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Prenatal polychlorinated biphenyl exposures in eastern Slovakia modify effects of social factors on birthweight

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Summary

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Polychlorinated biphenyls (PCB) were widely used for industrial purposes and consumer products, but because of their toxicity, production was banned by most industrialised countries in the late 1970s. In eastern Slovakia, they were produced until 1985. During 2002–04, a birth cohort of mothers ($n = 1057$) residing in two Slovak districts was enrolled at delivery, and their specimens and information were collected after birth. Congeners of PCBs were measured in maternal serum by high-resolution gas chromatography with electron capture detection. In this study, we used multiple linear regression to examine the effects of prenatal PCB exposure on birthweight adjusted for gestational age, controlling for inter-pregnancy interval, and maternal smoking, age, education, ethnicity, pre-pregnancy body mass index and height.

The association between total maternal serum PCB levels and birthweight was not statistically significant. However, an interaction model indicated that maternal PCB concentrations were associated with lower birthweight in Romani boys. Based on the fitted regression model, the predicted birthweight of Romani boys at the 90th percentile of maternal PCBs (12.8 ng/mL) was 133 g lower than the predicted birthweight at the 10th percentile of maternal PCBs (1.6 ng/mL). This is a similar magnitude of effect to that observed for maternal smoking and birthweight. These results suggest that higher levels of PCBs in maternal blood sera may inhibit growth in boys, particularly in those already affected by social factors related to ethnicity. This study is consistent with previous findings that boys are more susceptible than girls to growth restriction induced by *in utero* organochlorine exposures, and further indicates that high PCBs may magnify the influence of social disadvantage in this vulnerable group of boys.

Keywords: *PCBs*, *birthweight*, *ethnic origin*, *interaction*, *susceptibility*.

Introduction

Polychlorinated biphenyls (PCBs) are a family of chemical compounds that was introduced in 1929, and used for half a century in electrical transformers and capacitors, and as heat exchangers and hydraulic fluids. Because of their toxicity, production and use was banned by most industrialised countries in the late 1970s. These chemicals are known to cross the placenta, resulting in prenatal exposure to the fetus.[1] Postnatal exposure to the neonate occurs through breast milk.[2,3] The primary means for introduction of PCBs into the environment has been through improper disposal; secondary pathways include volatilisation of previously released material, and creation of PCBs during

combustion processes.[4] Because PCBs are very lipophilic and stable, bioaccumulation and biomagnification occur through the food chain. Body burdens of PCBs in humans have decreased slowly since they were banned; nevertheless levels are still relatively high in some areas of the world.

Between 1959 and 1985 in Slovakia, more than 21 000 tons of PCBs were produced as part of commercial mixtures at the Chemko Inc. chemical manufacturing facility in the Michalovce district. As a result of improper discharges into the environment, the local population has been exposed to higher levels of PCBs than those found in the general population of Slovakia or other countries, as documented by elevated blood and adipose PCB levels during the 1970s and early 1990s.[5] High levels of PCBs were found in home-made butter and eggs from producers in the Michalovce district.[6]

Exposure to high levels of heat-degraded PCBs from oil-poisoning accidents in Japan and in Taiwan resulted in adverse developmental effects on children exposed prenatally.[7,8] Among other problems in the Taiwanese babies, the proportions of low-birthweight and preterm births among the exposed newborns were higher than in unexposed births. Studies from fishing communities found that a high consumption of PCB-contaminated fish or increased levels of PCBs in cord serum were associated with greater risk of having a low-birthweight baby[9,10] or with lower mean birthweight or smaller mean head circumference.[11] In another study of fish consumers,[12] a positive association between prenatal PCB exposure and birthweight was observed, while studies of births in the Faroe Islands and in Great Lakes found no significant association.[13,14] These positive or null associations in some fishing community studies may be attributable to the beneficial effect on birthweight of n-3 fatty acids in fish, which promote growth[15] and hence counteract the negative effects of PCBs. At lower levels of PCB exposure in the general population, the findings have been contradictory, with some finding a reduction in intrauterine growth[16] and others showing null results.[17,18] One study with null results used measurements of PCBs in breast milk[18] as an indicator of prenatal PCB exposure.

A number of studies report a selective PCB effect on birthweights of male infants,[19–21] suggesting that male infants may be more vulnerable to prenatal PCB exposure than female infants. In the present study, we examined the effects of prenatal PCB exposure on birthweight overall and by gender, based on deliveries from 2002–04 in two districts of eastern Slovakia.

Materials and methods

Study population

This report is based on an on-going cohort study being conducted in eastern Slovakia by a bi-national team of investigators. Participants were mother–child pairs who were recruited from two districts: Michalovce with high PCB contamination in the environment from a chemical manufacturing plant, and Stropkov/Svidnik located 66 km to the north-east, having lower environmental levels of PCBs. Mothers were enrolled at the time they came to the hospital for delivery. Each district has only one hospital, and virtually all deliveries occur in these two hospitals. Births in the following categories were excluded from the study: (i) mothers with >4 previous births, (ii) mothers <18 years old, (iii) mothers who had resided in the district for <5 years, (iv) mothers who had a major illness during pregnancy, and (v) infants born with severe birth defects. Informed consent was obtained by trained nurses at the hospital before delivery. The protocols were approved by the Institutional Review Board of the University of California, Davis and the Slovak Medical University (SMU) prior to the initiation of the data collection.

Specimen collection

At delivery, maternal blood specimens (approximately 20 mL) were collected by a nurse using venipuncture into standard vacutainers (S-Monovette Serum SARSTEDT) and labelled by date and time of blood collection and study identification number. The tubes were stored in the refrigerator (5–10°C) and transported to the Department of Biochemistry where they were centrifuged and serum aliquots were divided into test tubes. Approximately 3 mL of blood serum were placed into an 8-mL clear screw cap glass vial (CHROMSERVIS) with solid screw cap with Teflon liner for analysis of PCBs. Samples were stored frozen (–20°C) and transported to Bratislava, where they were analysed at the Research Base of the SMU.

Total lipids were determined utilising the enzymatic 'summation' method according to Akins *et al.*[22]

PCB measurement

The Department of Toxic Organic Pollutants at the SMU performed the laboratory analyses. The concentrations of 17 congeners (#28, #52, #101, #105, #114, #118, #123, #138, #149, #153, #156, #157, #167, #170, #171, #180 and #189) were determined in the maternal serum samples by high-resolution gas chromatography with electron capture detection. Specimens were stored at –18°C.

In summary, the procedure included standardised extraction, clean-up and quantification accompanied by a quality assurance programme. Serum was treated with methanol to precipitate the proteins, and the resulting mixture was extracted with hexane. The extract was concentrated and then cleaned by passing through a Florisil-silica gel column. The eluate was then evaporated to a small volume and a known amount of PCB congeners was added as an internal standard. An aliquot of the mixture was injected and analysed on a chromatography system (HP 5890, Hewlett-Packard, Palo Alto, CA, USA) equipped with a Ni-63 electron capture detector using a 60-m DBP-5 capillary column (J&W Scientific, Folsom, MA, USA). Quantification was based on the calibration curve generated by authentic PCB reference standards vs. solution concentration. Quality control activities consisted of analyses of samples in batches of eight simultaneously with a quality control sample. Response for a particular congener had to be in the range of 90–110% using the concentration of the middle point of the calibration curves for that congener. The limit of detection for each analyte was determined as the mean of background noise plus three standard deviations from five reagent blank samples. The laboratory is certified by the World Health Organization and the German Agency for Occupational and Environmental Medicine (Deutsche Gesellschaft fur Arbeitsmedizin und Umweltmedizin e.V.).

To handle the problem of PCB measurements below the limit of detection (LOD), we calculated a combined PCB value in several ways, and then evaluated the sensitivity of our results to the choice of approach. Some of the low abundance congeners were below the LOD for a substantial proportion of the study population, other congeners were below LOD for a much smaller percentage of women, and still others were measured in all participants, which suggested several possible algorithms. (i) Only the congeners for which all measurements were above the LOD were summed. These were IUPAC #138, #153 and #180. (ii) Only the congeners for which at least 80% of the measurements were above the LOD were summed. These were PCB IUPAC #118, #138, #153, #156, #170 and #180. For those participants for whom the concentration of one of these six congeners was below LOD, imputation was carried out by dividing the value by square root of 2.[14,23] (iii) All measured PCB congeners were summed, regardless of the percentage that were above the LOD. In this case, the imputation for each congener was carried out as described above if fewer that 50% of the participants' measurements were below LOD; if greater that 50% of the individuals were classified as below LOD, then the value was divided by 2. Each of these three 'total PCB' metrics was either entered into the model directly with total lipids as a covariate, or divided by total lipids to obtain a lipid-adjusted measure. This provided six unique measures of total PCBs for which a multiple linear regression was conducted. For brevity, we present below only the results from the models in which we summed PCBs with 80% or more values above the LOD, and in which the PCBs were on a wet weight basis with the serum lipids as a covariate.

Data collection

The three major sources for data about covariates and the outcomes of pregnancy were an interview with the mother, the newborn's medical record, and the maternal pregnancy booklet. During the standard 5-day hospital stay, each mother was interviewed to obtain information on sociodemographic characteristics, past pregnancies, occupational history, medical conditions, medication history prior to and during the pregnancy, and living environment. The abstraction of the newborn's medical record included birthweight and gestational age. Maternal pre-pregnancy weight and height were extracted from the pregnancy booklet. The gestational age was based on ultrasound examination and the clinical judgment of the woman's physician, reported in the medical records. Usually, 2–3 ultrasounds were carried out during pregnancy.

Covariate definitions

The inter-pregnancy interval was defined as the interval between the previous infant's birth date and the reported date of the woman's last menstrual cycle before the current pregnancy. A previous livebirth or stillbirth occurring after 20 weeks of gestation was con-

Table 1. Participant characteristics of eastern Slovakian birth cohort 2002–2004

	Total		Eastern European		Romani	
Variable	*n*	%	*n*	%	*n*	%
District						
Total	1057	100.0	831	100.0	226	100.0
Michalovce	743	70.3	578	69.6	165	73.0
Svidnik	314	29.7	253	30.4	61	27.0
Maternal age (years)						
18–20	156	14.8	70	8.4	86	38.1
21–30	728	68.9	603	72.6	125	55.3
31+	173	16.4	158	19.0	15	6.6
Education						
University	79	7.5	79	9.5		
High School with graduation	482	45.6	476	57.3	6	2.7
Lower education	496	46.9	276	33.2	220	97.3
Marital status						
Married	970	91.8	776	93.4	194	85.8
Never married	63	6.0	34	4.1	29	12.8
Widowed	10	0.9	9	1.1	1	0.4
Divorced/separated	14	1.3	12	1.4	2	0.9
Romani						
Eastern European	831	78.6	831	100.0		
Romani	226	21.4			226	100.0
Smoking						
No	676	64.0	568	68.4	108	47.8
Yes	381	36.0	263	31.6	118	52.2
Previous children						
No previous children	437	41.3	362	43.6	75	33.2
1 previous child	378	35.8	316	38.0	62	27.4
2 previous children	172	16.3	114	13.7	58	25.7
3-4 previous children	70	6.6	39	4.7	31	13.7
Child's gender						
Female	512	48.4	422	50.8	90	39.8
Male	545	51.6	409	49.2	136	60.2
	Mean	StdDev	Mean	StdDev	Mean	StdDev
Birthweight (grams)	3325	497	3407	480	3023	438
BMI (maternal)	22.3	5.2	22.4	5.4	21.8	4.0
Mother's height (cm)	164.3	6.5	165.4	6.0	159.9	6.4
Mother's weight (kg)	59.9	11.4	60.9	11.1	55.8	11.8
Gestation (weeks)	39.6	1.3	39.7	1.2	39.3	1.5
Total serum lipids (mg/mL)	10.2	2.0	10.4	2.0	9.5	1.8
PCBs ng/mL (6 major congeners)	6.3	8.3	6.6	9.0	5.1	4.8
Log PCB (ng/mL)	1.5	0.7	1.6	0.7	1.3	0.8

StdDev, standard deviation; BMI, body mass index; PCB, polychlorinated biphenyl.

sidered an eligible event for the calculation of this interval. Previous pregnancies that ended in abortion or miscarriage with gestation <20 weeks were not considered eligible events. In these cases, the interval extended back in time until the next previous occurrence of a livebirth or stillbirth. This interval was then categorised into: 5 months or fewer, 6–17 months, 18–23 months and ≥24 months between pregnancies,

with primiparae, i.e. women having no previous births, serving as the referent group. Maternal pre-pregnancy body mass index (BMI) was defined as weight (kg) divided by height (cm) squared. Because mothers who smoke during pregnancy have been reticent to report it, we used pre-pregnancy smoking history (yes/no) rather than reports of smoking during pregnancy. Education was coded as the total number of years of schooling completed. Romani ethnicity was attributed to the mother if the ethnic origin of either of her parents was Romani or if the Romani language was spoken in the home, or if she was planning to raise her child with the Romani language. This definition of Romani was subsequently independently confirmed by a Slovak member of the research team who used additional information such as the family's last name for classification.

Data analysis

Initial bivariate regressions were performed to explore the relationships between the outcome and predictor variables. These bivariate regressions guided the selection of variables to include in the final multivariable model.

We used SAS PROC GLM[24] to fit a multiple linear regression model to the data. The model predicted birthweight as a function of mother's home district, inter-pregnancy interval, maternal BMI, height, smoking status, years of education, mother's age, Romani ethnicity, maternal PCB serum concentration, and the child's gestational age and gender. The continuous variables were centred on their respective means to assist interpretation of interactions. Gender was considered a potential effect modifier based on the literature regarding PCB exposure and growth or development;[19] because of strong cultural and life style differences by ethnicity[25] we also evaluated this variable as an effect modifier. The PCB measurements were centred on their mean. Predicted birthweights were calculated for the 90th and 10th percentiles of the PCB distribution. Adjusted differences and their 95% confidence intervals were calculated for specific contrasts.

Either maternal height or weight, which were used to calculate maternal BMI, was missing from 4% of the medical records. As a result, 45 maternal heights and 58 maternal BMI measures were missing. To address this issue we used multiple imputation (SAS PROC MI). The method uses Markov Chain Monte Carlo sampling and regression imputation. (See SAS PROC MI and

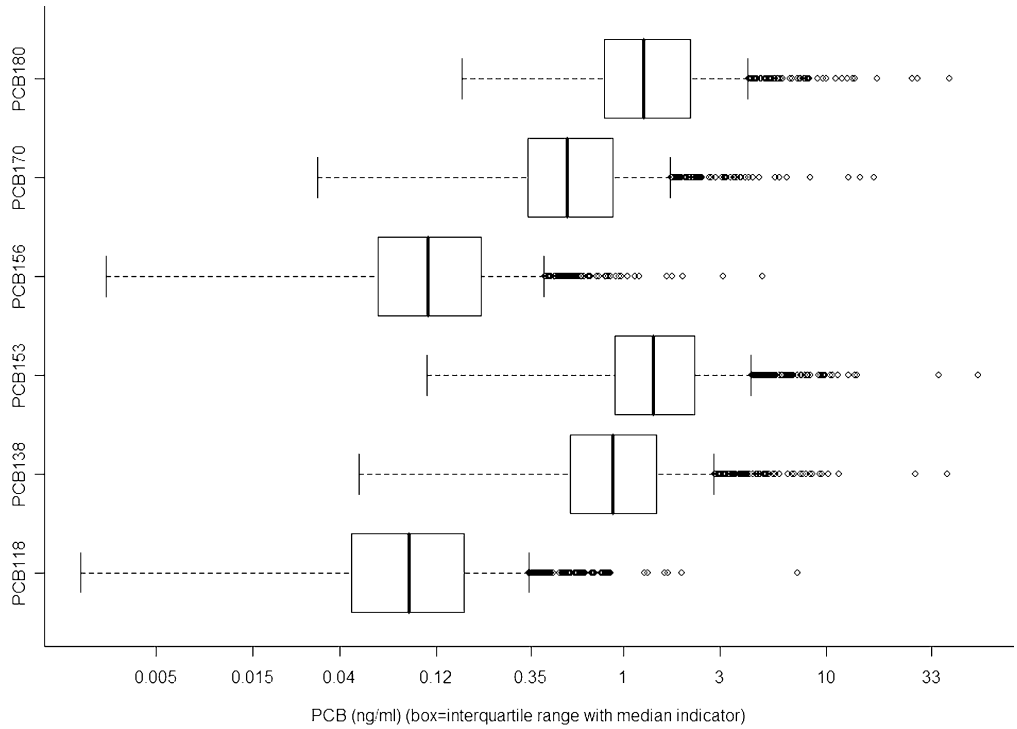

Figure 1. Maternal serum polychlorinated biphenyl (PCB) exposure: six major congeners.

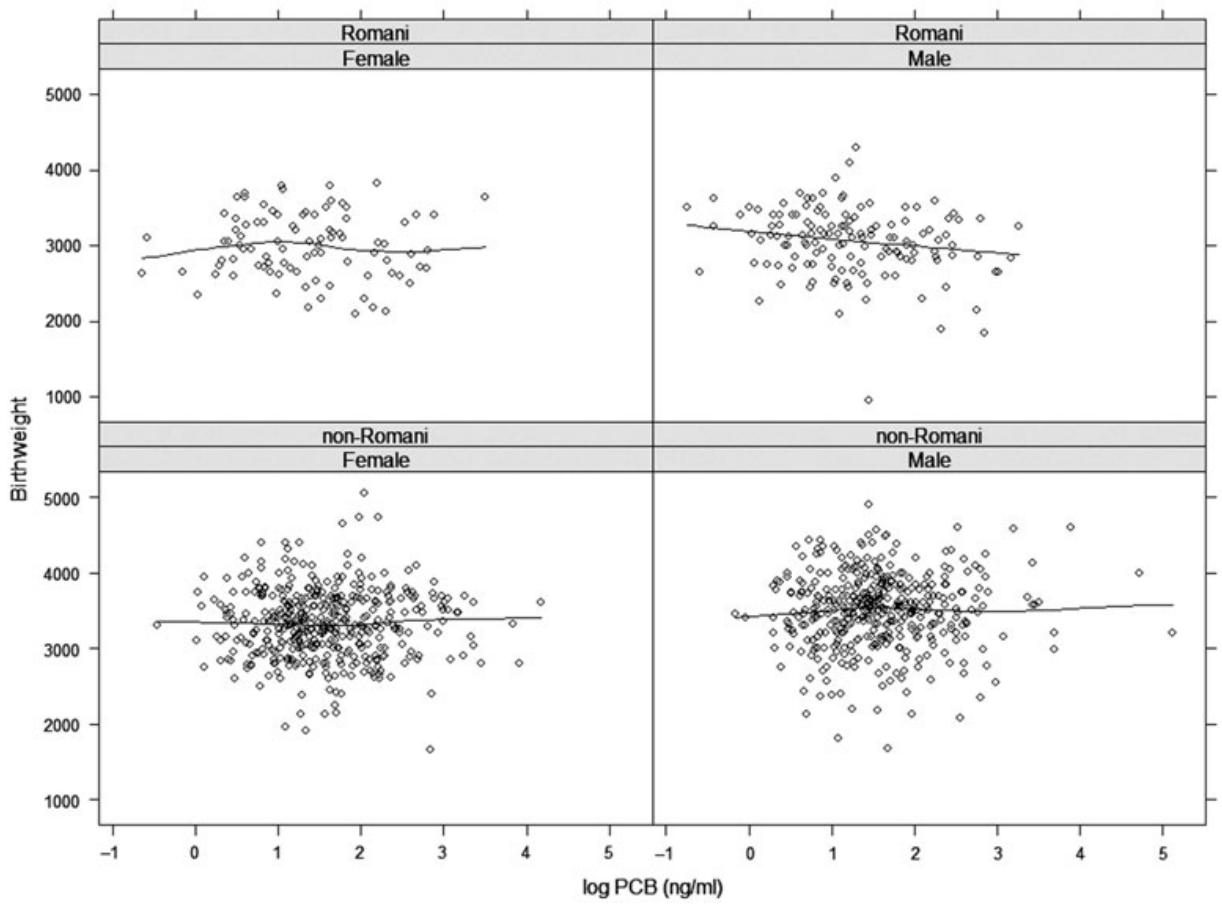

Figure 2. Smoothed trend curve for birthweight vs. log maternal serum polychlorinated biphenyls (PCBs) by child's ethnicity and gender.

MIANALYZE for further details.) The imputed values for the missing maternal height and weight variables were used to calculate the maternal BMI value used in the regression model.

Results

During the period from October 2002 to December 2004, a total of 2654 women were invited to participate in this study. Of these, 1134 (42.7%) were eligible, 494 (18.6%) were not eligible and 1026 (38.7%) with unknown eligibility declined to participate. Of the total 1134 participants, 811 (71.5%) were from Michalovce and 323 (28.5%) from Svidnik. From this total, 1057 had maternal serum determination and sufficiently complete records to be included in the analysis, with 512 (48.4%) newborns being female and 545 (51.6%) male. The mothers ranged in age from 18 to 43 years. The average birthweight was 3325 g (range 950–5060 g). The PCB values ranged from 0.5 to 166 ng/mL wet weight.

The overall cohort is described in Table 1. Those of Romani ethnicity represented 21% of the study population. Thirty-six per cent of the women in the study smoked prior to this pregnancy, 41% were primiparae and, among those with at least one previous child, 39% had an inter-pregnancy interval >24 months, and 3%, <5 months. The mean gestational age was 39.6 weeks and the mean birthweight was 3325 g.

The full distributions for the six PCB congeners that were summed to create the maternal PCB exposure variable are plotted on a log scale in Fig. 1. The unadjusted relationships between birthweight and maternal PCB exposure stratified by the child's gender and ethnicity are provided in Fig. 2. In the Romani boys, increasing PCBs were associated with lower birthweight throughout the distribution.

The final regression model for PCB exposure and birthweight is shown in Table 2. We modelled birthweight in grams with adjustment for district, interpregnancy interval, maternal BMI, maternal height, smoking, years of education, maternal age, gestational

Table 2. Multivariable linear regression: final model predicting birthweight (g) $n = 1057$

Parameter	b	SE	*t* value	P
Intercept	3215.5	28.9	110.94	<0.001
District: Svidnik vs. Michalovce	79.0	26.9	2.93	0.003
Inter-pregnancy Interval: ≤5 months vs. primiparae	–4.9	74.5	–0.07	0.947
Inter-pregnancy Interval: 6–17 months vs. primiparae	38.2	42.1	0.91	0.364
Inter-pregnancy Interval: 18–24 months vs. primiparae	226.6	60.4	3.75	<0.001
Inter-pregnancy Interval: >24 months vs. primiparae	59.0	30.6	1.93	0.054
BMI (maternal kg/cm^2)	15.5	3.3	4.72	<0.001
Mother's height (cm)	15.9	2.1	7.55	<0.001
Smoking vs. non-smoking	–40.5	25.4	–1.59	0.112
Education (years)	23.9	6.8	3.51	<0.001
Mother's age (years)	–3.7	3.2	–1.17	0.241
Gestation (weeks)	169.5	9.7	17.46	<0.001
Ethnicity: Romani vs. Slovak/other	–116.0	42.3	–2.74	0.006
Male vs. female	186.2	24.5	7.60	<0.001
Total lipid(mg/mL)	15.4	6.1	2.51	0.012
PCB (ng/mL)	0.6	1.5	0.38	0.705
PCB (ng/mL)*Romani boys	–13.2	6.7	–2.16	0.031

b: grams change in birthweight per unit change in variable; SE: standard error.
BMI: body mass index; PCBs wet weight polychlorinated biphenyls (ng/mL);
Smoking, smoking history before pregnancy.
Centred variables: maternal BMI, maternal height, education, age, gestation, lipid and PCB.

age, ethnicity and child's gender. Higher maternal BMI and height, greater maternal education and a longer length of gestation each independently contributed to larger birthweight. Mothers who smoked before pregnancy gave birth to smaller babies than those who did not smoke. For mothers with an inter-pregnancy interval of 18–24 months between births, the birthweight of the recent child was, on average, 226 g larger than the birthweight of babies that were first born.

Several two-way interactions were explored during the initial analysis. These interactions were between (i) Romani ethnicity and gender, (ii) Romani ethnicity and PCB, and (iii) gender and PCB. Of these, only the interaction between Romani ethnicity and gender was significant (data not shown). These separate two-way interactions were also modelled in pairs and used all together in one model. In all of the modelling (individually, in pairs and all together), the Romani-gender interaction remained significant. Given these results and the crude inverse relationship between maternal PCB levels and birthweight among Romani boys, we created a three-way interaction term to represent the Romani boys and maternal PCB. The reference group for Romani boys was comprised of Romani girls and Slovak/other Eastern European boys and girls. This three-way interaction term was significant in our model, indicating that birthweight in Romani boys was 13 g lower for each ng/mL increase in maternal PCB. The regression model was then used to calculate the predicted birthweight of boys at the 90th PCB percentile (12.8 ng/mL) and the 10th PCB percentile (1.58 ng/mL). An increase in maternal PCBs from the 10th percentile to the 90th percentile level resulted in a lower predicted birthweight of 3129 vs. 3262 g, for a difference of 133 g or a 4% decrease in weight.

The results from our sensitivity analysis, in which we calculated total PCBs using different approaches to the values <LOD and different adjustments for lipids, were not essentially altered (data not shown). Additionally, when we excluded subjects with imputed height and maternal BMI, the results again did not change.

Discussion

The PCB exposure levels in this population were relatively high in comparison with most other populations in the world today. In studies of early childhood development in general populations from the 1990s, the median concentration of PCB 153, representing the major congener in humans, ranged from 30 ng/g serum lipids in Massachussetts to 140 ng/g serum lipids in Germany, with a high of 450 ng/g in the Faroe Islands, where whale meat and blubber are commonly

consumed.[13,26–28] In the present study, the median concentration of PCB 153 was 140 ng/mg serum lipid. The typical half-life of PCBs is 10 years, implying that levels will continue to decrease as time since exposure increases. These levels imply that the residents in eastern Slovakia may be an ideal population for assessing the upper limits of adverse health and developmental outcomes resulting from PCB exposure, especially in populations with typical western diets.

While we did observe an association between birthweight and prenatal PCB exposure in the Romani population, we did not observe this association in the Slovak/other eastern European population, indicating inconsistency with some studies that observed associations at lower exposures. On the one hand, findings in other studies could have been artefacts owing to measurement error, bias, confounding or random sampling fluctuations. On the other hand, we cannot rule out the possibility that other unmeasured factors or biases may have diluted the actual association in the Slovak population. Notably, the observation of an interaction between PCBs and ethnicity that was limited to boys is in the same vein as several published reports of a PCB-associated reduction in birthweight that was stronger in, or limited to, males.[9,19–21] These studies are summarised in Table 3.

Whereas the present study did not observe lower birthweight with increasing PCB levels in Slovak males, Slovak females or Romani females, the association among the Romani boys indicates that some combination of co-factors, rather than male sex alone, may have led to heightened susceptibility in this population. Romani infants are usually smaller in birthweight than Slovak/other eastern European infants.[29] When the regression model was used to calculate the predicted birthweight of Romani boys, an increase from the 10th to 90th percentile of maternal PCBs corresponded to a decline of 133 g, which is similar to typical reductions in birthweight observed for smokers vs. non-smokers in many western countries.[30,31] These results suggest that higher levels of PCBs in maternal blood sera may inhibit growth in boys already affected by social factors. The Romani, historically known as gypsies, were recognised by the government of the Slovak Republic in the early 1990s as a national minority group.[29] The interaction observed among sex, PCB exposure and ethnicity may suggest that one or more characteristics of the Romani such as higher smoking intensity (note: we adjusted for smoking as a yes/no variable, but not for cigarettes per day), high-fat diets, poor hygienic conditions and lower prevalence of vaccination[29,32] may play a role in exacerbating susceptibility to *in utero* PCB exposures, especially among males. Male infants, in spite of their larger size, are often more vulnerable than girls, for instance, having greater mortality among preterm deliveries.[33] Thus, our findings may reflect a three-way interaction of genetic predisposition with environmental chemicals and social factors.

The relationship among PCBs, gestational age and birthweight was explored. A model in which gestation was used as an outcome allowed us to examine the association between PCBs and gestational age. In this model, PCBs did not significantly predict gestational age ($P = 0.61$). Next, we fitted our original regression model for birthweight (Table 2) without preterm infants (those with gestational lengths <37 weeks). The regression coefficients for the PCB*Romani boys interaction terms for two models were 13.2 and 12.5; thus, exclusion of preterm infants changed the regression coefficients only slightly, about 5%.

We found a smaller effect of maternal smoking (40 g difference) than is typical in studies of birthweight. This result reflects the fact that the number of cigarettes smoked was quite low. For instance, among those who reported smoking before pregnancy, half reported smoking five or fewer cigarettes per day, and one-third reported 6–10 cigarettes per day; by comparison, smokers in the US often smoke 20 cigarettes per day. As expected, birthweight increased with greater education, maternal BMI, gestational age and maternal height. Inter-pregnancy intervals also influenced birthweight, i.e. mothers with short or long intervals had lower-birthweight babies than those with the optimal inter-pregnancy intervals of 18–23 months.[34,35] These observed findings are consistent with the published literature.[13,19,36]

To our knowledge, the current study is the largest of the birth cohort studies to examine birthweight in relation to PCB exposures, and one of very few with both congener-specific PCB measurements in maternal serum and extensive individual confounder information, such as maternal BMI and maternal education. Of note, other similar studies did not control for inter-pregnancy interval, which was associated with both PCBs and birthweight in this study.

Unlike the Faroe Island study, the heavy metal concentrations measured in maternal serum were very low (data not shown) in the present study, and unlike many PCB-exposed groups, fish was not a major food source in this study population.[37] Therefore, it is

Table 3. Gender-specific birthweight effects reported in the literature

Author	Location	Exposure measure	General conclusions	Gender-specific results in relation to PCB exposure
Dewailly *et al.*[51]	Quebec, Canada	Breast milk	Correlations between TSH level, weight and head circumference with PCB were negative for boys and positive for girls	Pearson coefficients for boys ranged between –0.23 and –0.41 for PCB and height
Rylander *et al.*[9]	Sweden	Estimated fish consumption	Decreased birthweight among those who eat larger amounts of fish	The OR for a fish-eating population for low birthweight = 1.45. The boys subgroup OR = 1.95
Rylander *et al.*[20]	Sweden	Estimated fish consumption	Median birthweight for study group = 2450 g (higher exposure) Median birthweight for control group = 3680 g (lower exposure)	For high fish consumers, overall adjusted OR for low birthweight = 1.9. The OR for boys = 3.4
Baibergenova *et al.*[21]	New York, USA	Exposure classified by residence in a zip code with known PCB contamination	PCB-zipcode mean birthweight = 3367.7 g Control-zipcode mean birthweight = 3408.3 g Difference = 21.6 g	Boys in PCB-exposed zipcodes had a 6% increased risk for low birthweight Girls had a 3% increased risk for low birthweight
Hertz-Picciotto *et al.*[19]	San Francisco Bay Area, USA	Blood serum	Increased maternal serum PCBs were associated with reduced birthweight in boys. PCBs were not associated with a reduction in gestational duration	Boys at the higher end of the PCB range had lower birthweights by 290 g. In girls, the corresponding reduction in birthweight was 28 g

OR, odds ratio; TSH, thyroid stimulating hormone; PCB, polychlorinated biphenyl.

unlikely that fish consumption or metal exposures influenced our findings for PCBs in relation to birthweight.

In a Wisconsin study,[12] increased birthweight was found to be associated with PCB exposure, but this study has been criticised for inappropriately controlling for maternal body weight gain, a variable that is a component of the actual outcome, baby's weight.[19] Previous studies with fishermen's families in Sweden consistently showed adverse effects of PCBs on birthweight. However, most of these studies did not actually measure PCB levels.[9,10,20] Other epidemiological studies from fishing communities such as Lake Michigan and the Faroe Islands reported varying results on birthweight in relation to PCB exposures.[11,13,14] If the principal targets for PCB growth inhibition are males with social disadvantage, such mixed findings might be expected.

Longnecker *et al.*[38] reported a null association between maternal levels of PCBs and birthweight, and commented that the concentration of PCB #118 was higher in their study population than in other previous studies. In the Slovak population, PCB #138, #153 and #180 were the major congeners, representing a range of 38–87% of the total PCBs for each woman, and PCB #118 constituted, on average, 2.3% of the sum. PCB #153 was, as is true in general, the most abundant (as median, 30.5 % of total PCBs in this population). PCB #153 has been found by some authors to exhibit oestrogenic effects.[39,40] Considering the fact that oestrogens during pregnancy seem to increase birthweight,[41,42] possible oestrogenic effects of PCB #153 could counteract the effect of other PCB congeners and finally result in the null change in birthweight found in girls in our study. However, anti-oestrogenic activity of PCB #153 was also suggested by other authors.[43,44]

The principal weakness in this study was the proportion of refusals at the recruitment stage of 51% in Michalovce and 27% in Svidnik, comparable with those currently commonplace in the US.[19] The percentage of refusals who were actually eligible is unknown. Limited information on their characteristics was available and indicated no difference in ethnicity, but a lower average birthweight (by 191 g) than those enrolled in the study (data not shown). This was at least partially a result of non-participation of mothers of extremely small babies likely to have required medical intervention. For example, the 5th percentile for the refusals was 2085 g vs. 2500 g for participants. Having relatively bigger babies participating may contribute to masking a possibly real association between high prenatal PCB exposure and lower birthweight, particularly as our finding was strongest in those with the greatest social disadvantage, who may have been under-represented. If so, the findings in the total population could be more dramatic than we observed.

Some of the studies reporting sex-specific effects of PCBs (Table 3) used a surrogate for exposure such as residential information[21] or the number of fish meals consumed,[9,20] which may entail greater errors in exposure classification. In the present study, maternal serum samples were collected immediately after delivery. The correlation was extremely high between our non-lipid-adjusted and lipid-adjusted PCB concentrations, and our results were not affected by alternative parameterisations of PCB exposures.

Polychlorinated biphenyls and DDE (dichlorodiphenyl dichloroethylene) have similar lipophilic properties, but may be associated with opposite effects on birthweight. Oestrogenicity of DDE might increase birthweight as suggested by others,[45,46] and oestrogen levels during pregnancy have been shown to be associated with increasing birthweight.[41,42] In a sensitivity analysis with DDE included as a covariate in the model, we attempted to separate its effects from those of PCBs, but DDE was not associated with birthweight in this population.

It has also been argued that it is the metabolites of PCBs, rather than the parent compounds, which are responsible for toxicity observed in association with PCB exposures. Soechitram *et al.*[47] reported that placental transfer was more efficient for hydroxylated PCB metabolites than for the parent compounds. We found measurable levels of hydroxylated and methysulfonyl PCB metabolites from the maternal and cord samples taken from the participants in this study.[48–50] Because many PCB congeners, as well as dioxin, were not measured, we may have missed other critical compounds that may adversely affect growth.

This study has several strengths: the large sample size; the use of maternal serum specimens for direct PCB analyses rather than a surrogate for PCB exposure such as fish consumption; tight quality control of those laboratory measurements by experienced personnel; and wide variability in PCB concentration in the maternal serum relative to other studies of general populations.

In conclusion, PCBs may have a growth-inhibiting effect on male infants from disadvantaged groups,

although our data did not support a global association between prenatal PCB exposure and birthweight. The roles of metabolites, other PCB congeners or other chemicals that interact with the hormonal milieu remain to be investigated in future studies, as well as other mediators such as micro- or macronutrients in the diet, immune dysregulators and exposures to pathogenic micro-organisms. Nevertheless, evidence from this and other studies[9,19,20,21,51] suggests that males are more vulnerable to *in utero* PCB exposures, and this effect appears to be magnified by social factors.

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