminoma and 36 had nonseminoma testicular cancer (28 embryonal cancer and 8 teratoma).

Table 2 displays results on concentrations of organochlorines for the sons. The only significant difference was an increased concentration of *cis*-nonachlordane in cases with testicular cancer. This yielded OR = 2.6 (95% CI, 1.2–5.7) if the median concentration among the controls was used as cutoff value (Table 3). Sum of PCBs did not differ between cases and controls. For other studied organochlorines, somewhat increased concentrations were found among the cases yielding increased ORs, although not significantly so.

Analysis according to histopathology gave only significantly increased risk for *cis*-nonachlordane among seminoma cases with OR = 4.8 (95% CI, 1.4–16) (Table 4).

Mothers to cases and controls. Both groups of mothers were of similar age overall, although mothers to seminoma cases tended to be somewhat older (Table 1). Median duration of breast-feeding in total before blood sampling (all children including the studied child) was 8 months both for case and control mothers ($p = 0.91$).

Table 5 displays the results of concentrations of organochlorines in mothers of cases and controls. Significantly increased concentrations were found for the sum of PCBs, HCB, *trans*-nonachlordane, *cis*-nonachlordane, and sum of chlordanes.

Table 6 shows results of calculations of OR and CI in the group of mothers. For sum of PCBs, OR = 3.8 (95% CI, 1.4–10); for HCB, OR = 4.4 (95% CI, 1.7–12); for *trans*-nonachlordane, OR = 4.1 (95% CI, 1.5–11); and for *cis*-nonachlordane, OR = 3.1 (95% CI, 1.2–7.8). For *cis*-chlordane, a borderline significant result was obtained with OR = 2.5 (95% CI, 0.99–6.1). The other organochlorines also yielded increased ORs, although not significantly so.

We made further calculations for mothers, separated according to histopathology type of the testicular cancer in the respective child (Table 7). The results were similar to the whole study group.

Discussion

The organochlorines studied are fat-soluble chemicals that bioaccumulate in the human body. The half-life for PCBs has been estimated to be between 7 and 30 years in human serum (Wolff et al. 1992). For *p,p´*-DDE, the half-life in plasma is approximately 10 years (Hunter et al. 1997), and for chlordanes, half-life is 10–20 years (Dearth and Hites 1991). For HCB, no half-life figure in humans is documented.

Because of the long half-life, it would be possible to estimate previous exposure by measurement of lipid-based concentrations of certain organochlorines. Because median time from the fetal period until blood sampling was similar for the cases (30 years) and for the controls (31 years) in the data presented here, previous differences in exposure may be reflected in current blood levels. Of course, there may be individual differences in exposure and metabolism patterns over the years, but we lack such data. The results certainly indicate that further studies are necessary, perhaps with a different design, use of early blood samples if existing, and the like. It cannot be completely excluded that the real etiologic agent is something unknown related to these factors. However, the results for control mothers seem to be in reasonable agreement with those found in another study in Sweden (Hardell et al. 2001).

Table 4. OR (95% CI) for cases with testicular cancer, seminoma, and nonseminoma, respectively.[a]

	Seminoma		Nonseminoma	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
Sum of PCBs	13/30	1.1 (0.4–3.5)	15/30	1.1 (0.4–3.0)
HCB	14/30	1.6 (0.6–4.5)	21/30	1.8 (0.7–4.4)
p,p´-DDE	14/30	1.5 (0.5–4.5)	20/30	1.9 (0.8–4.7)
cis-Heptachlordane	12/29	1.4 (0.5–3.7)	22/29	2.1 (0.9–5.1)
cis-Chlordane	7/26	0.7 (0.2–1.9)	20/26	1.9 (0.8–4.7)
Oxychlordane	11/29	1.0 (0.4–2.8)	20/29	1.9 (0.8–4.7)
MC6	12/30	0.9 (0.3–2.7)	18/30	1.8 (0.7–4.9)
trans-Nonachlordane	11/30	0.7 (0.2–2.1)	16/30	1.2 (0.4–2.9)
cis-Nonachlordane	18/29	4.8 (1.4–16)	22/29	2.0 (0.8–4.7)
Sum of chlordanes	11/30	0.8 (0.3–2.4)	20/30	1.8 (0.7–4.4)

[a]The median concentration of the chemicals in the controls was used as cutoff value; numbers greater than the median (expressed in nanograms per gram lipid) are shown for cases and controls, and adjustment was made for age and BMI.

Table 5. Concentrations of organochlorine compounds (nanograms per gram lipid) in mothers of cases with testicular cancer and controls.

	No.	Mean	Median	Minimum	Maximum	*p*-Value[a]
Sum of PCBs[b]						
Case mothers	43	859	792	236	2,114	0.0006
Control mothers	41	592	563	141	1,193	
HCB						
Case mothers	44	47	39	12	120	0.005
Control mothers	45	34	31	8.9	81	
p,p´-DDE						
Case mothers	44	566	315	109	3,339	0.48
Control mothers	45	428	324	51	1,431	
cis-Heptachlordane						
Case mothers	44	1.2	1.0	0.3	5.1	0.12
Control mothers	45	1.0	0.8	0.3	7.6	
cis-Chlordane						
Case mothers	44	0.9	0.7	0.2	7.9	0.24
Control mothers	45	0.7	0.7	0.2	1.5	
Oxychlordane						
Case mothers	44	14	10	1.9	50	0.24
Control mothers	45	10	9.4	1.4	32	
MC6						
Case mothers	44	5.1	4.2	0.7	13	0.09
Control mothers	45	3.6	3.8	0.6	7.8	
trans-Nonachlordane						
Case mothers	44	22	17	2.4	64	0.02
Control mothers	45	15	13	0.6	42	
cis-Nonachlordane						
Case mothers	44	1.8	1.3	0.4	9.2	0.008
Control mothers	45	1.1	1.0	0.4	2.8	
Sum of chlordanes						
Case mothers	44	46	34	14	131	0.04
Control mothers	45	32	31	5.8	76	

[a]Wilcoxon *p*-value. [b]One case and four controls were not analyzed for certain PCB congeners because of technical reasons.

Collection of blood was made during the same period for cases and controls. In this way, any change over time of organochlorines in the population was eliminated. All blood was drawn before treatment of the cases with chemotherapy or radiotherapy to eliminate any potential influence of treatment on the results. When a case pair was recruited, a control subject was selected at random in the same age group (5-year intervals). Furthermore, as an inclusion criterion, the control mother was in the same age group as the respective case mother. The study was well balanced for age in the case and control series.

BMI might influence the concentration of organochlorines. Furthermore, the concentrations increase with age. All results were adjusted for BMI and age (Hardell et al. 2001).

No subject reported occupational exposure to the studied chemicals.

In this study, only one type of chlordane, *cis*-nonachlordane, was significantly increased among the cases. Most other studied organochlorines showed some but not significant increases.

Interestingly, significantly increased concentrations were found among case mothers for the sum of PCBs, HCB, *cis*-nonachlordane, and the sum of chlordanes. Analysis according to the histopathology of the sons (seminoma or nonseminoma) yielded similar risk patterns. Seminoma and nonseminoma both start as carcinoma *in situ* (Skakkebæk et al. 1987). Epidemiological studies (Andersen et al. 1999; Møller 1989; Møller and Skakkebæk 1997; Skakkebæk 1987) and biological evidence (Jørgensen et al. 1995; Skakkebæk et al. 1987) indicate that carcinoma *in situ* starts during the fetal period. Thus, biologically it would be relevant to study concentrations of endocrine disruptors in the mothers.

The concentration of POPs in mothers' milk reflects the body burden. Decreasing concentrations of certain organochlorines such as PCBs have been found in Swedish breast milk since the 1980s. The highest concentrations were found in early 1970s (Norén and Meironyté 1998). Because the median age among the cases was 30 years, most of them were born during the period with high concentration in the population. Fetuses and nursing infants receive significant exposures to POPs as well as the largest body burdens (Hooper and McDonald 2000). Prenatal and lactation exposure appears to be an important source of the adverse health effects of POPs seen in infants, such as cognitive motor deficits (Patandin et al. 1999).

During lactation, the concentration of POPs decreases in the mother (Lindström 1988; Lindström et al. 1994), but the period when breast-feeding took place did not differ for cases and controls. Furthermore, total time of breast-feeding for all children was similar among case and control mothers. In contrast, higher concentrations of POPs give higher exposure during breast-feeding in spite of similar duration of breast-feeding. Because of the long half-life for the studied chemicals, it is postulated that the increased concentration among the case mothers indicates higher exposure during the fetal and postnatal period for cases than for controls. One explanation for the differences found would be if exposure to POPs, mainly through the food chain, differs later in life among case mothers.

In one study, neonates born to mothers who were active smokers had highest PCB and HCB concentrations compared with children of mothers exposed to second-hand smoke or nonsmoking mothers (Lackman et al. 2000). However, in our study, smoking habits in mothers did not change the results (data not shown).

Some POPs, such as PCBs, especially the hydroxylated metabolites, and chlordanes, have been postulated to be endocrine disruptors (Andersen et al 1999; Andersson et al. 1999; Willingham and Crews 1999; Willingham et al. 2000). PCBs reverse gonadal sex in turtle (Bergeron et al. 1994), and abnormalities of reproductive development has been described in juvenile alligators living in a contaminated environment in Florida (Guillette et al. 1994, 1996). HCB has endocrine-disrupting properties (Colborn et al. 1993). In addition, *p,p′*-DDE has been postulated to be an environmental endocrine disruptor (Willingham and Crews 1999). In this study, no significant differences were found for *p,p′*-DDE.

In summary, according to current hypotheses, testicular cancer is initiated during the fetal period (Sharpe and Skakkebæk 1993; Skakkebæk et al. 1987, 2001). Our results show that the concentrations of certain POPs are higher in mothers to patients with testicular cancer, but the etiologic significance of this finding needs to be further explored.

Table 6. OR (95% CI) for mothers of cases with testicular cancer, all types combined.[a]

	Cases/controls	OR (95% CI)
Sum of PCBs[b]	34/20	3.8 (1.4–10)
HCB	35/22	4.4 (1.7–12)
p,p′-DDE	22/22	1.3 (0.5–3.0)
cis-Heptachlordane	27/21	2.1 (0.8–5.0)
cis-Chlordane	22/15	2.5 (0.99–6.1)
Oxychlordane	28/22	2.6 (0.9–7.1)
MC6	25/22	1.3 (0.5–3.2)
trans-Nonachlordane	34/22	4.1 (1.5–11)
cis-Nonachlordane	32/22	3.1 (1.2–7.8)
Sum of chlordanes	27/22	1.9 (0.7–5.0)

[a]The median concentration of the chemicals in the mothers of controls was used as cutoff value; numbers greater than the median (expressed in nanograms per gram lipid) are shown for cases and controls, and adjustment was made for age and BMI. [b]One case and four controls were not analyzed for certain PCB congeners because of technical reasons.

Table 7. OR and 95% CI for mothers of cases with testicular cancer, seminoma, and nonseminoma, respectively.[a]

	Seminoma		Nonseminoma	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
Sum of PCB[b]	11/20	3.1 (0.7–14)	23/20	4.3 (1.3–14)
HCB	10/22	2.1 (0.6–8.2)	25/22	9.0 (2.4–33)
p,p′-DDE	7/22	1.0 (0.3–3.7)	15/22	1.4 (0.5–4.0)
cis-Heptachlordane	9/21	3.2 (0.8–13)	18/21	1.8 (0.7–4.7)
cis-Chlordane	8/15	4.3 (1.1–17)	14/15	2.1 (0.7–5.7)
Oxychlordane	11/22	3.3 (0.7–16)	17/22	2.5 (0.8–7.9)
MC6	9/22	1.3 (0.4–5.0)	16/22	1.3 (0.5–3.6)
trans-Nonachlordane	10/22	1.9 (0.5–7.5)	24/22	5.6 (1.7–19)
cis-Nonachlordane	11/22	4.1 (0.96–18)	21/22	2.8 (1.01–7.8)
Sum of chlordanes	9/22	1.2 (0.3–4.8)	18/22	2.4 (0.8–7.3)

[a]All control mothers used for comparison. The median concentration of the chemicals in the mothers of controls was used as cutoff value; numbers greater than the median (expressed in nanograms per gram lipid) are shown for cases and controls, and adjustment was made for age and BMI. [b]One case and four controls were not analyzed for certain PCB congeners because of technical reasons.

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