Relationship of Lead, Mercury, Mirex, Dichlorodiphenyldichloroethylene, Hexachlorobenzene, and Polychlorinated Biphenyls to Timing of Menarche Among Akwesasne Mohawk Girls

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ABSTRACT. *Background.* **Children are commonly exposed at background levels to several ubiquitous environmental pollutants, such as lead and persistent organic pollutants, that have been linked to neurologic and endocrine effects. These effects have prompted concern about alterations in human reproductive development. Few studies have examined the effects of these toxicants on human sexual maturation at levels commonly found in the general population, and none has been able to examine multiple toxicant exposures. The aim of the current investigation was to examine the relationship between attainment of menarche and levels of 6 environmental pollutants to which children are commonly exposed at low levels, ie, dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), mirex, lead, and mercury.**

Methods. **This study was conducted with residents of the Akwesasne Mohawk Nation, a sovereign territory that spans the St Lawrence River and the boundaries of New York State and Ontario and Quebec, Canada. Since the 1950s, the St Lawrence River has been a site of substantial industrial development, and the Nation is currently adjacent to a US National Priority Superfund site. PCB, *p,p′*-DDE, HCB, and mirex levels exceeding the US Food and Drug Administration recommended tolerance limits for human consumption have been found in local animal species. The present analysis included 138 Akwesasne Mohawk Nation girls 10 to 16.9 years of age. Blood samples and sociodemographic data were collected by Akwesasne community members, without prior knowledge of participants' exposure status. Attainment of menses (menarche) was assessed as present or absent at the time of the interview. Congener-specific PCB analysis was available, and all 16 PCB congeners detected in >50% of the sample were included in analyses (International Union of Pure and Applied Chemistry numbers 52, 70, 74, 84, 87, 95, 99, 101 [+90], 105, 110, 118, 138 [+163 and 164], 149 [+123], 153, 180, and 187). Probit analysis was used to determine the median age at menarche for the sample. Binary logistic regression analysis was used to determine predictors of menarcheal status. Six toxicants (*p,p′*-DDE, HCB, PCBs, mirex, lead, and mercury) were entered into the logistic regression model. Age, socioeconomic status (SES), and BMI were tested as potential cofounders and were included in the model at $P < .05$. Interactions among toxicants were also evaluated.**

Results. **Toxicant levels were measured in blood for this sample and were consistent with long-term exposure to a variety of toxicants in multiple media. Mercury levels were at or below background levels, all lead levels were well below the Centers for Disease Control and Prevention action limit of 10 μg/dL, and PCB levels were consistent with a cumulative, continuing exposure pattern. The median age at menarche for the total sample was 12.2 years. The predicted age at menarche for girls with lead levels above the median (1.2 μg/dL) was 10.5 months later than that for girls with lead levels below the median. In the logistic regression analysis, age was the strongest predictor of menarcheal status and SES was also a significant predictor but BMI was not. The logistic regression analysis that corrected for age, SES, and other pollutants (*p,p′*-DDE, HCB, mirex, and mercury) indicated that, at their respective geometric means, lead (geometric mean: 0.49 μg/dL) was associated with a significantly lower probability of having reached menarche ($\beta = -1.29$) and a group of 4 potentially estrogenic PCB congeners (E-PCB) (geometric mean: 0.12 ppb; International Union of Pure and Applied Chemistry numbers 52, 70, 101 [+90], and 187) was associated with a significantly greater probability of having reached menarche ($\beta = 2.13$). Predicted probabilities at different levels of lead and PCBs were calculated on the basis of the logistic regression model. At the respective means of all toxicants and SES, 69% of 12-year-old girls were predicted to have reached menarche. However, at the 75th percentile of lead levels, only 10% of 12-year-old Mohawk girls were predicted to have reached menarche; at the 75th percentile of E-PCB levels, 86% of 12-year-old Mohawk girls were predicted to have reached menarche. No association was observed between mirex, *p,p′*-DDE, or HCB and menarcheal status. Although BMI was not a significant predictor, we tested BMI in the logistic regression model; it had little effect on the relationships between menarcheal status and either lead or E-PCB. In models testing toxicant interactions, age, SES, lead levels, and PCB levels continued to be significant predictors of menarcheal status. When each toxicant was tested in a logistic regression model correcting only for age and SES, we observed little change in the effects of lead or E-PCB on menarcheal status.**

Conclusions. **The analysis of multichemical exposure among Akwesasne Mohawk Nation adolescent girls suggests that the attainment of menarche may be sensitive to relatively low levels of lead and certain PCB congeners. This study is distinguished by the ability to test many toxicants simultaneously and thus to exclude effects from unmeasured but coexisting exposures. By testing several**

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PCB congener groupings, we were able to determine that specifically a group of potentially estrogenic PCB congeners affected the odds of reaching menarche. The lead and PCB findings are consistent with the literature and are biologically plausible. The sample size, cross-sectional study design, and possible occurrence of confounders beyond those tested suggest that results should be interpreted cautiously. Additional investigation to determine whether such low toxicant levels may affect reproduction and disorders of the reproductive system is warranted. *Pediatrics* **2005;115:e127–e134. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-1161;** ***puberty, sexual maturation, endocrine disruptors, Native Americans, Haudenosaunee, Iroquois.***

ABBREVIATIONS. HCB, hexachlorobenzene; NHANES III, Third National Health and Nutrition Examination Survey; PCB, polychlorinated biphenyl; POP, persistent organic pollutant; *p,p′*-DDE, dichlorodiphenyldichloroethylene; SES, socioeconomic status; E-PCB, sum of 4 potentially estrogenic polychlorinated biphenyl congeners.

Today, children in many countries are chronically exposed at background levels to a range of common pollutants, including lead and persistent organic pollutants (POPs). POPs, such as mirex, dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs), are environmentally persistent chemicals that tend to be lipophilic and bioaccumulate.[1] Lead and POPs have been shown to affect the endocrine system in laboratory animals and in wildlife.[2–4]

The endocrine-disrupting effects of these environmental pollutants in animal studies have prompted interest in possible reproductive effects.[5] Recent observations[6] of pubic hair and breast development among many girls <7 years of age, especially black girls, have been viewed by some as evidence of an ongoing secular trend.[7] It has been suggested that a secular trend toward earlier maturation in the past few decades has been fueled by exposure to POPs.[8] Animal studies indicate that POPs and lead have a variety of adverse effects on reproductive development and functioning.[9–13]

Comparatively little research has investigated the relationship of pollutants to human sexual maturation. Perinatal exposure to POPs has been associated with earlier age at menarche and pubic hair staging among girls[14] and higher rates of precocious puberty,[15] as well as delayed sexual maturation among both boys and girls[16] and smaller penis size.[17] Recent analyses of lead have found delays of menarche and sexual maturation (Tanner staging) among girls with relatively low lead levels.[18,19]

Although each exposure may produce different effects and most populations are exposed to mixtures of chemical pollutants, to date studies of pollutants and human sexual maturation have not examined the concurrent effects of the most common pollutants to which children may be exposed. Here we assess the relationship between attainment of menarche and PCBs, *p,p′*-DDE, HCB, mirex, lead, and mercury among Mohawk girls, to determine the effects, if any, of low levels of these pollutants on female sexual maturation.

METHODS

Setting

The study was conducted with residents of the Akwesasne Mohawk Nation. The Nation is a sovereign territory lying on both sides of the St Lawrence River and spanning the boundaries of New York State and Ontario and Quebec, Canada. Since the 1950s, the St Lawrence River has been a site of substantial industrial development. Today, several industrial complexes are in close proximity to Akwesasne, including a US National Priority Superfund site (General Motors Central Foundry Division) and 2 New York State Superfund sites (Reynolds Metal Company and Aluminum Company of America). Exposure assessment studies in the 1990s found some local animal species to have PCB, *p,p′*-DDE, HCB, and mirex levels above the US Food and Drug Administration human consumption tolerance limits.[20,21] Current levels of POPs in this study sample of 10- to 16.9-year-old participants largely reflect prenatal and lactational exposure[22] that is consistent with known levels of contaminants among adult women, attributable to past maternal consumption of locally caught, contaminated fish.[23] The history of local environmental pollution, the traditional reliance on locally caught fish and game, and the concerns of community members about the health effects of environmental pollutants prompted human health studies at Akwesasne, including an investigation of adolescent development.

Participants

The study participants, recruitment and data collection protocols, laboratory analysis methods, and substitution methods for toxicant levels below the laboratory minimum detection limits were previously described in detail.[22] Briefly, all data were collected by Akwesasne community members, without prior knowledge of participants' exposure status. All study protocols were approved by the institutional review board at the University at Albany, State University of New York, and informed consent was obtained from all participants. The target population was defined as residents of Mohawk households located in the Akwesasne Mohawk Nation and in neighboring communities within 10 miles of Akwesasne. Of the households meeting the eligibility requirements for the study, 294 mother/adolescent pairs enrolled in the study. Of these, 19 were lost to follow-up, 1 was found to be ineligible, and 3 had blood samples that were broken in transit, for a final sample size of 271 (140 females and 131 males). Of the 140 eligible female participants who finished the study, 138 had complete data for this analysis.

Data Collection and Variables

Menarche

Menarche was measured as present or absent, based on self-report at the time of the interview and blood sample collection.

Socioeconomic Status

A weighted index of socioeconomic status (SES) was created in consultation with Native American informants from the community. The index used data from interviews with mothers and included: employment, marital status, education, house size and condition, number of motor vehicles, and age of newest motor vehicle.

Pollutants

Fasting blood specimens were collected at first rising by trained Mohawk staff members. Toxicants analyzed were lead, mercury, PCBs, *p,p′*-DDE, HCB, and mirex. Analyses of PCBs and organochlorine pesticides were conducted at the University at Albany School of Public Health Analytical Laboratory. High-resolution, ultratrace, congener-specific analysis was performed with parallel dual-column (splitless injection) gas chromatography with electron capture detection.[24] This method quantitates up to 83 individual PCB congeners and 18 PCB congeners as pairs and triplets, as well as *p,p′*-DDE, HCB, and mirex. Lead and mercury analyses were conducted by Le Centre de Toxicologie due Quebec (Sainte-Foy, Quebec, Canada). Mercury analysis was based on cold-vapor atomic absorption spectrometry. Lead was analyzed with Zeeman-corrected graphite furnace atomic absorption spectrometry.[25]

The US Environmental Protection Agency recommended meth-

od[26] was used to impute values below the minimum detection limits for lead, mercury, PCBs, and HCB, all of which had rates of detection of ≥50%. Lead, mercury, PCBs, *p,p′*-DDE, and HCB were natural-logarithmically transformed, because of the skewness of the toxicants. Mirex levels were categorized into 3 groups because of the high rate of undetectable levels, ie, nondetects (below the minimum detection limit of 0.02 ppb; 51.4% of the sample), low detects (0.02–0.03 ppb; 20.3% of the sample), and high detects (0.04–1.17 ppb; 28.3% of the sample).

We considered the 16 PCB congeners with a >50% rate of detection in our sample for hypothesis testing. Three representations of PCB burden were tested, on the basis of the PCB congener groups described by Wolff and co-workers,[27,28] ie, estrogenic/neurotoxic, antiestrogenic/dioxin-like, and enzyme-inducing (Table 1).

Statistical Analyses

We used probit and logistic regression to model the probability that study participants reported the presence (scored 1) or absence (scored 0) of menarche and how that probability was altered by changes in relevant independent variables. The motivation for both models can be found in the generalized linear model approach to describing models of binary outcomes. The cumulative distribution functions of the probit and logistic regression models are quite similar and generate predicted probabilities that are almost identical. The choice is often dictated by disciplinary preference rather than inferential properties.[29,30] We used the probit model to estimate the median age at menarche but we used logistic regression to interpret the effects of toxicants on the attainment of menarche, because of its greater flexibility in describing model results.

The median age at menarche was estimated to establish the comparability of the sample to other populations, as well as to compare the effect of high versus low lead levels on age at menarche without adjustment for additional toxicants or covariates. The median age at menarche for the sample was estimated with probit analysis[31] using the status quo method.[32] With the status quo method, age and the presence or absence of menarche are assessed at the time of the interview. The combination of probit analysis and the status quo method is the best method for estimating the median age at menarche for a population, because it eliminates recall bias and solves the problem posed by individuals who have not yet reached menarche.[33] Instead of recall of the age at menarche, the probit transformation is applied to the distribution of the percentage of postmenarcheal girls in each age group. The age corresponding to 50% of the probit distribution is the predicted median age at menarche for the population.

Binary logistic regression analysis was used to test the influence of 6 toxicants (mirex, *p,p′*-DDE, HCB, PCBs, lead, and mercury) on the likelihood (log odds) of having attained menarche (yes/no), controlling for other influences on menarche. The control variables (age, SES, and BMI) tested for inclusion in the model were retained in the model if they reached statistical significance ($P < .05$). Any variable not included on the basis of statistical significance was reconsidered, to estimate the effects on results and to determine whether its exclusion would alter any observed effects of toxicants. All continuous independent variables were mean-centered. Squared terms were included to test for nonlinear effects. All statistical testing was performed with SPSS software, version 11.5.0 (SPSS, Chicago, IL). An α level of .1 was chosen for inclusion of nonlinear terms. An α level of .05 was used for testing of all other effects. Squared terms that were maintained in the model and their corresponding variable main effects were tested at several toxicant levels.

In addition, a logistic regression model with age, SES, and only 1 toxicant was run for each toxicant. We compared the results of the single-toxicant models with the model containing all 6 toxicants simultaneously. The purpose of this comparison was to test the influence of additional toxicants in the multiple-toxicant model on the observed effect of a given toxicant.

RESULTS

Toxicant Levels

Toxicant levels for this sample of Mohawk girls are shown in Table 2. Levels were consistent with long-term exposure to a variety of toxicants in multiple media. Mercury levels in the current study were at or below background levels observed for the general population (0.1–0.8 μg/dL).[34] The highest lead level was less than one half the Centers for Disease Control and Prevention action limit of 10 μg/dL. PCB levels were consistent with a pattern of both cumulative and recent exposure.[22]

Probit Analysis

Girls included in this analysis ranged in age from 10 through 16.9 years; 83 (59.7%) had reached menarche. With probit analysis, the median predicted age at menarche for the total sample was 12.2 years (95% confidence interval: 11.9–12.5 years). This group of Mohawk girls was comparable to a larger sample of 10- to 16-year-old American girls from the Third National Health and Nutrition Examination

TABLE 1. Structures, Rates of Detection, and Groupings[27,28] of the 16 PCB Congeners With Rates of Detection of ≥50% in Our Sample (n = 138)

PCB IUPAC No.	Structure	Rate of Detection, %	Wolff Group*
118	2,3′,4,4′,5	98.6	2
153	2,2′,4,4′,5,5′	97.8	3
110	2,3,3′,4′,6	96.4	
99	2,2′,4,4′,5	95.7	3
180	2,2′,3,4,4′,5,5′	89.9	3
138 [+163 and 164]	2,2′,3,4,4′,5′ + 2,3,3′,4′,5′,6 + 2,3,3′,4′,5,6	88.4	2 [138]†
101 [+90]	2,2′,3,4′,5 + 2,2′,4,5,5′	86.2	1 [101]†
95	2,2′,3,5′,6	83.3	
52	2,2′,5,5′	82.6	1
87	2,2′,3,4,5′	81.2	
74	2,4,4′,5	76.1	2
105	2,3,3′,4,4′	62.3	2
149 [+123]	2,2′,3,4′,5′,6 + 2,3′,4,4′,5′	60.9	
70	2,3′,4′,5	59.4	1
187	2,2′,3,4′,5,5′,6	55.8	1
84	2,2′,3,3′,6	54.3	

IUPAC indicates International Union of Pure and Applied Chemistry.
* The PCB congener groups described by Wolff and co-workers[27,28] are as follows: 1, estrogenic/neurotoxic (E-PCB); 2, antiestrogenic (dioxin-like); 3, enzyme-inducing.
† Numbers in parentheses refer to the PCB congener in the summation that determines the Wolff group.

TABLE 2. Descriptive Statistics for Variables Included in the Logistic Regression Model ($n = 138$)

Variables	Mean*	SD	Range†
Age, y	12.9	1.92	10–16.9
SES index‡	25.0	5.23	9–37
Mercury, μg/dL	0.09	0.084	0.02–0.51
Blood lead, μg/dL	0.49	0.905	0.07–4.40
E-PCB, ppb	0.12	0.083	0.06–0.47
p,p′-DDE, ppb	0.35	0.347	0.09–2.93
HCB, ppb	0.03	0.013	0.02–0.11

* The geometric mean is given for toxicants.
† Minimum values given for mercury, lead, and HCB are the substitution values for nondetects.
‡ A weighted index of SES was created based on data from interviews with mothers and included employment, marital status, education, house size and condition, number of motor vehicles, and age of newest motor vehicle.

Survey (NHANES III),[18] in terms of the distribution of menarcheal status according to age (Table 3).

Girls at or above the median blood lead level of 1.2 μg/dL had a predicted age at menarche of 12.7 years (95% confidence interval: 12.2–13.1 years), without adjustment for any other factors that can influence age at menarche. Girls below the median lead level had a predicted age at menarche of 11.8 years (95% confidence interval: 9.9–12.8 years), 10.6 months earlier than that for girls with higher lead levels.

Logistic Regression

Binary logistic regression analysis predicting menarcheal status (premenarcheal or postmenarcheal) was used to consider simultaneously the effects of multiple toxicants while controlling for other influences on menarche (Table 4). The effect associated with each predictor variable takes into account all other variables in the model. As expected, age was the strongest predictor of attainment of menarche in the binary logistic regression analysis and was positively associated with having reached menarche. Lower SES was related to higher odds of having reached menarche. In other words, same-aged girls with lower SES were more likely to have reached menarche than were girls with higher SES. Mirex, *p,p′*-DDE, and HCB were unrelated to menarcheal status. The effect of BMI was tested, but BMI was found not to be a significant predictor in the model, perhaps because of its association with age ($r = 0.322$, $P < .001$), which is included in the model.

A nonlinear effect was observed for lead, as indicated by the squared term in Table 4. At the geometric mean (0.49 μg/dL) and 75th percentile (1.66 μg/dL), lead was associated with a lower likelihood of having attained menarche, after adjustment for age, SES, and other toxicants. This main effect of lead was not significant below the geometric mean. A 100% increase in lead levels above the mean, from 0.49 to 0.98 μg/dL, decreased the odds of reaching menarche by 72% when all other variables were held constant at their respective means. The odds of reaching menarche decreased by 98% with a doubling of lead levels above the 75th percentile, from 1.66 to 3.32 μg/dL.

The estrogenic PCB (E-PCB) group described by Wolff and co-workers[27,28] was associated with a higher likelihood of having reached menarche, after adjustment for age, SES, and other toxicants. A 100% increase in E-PCB levels above the mean, from 0.12 to 0.24 ppb, was associated with 8.4 times greater odds of having reached menarche. Using the same logistic regression analytic technique, we tested the effects of the 2 other classes of PCBs (antiestrogenic and enzyme-inducing) described by Wolff and co-workers.[27,28] No relationship was observed between menarche and the PCB groups that Wolff and co-workers[27,28] classed as antiestrogenic or enzyme-inducing.

A nonlinear effect of mercury was observed, as indicated by the squared term in Table 4. The main effect of mercury was not significant at the mean and was marginally significant ($\beta = 2.57$, $P = .08$, 2-tailed test; results not shown) at the geometric 95% percentile of mercury levels (0.28 μg/dL).

Figures 1 and 2 show predicted probabilities of 12-year-old Mohawk girls reaching menarche at the geometric mean lead level and above (Fig 1) and at all levels of E-PCB (Fig 2), controlling for age, SES, and all other toxicants. Predicted probabilities for lead are not shown below the geometric mean because lead was a significant predictor of menarcheal status only at the geometric mean and above. Calculations were based on the mean-centered logistic regression model presented in Table 4, with all other variables held constant at their respective means. Approximately 69% of 12-year-old girls were predicted to have reached menarche. As lead levels increased to the 75th percentile, 10% of 12-year-old Mohawk girls were predicted to have reached menarche. As the levels of E-PCB increased, the predicted probability of 12-year-old Mohawk girls having reached menarche increased from 52% at the 25th

TABLE 3. Number and Percentage of Akwesasne Mohawk Girls Who Have Reached Menarche, According to Annual Age Group (10–16 Years of Age), in the Present Study ($n = 138$), Compared With the NHANES III[18] Dataset ($n = 1235$)

Age, y	Akwesasne Mohawk Girls		NHANES III	
	No. With Menses/No.	% With Menses	No. With Menses/No.	% With Menses
10	1/20	5.0	7/192	3.6
11	0/19	0.0	45/211	21.3
12	10/22	45.5	106/186	57.0
13	21/26	80.8	141/169	83.4
14	16/17	94.1	152/162	93.8
15	19/19	100.0	153/154	99.4
16	15/15	100.0	161/161	100.0

TABLE 4. Binary Logistic Regression Analysis Predicting Menarcheal Status (Premenarcheal or Postmenarcheal) (n = 138)

Variables	β	SE	P	Exp(β)
Mean-centered model constant	2.90	1.573	0.07	18.21
Mirex (nondetects vs low detects)	0.73	0.955	0.45	2.07
Mirex (high detects vs low detects)	−0.13	1.052	0.91	0.88
Mean-centered				
Age, y	2.44	0.496	0.00	11.51
SES index	−0.17	0.073	0.02	0.85
p,p'-DDE, ppb*	−0.37	0.837	0.66	0.69
HCB, ppb*†	0.12	1.315	0.93	1.12
E-PCB, ppb*†	2.13	1.017	0.04	8.39
Mercury, μg/dL*†	0.16	0.577	0.78	1.17
Mercury level squared*†	1.08	0.608	0.08	2.94
Lead, μg/dL*†	−1.29	0.494	0.01	0.28
Lead level squared*†	−1.01	0.569	0.08	0.36
75th percentile model constant	0.26	0.827	0.76	1.29
75th percentile-centered				
Mercury, μg/dL*†	1.36	0.897	0.13	3.91
Lead, μg/dL*†	−3.75	1.822	0.04	0.02

Cox and Snell R^2 = 0.61; Nagelkerke R^2 = 0.83.
* Natural-logarithmically transformed.
† Values below the detection limit were replaced with the use of formulae described previously.[22]

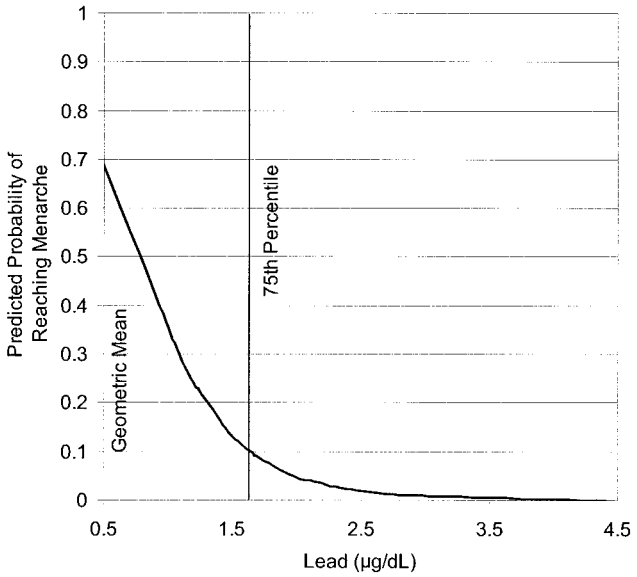

Fig 1. Predicted probability of 12-year-old Mohawk girls reaching menarche at different levels of lead greater than or equal to the geometric mean, with all other variables held constant at their respective means (calculations based on the mean-centered model in Table 4). The predicted probability curve crosses the y-axis at the geometric mean lead level (0.5 μg/dL), and the vertical line indicates the 75th percentile of lead levels (1.7 μg/dL).

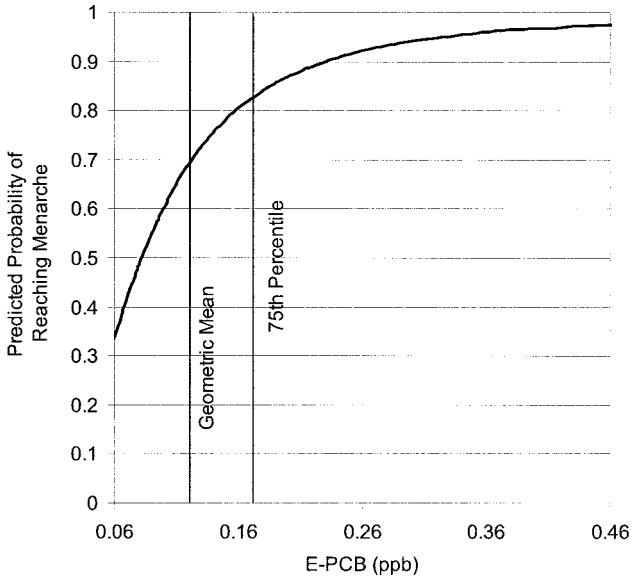

Fig 2. Predicted probability of 12-year-old Mohawk girls reaching menarche at different levels of E-PCB, with all other variables held constant at their respective means (calculations based on the mean-centered model in Table 4). The vertical lines indicate the geometric mean (0.12 ppb) and 75th percentile (0.17 ppb) of E-PCB levels.

percentile of E-PCB levels to 69% at the mean and to 86% at the 75th percentile.

To substantiate the interpretation of coefficients in the logistic regression model containing all 6 toxicants (Table 4), single-toxicant logistic regression models that corrected only for age and SES were considered. We compared these single-toxicant models with the model shown in Table 4 and found little change in the measure of effect (β coefficients), with the exception of mercury (results not shown). Neither main nor nonlinear effects of mercury were found in the model containing only age, SES, and mercury. Also, adding BMI to the logistic regression model (Table 4) had little effect on the relationships between menarche and either lead or E-PCB, as indicated by the small changes in β coefficients (lead: without BMI, $\beta = -1.29$; with BMI, $\beta = -1.10$; E-PCB: without BMI, $\beta = 2.13$; with BMI, $\beta = 1.98$).

We assessed nonadditivity in the effects of toxicants in the 6-toxicant model. Interactions among all pairs of the 6 toxicants were tested. Three statistically significant interactions were detected, namely, lead and E-PCB, lead and HCB, and lead and mirex (results not shown). In the 3 models with significant interaction terms, age, SES, E-PCB, and lead remained significant at $P < .05$. To estimate how the effects of toxicants were conditioned by levels of the other toxicants, predicted probability plots were compared, those based on the presented model without interactions (Table 4) and those based on models

including interactions. The patterns of effects for each toxicant were indistinguishable across models.

DISCUSSION

Analysis of multichemical exposure among Akwesasne Mohawk adolescent girls provides evidence that lead and certain PCB congeners may affect attainment of menarche. These associations were observed after controlling for potential confounders. No association was observed between mirex, *p,p′*-DDE, or HCB and menarcheal status.

In this study, lead was associated with a later median predicted age at menarche, without controlling for other variables, as well as a reduced likelihood of having reached menarche, controlling for other toxicants, age, and SES. This finding is consistent with analyses of the NHANES III data that showed 1- to 6-month delays in attainment of menarche and Tanner breast and pubic hair stages among girls 8 to 18 years of age with only modest increases in blood lead levels from 1 to 3 μg/dL.[18,19] The NHANES III effects were seen after adjustment for socioeconomic factors and body size, which suggests that lead might have delayed maturation through changes in the endocrine system, rather than as a consequence of delayed growth. Although the effect we observed was larger, it was stable across analyses, both with and without control for SES, BMI and other toxicants. One possible explanation for the larger effect size is our greater control for other toxicant levels, which could have maximized the observed effect of lead.

The plausibility of the observed relationships between higher lead levels and a lower likelihood of having reached menarche and delayed menarche is supported by animal studies showing that lead exposure delayed pubertal development and reduced reproductive organ growth.[10–12] Two biological mechanisms might be at work here. Lead might alter sexual maturation through direct effects on all levels of the hypothalamic-pituitary-gonadal axis.[3,13] Lead might also indirectly affect sexual maturation by restricting or delaying growth. Several cross-sectional studies found higher lead levels to be associated with decreased weight and height.[35–38] Body size, in turn, has been directly related to the timing of sexual maturation.[39,40]

In addition to the effect of lead, a group of 4 potentially estrogenic PCB congeners was found to be associated with a greater likelihood of having attained menarche, controlling for age, SES, lead, mercury, *p,p′*-DDE, HCB, and mirex. Few other studies are available for comparison, and among these there is some variation in results. Among breastfed girls, perinatal polybrominated biphenyl exposure was associated with earlier pubic hair staging, and in utero polybrominated biphenyl exposure was associated with earlier menarche.[14] Similarly, significantly higher concentrations of *p,p′*-DDE were found among girls with precocious puberty who immigrated to Belgium from developing countries.[15] Among girls in a North Carolina cohort, there was some evidence that PCB exposure was associated with younger ages at attainment of pubertal stages, but results were not conclusive because of the small sample size.[41] These associations of POP exposure with earlier sexual maturation are consistent with the present study. However, a study of Flemish adolescents found an association between dioxin-like compounds and delayed attainment of Tanner adult breast stage, and neither PCB levels nor polychlorinated aromatic hydrocarbon exposure was significantly correlated with the timing of menarche or pubic hair staging among girls.[14,16,42] These differences in results (delay, acceleration, or no effect) might be attributable to exposure to different types and/or combinations of POPs, which can have estrogenic, androgenic, and antiestrogenic effects. In addition, some of these studies focused on 1 or 2 toxicants or classes of toxicants in their analyses, leaving open the possibility that other covarying but unmeasured toxicants modify the observed effects.

PCBs and related compounds have been associated with several other effects related to the reproductive system, including decreased implantation rate, litter size, and sexual receptivity among animals, as well as reduced fecundity, menstrual irregularity, decreased sperm motility, and hormonal changes among humans.[9] Although these effects are consistent with our results, the exact mechanisms underlying the effects of POPs are unclear. Furthermore, >1 pathway may be involved, depending on the combination, concentration, and timing of POP exposure, as well as the species affected. Although the specific mechanisms of effects are not known, modification of the hypothalamic-pituitary-gonadal axis has been proposed as a likely means of action.[43–46]

The results for mercury reported here cannot be assessed against other research because no investigations of the relationship between mercury levels and sexual maturation among humans have been published. Animal studies indicate the possibility of endocrine and reproductive effects of mercury.[47,48]

The relationship observed here between lower SES and earlier menarche requires interpretation. Higher SES has been shown to be associated with earlier menarche.[49,50] However, this negative relationship was observed in settings in which nutritional status is highly variable and strongly related to socioeconomic factors. Other studies that found no relationship involved well-off populations without threats to maturation from nutritional stress.[51,52] The Akwesasne Mohawk sample demonstrates no evidence of nutritional deficiency that would affect menarche,[53,54] and the observed relationship between menarche and SES fits the pattern observed for well-off samples.

Here our focus was not on the relationship of SES to menarche but on the role of SES in influencing the relationship of lead and PCBs to menarche. In the present study, the removal of SES from the logistic regression model had little impact on the effects of age and toxicants on menarche (on the basis of changes in β coefficients) or on the pseudo-variance explained (2.8% reduction in both Cox-Snell and Nagelkerke pseudo-R^2).

This study is distinguished by its ability to con-

sider multiple common toxicants simultaneously and to include congener-specific PCB analysis. Despite relatively low toxicant levels in our sample, both lead and E-PCB were found to be associated with attainment of menarche after controlling for age, SES, and other toxicants. By testing several PCB congener groupings, we were able to determine that specifically a group of potentially estrogenic PCB congeners affected the odds of reaching menarche. Although a nonlinear effect of mercury on menarche was suggested, with the effect strengthening at higher levels, mercury did not reach statistical significance in our model. Additional tests of single-toxicant models suggested that the results observed for lead and E-PCB were robust and not influenced by the other model variables, as indicated by the stable β coefficients. Body size had little impact on the associations of lead or E-PCB levels with menarcheal status in this sample. Inclusion of terms representing interactions among the toxicants did not substantially change the effects of each toxicant. The small sample size and very small number of participants at the extremes of the interaction space limit our ability to generalize from analyses of interactions.

CONCLUSIONS

We observed an association between low levels of 4 potentially estrogenic PCB congeners and lead and the attainment of menarche, controlling for SES, age, and other toxicants. These findings are consistent with the literature and are biologically plausible. However, cautious interpretation is warranted because of the sample size, the cross-sectional study design, and the possible occurrence of confounders beyond those tested, including genetic factors. In this, as in other observational studies, the results are derived from a specific range of values, and it is inappropriate to generalize the effects to levels above or below the values in this sample. At much higher or lower levels of lead and/or PCBs, different effects may occur. Findings reported here suggest that the timing of sexual maturation may be sensitive to relatively low levels of lead and certain PCB congeners. Additional investigation to determine whether such low levels may affect reproduction and disorders of the reproductive system is warranted.

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