

The Yugoslavia Prospective Study of Environmental Lead Exposure

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The Yugoslavia Prospective Study of environmental lead exposure has studied the associations between exposure to lead and pregnancy outcomes; childhood neuropsychological, behavioral, and physical development; and hematologic, renal, and cardiovascular function. The cohort comprises 577 children born to women recruited at midpregnancy in two towns in Kosovo, Yugoslavia; one town is the site of a lead smelter, refinery, and battery plant and the other is 25 miles away and relatively unexposed. A sample of these children has been followed at 6-month intervals through 7.5 years of age. Blood lead concentrations ranged from 1 to 70 µg/dl. Exposure to lead was not associated with adverse pregnancy outcomes. Exposure was associated with modest decrements in intelligence, small increases in blood pressure, higher risks of proteinuria, small increases in behavior problems, and perturbed hematopoiesis. Only at low level exposures (i.e., <16 µg/dl) were small associations with decreased height found. We discuss methodological problems that may hinder causal interpretation of these data, namely, use of blood lead concentration as an exposure measure, confounding, and town-specific associations. We conclude that while reported associations are small, collectively they lend support to the notion that lead is a toxicant with numerous adverse health effects. *Key words:* blood pressure, childhood, development, height, lead. *Environ Health Perspect* 107:9–15 (1999). [Online 4 December 1998]

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American public health scientists and policy makers were slow to recognize the hazards of environmental lead (Pb) exposure. Adverse effects of occupational Pb exposure on neurologic, hematologic, and renal function have been known for centuries (1,2). By 1922, seven countries had banned Pb-based paint based on reports of encephalopathy in children. Only in the 1920s, when tetraethyl lead was introduced as a gasoline antiknock additive (3), did many begin to entertain the possibility that environmental Pb exposure might adversely impact health.

Modern day concerns regarding environmental Pb exposure likely originated with Byers and Lord's (4) classic description of the late neurological and neurodevelopmental effects in child survivors of Pb encephalopathy. Subsequently, cross-sectional studies examined associations between Pb exposure and development in asymptomatic children (5–8). In essence, many attempted to answer a question posed in a 1978 editorial in the *Lancet*: "Could exposure to lower amounts of inorganic lead, at times when the nervous system may be especially susceptible, during prenatal life and early childhood, result in mental handicap?" (9). In 1979, a report from Boston, Massachusetts (10), described associations between tooth Pb level and classroom performance. This provided impetus for four long-term prospective studies (11–14), which monitored exposure and outcome over time in Boston (11), Cincinnati, Ohio (12), Port Pirie, Australia (13), and Kosovo, Yugoslavia

(14). A study in Cleveland, Ohio (15), originally designed to examine associations between prenatal alcohol exposure and child development, has also examined these associations.

In February 1978, one of us (JG) visited a town in Yugoslavia, which was the site of a Pb smelter, refinery, and battery plant, and was known to have a serious occupational Pb poisoning problem. The town, Kosovska Mitrovica (K. Mitrovica), was subsequently described as a region with high blood Pb (BPb) concentrations in both children and adults (16). Residents of a comparable town (Pristina), 25 miles to the south, had low BPbs because Pb-based paint was banned in Yugoslavia in 1922 and automobile traffic was minimal.

In this paper, we summarize results from this Yugoslavia site on the relations of Pb exposure to pregnancy outcomes and infant and childhood development, and consider methodological problems that may influence interpretation of results (14,17–21). This study, in which high exposure is not limited to children of low socioeconomic status (SES), is characterized by its breadth of outcomes, including pregnancy; childhood neuropsychological, behavioral, and physical development; and hematologic, renal, and cardiovascular function. The range of BPbs, from 1 to 70 µg/dl, is extremely unusual. Launched in 1985, the study continues today as the children reach their twelfth birthdays.

Materials and Methods

Sample Selection

Details of the sample selection were presented previously (18,19). Briefly, between May 1985 and December 1986, women between 12 and 20 weeks of gestation presenting for their first prenatal visit at the two government clinics were asked to participate in a study of pregnancy outcome. A total of 1,502 women were recruited at midpregnancy: 900 women in Pristina and 602 women in K. Mitrovica, with the inequality reflecting differences between towns and clinics.

Of the women on whom data were obtained at midpregnancy, 1,008 (67%) were subsequently identified in the delivery room. Women not identified were traced using the visiting nurse service or via intensive follow-up by one of the staff physicians. In total, we had data on outcome of pregnancy for 1,357 (90.3%) women.

We selected infants for follow-up based on BPb in the umbilical cord, town of residence, and parental education. Infants were excluded if they met any of the following criteria: gestation <28 weeks or >44 weeks, major central nervous system defects, chromosomal abnormalities, multiple births, and residence more than approximately 10 km from the pediatric clinic in either town. Five groups of infants were selected. In K. Mitrovica, selection of infants in three strata was based on cord BPb: low (<15 µg/dl; *n* = 78), middle (15–20 µg/dl; *n* = 99), and high (>20 µg/dl; *n* = 217). In Pristina, two groups were selected: one (*n* = 221) was frequency matched on maternal and paternal education to the high cord BPb group in K. Mitrovica, while a second (*n* = 91) was selected from among those with the highest cord BPbs. The design achieved broad representation across exposure and SES. Thus, 706 infants were selected for follow-up at 6-month intervals.

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Of these, parents of 577 consented to at least one visit by 7.5 years of age. The number of children presenting for each follow-up visit varied (Table 1).

Data Collection

All mothers were interviewed at midpregnancy, delivery, and at each follow-up visit by trained bilingual (Serbo-Croatian and Albanian) nurses. Beginning at age 6 months and at each follow-up visit, height and weight measurements were obtained. Beginning at 5.5 years of age, blood pressure measures were obtained using automated monitors, and urine samples were obtained for dipstick analyses (Bili-Labstix, Ames, Miles Laboratories, Elkhart, IN).

Hematological assays. Venous blood samples from the mothers at midpregnancy and at delivery, from the umbilical cords, and from the child at each follow-up visit were obtained for the measurements of BPb (22), erythrocyte protoporphyrin (EP) (23), hemoglobin (Hgb), and serum ferritin (SF) (24). Samples were appropriately stored and transported to Columbia University for biochemical assays. The Columbia laboratory participates in the Centers for Disease Control and Prevention quality control program for BPb analyses; during the course of the study, the intraclass correlation coefficient for agreement was 0.97. We established a serum bank from the remainder of the samples, which was useful for testing ancillary hypotheses such as those regarding the associations between Pb and hematopoiesis (25–27).

Outcome measures. Primary outcomes were defined at specific ages. These included measures of intelligence at ages 2, 4, and 7 years; physical growth from birth through age 4 years; blood pressure and renal function at age 5.5 years; and behavior problems at age 3 years. We have also explored whether Pb exposure is associated with decreased synthesis of erythropoietin (EPO), a hormone produced in the proximal

tubules of the kidney, which regulates the production of red blood cells (28).

Developmental measures. The Bayley Scales of Infant Development (29) is a widely used instrument to assess cognitive function in children up to 2 years of age. We employed the Mental Development Index (MDI) of Bayley Scales at ages 6, 12, 18, and 24 months, with the last time point as the primary outcome measure.

At age 4 years, we employed the McCarthy Scales of Children's Abilities (30), used in other studies of Pb exposure (11,13). Its General Cognitive Index (GCI) is interpretable as a measure of the intelligence quotient (IQ); the Perceptual Performance, Verbal, Quantitative, Memory, and Motor scales are specific subscales.

We employed the Wechsler Intelligence Scale for Children-III (WISC-III) (31) in 7-year-old children. We administered five verbal subtests (information, similarities, arithmetic, comprehension, and digit span) and five performance subtests (picture completion, coding, picture arrangement, block design, and object assembly). The vocabulary subtest was not administered due to limitations imposed by translation. The WISC-III results in scores for Total IQ, Verbal IQ, and Performance IQ.

Behavior. Behavior was assessed at 3 years of age using the Child Behavior Checklist (CBCL 2/3) (32), which generates six subscales: Destructive, Aggressive, Somatic Problems, Withdrawn, Anxious-Depressed, and Sleep Problems.

Blood pressure and renal function. Blood pressure was measured six times during the

course of each visit, three at the beginning and three at the end. The mean of the last two measures was used to approximate resting blood pressure. Proteinuria was assessed using urinary dipsticks. Because the numbers of children with proteinuria was small, we grouped proteinuria into "any" (including trace levels) versus none.

Erythropoietin production. Sera were analyzed using an enzyme immunoassay (Amgen, Thousand Oaks, CA). In our laboratory, the limit of detection was 2 mU/ml and the coefficient of variation was 9.8%.

Covariate assessment. We measured variables known to be associated with one or more outcomes and with exposure; these included SES, operationalized as maternal education and intelligence, ethnicity, and quality of the childrearing environment. The latter was measured between ages 2 and 3 using an adaptation of the preschool Home Observation for Measurement of the Environment (HOME) (33). Maternal intelligence was assessed using Raven's Progressive Matrices (34), a nonverbal test that is relatively culture free. Physical characteristics of the mother were assessed for specific analyses, e.g., height and blood pressure. Infant temperament, a predictor of childhood behavior (35), was measured at age 2 years using the Infant Characteristics Questionnaire (36), a maternal report instrument; the subscale Difficult Temperament was used in the analysis of child behavior problems.

Statistical Analyses

Associations between Pb exposure and health outcomes were estimated using ordinary

Table 1. Number of children assessed at each age in Kosovska Mitrovica and Pristina, Yugoslavia

Age	Kosovska Mitrovica	Pristina
6 months	237	226
12 months	198	200
18 months	196	185
24 months	211	190
30 months	189	192
36 months	191	183
42 months	169	182
48 months	156	175
54 months	139	163
60 months	153	159
66 months	133	146
72 months	128	134
78 months	130	137
84/90 months	151	150

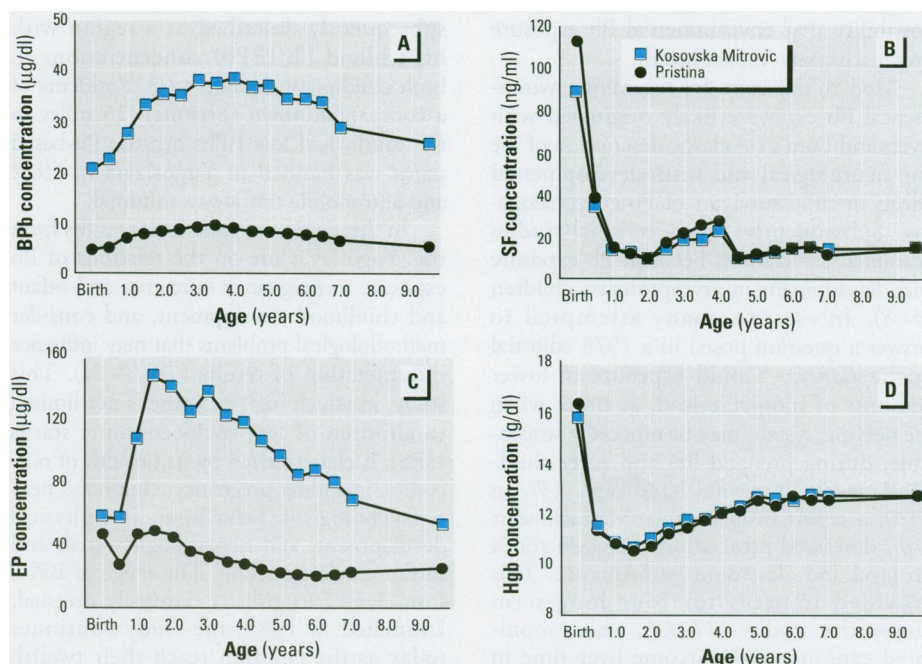


Figure 1. Mean concentrations of (A) blood lead (BPb), (B) serum ferritin (SF), (C) erythrocyte protoporphyrin (EP), and (D) hemoglobin (Hgb) for Kosovska Mitrovica and Pristina by age.

least-squares regression (37) and unconditional maximum likelihood logistic regression analyses (38), depending on whether the outcome variable was continuous (i.e., MDI, GCI, IQ, blood pressure, height) or categorical (i.e., proteinuria), respectively. We used two primary measures of exposure: individual BPb, measured concurrently to the outcome measure, and cumulative BPb, estimated using the trapezoidal approximation of the area under the BPb versus time curve. To elucidate critical time periods of exposure, we also estimated associations with each prior BPb measure and with cumulative exposure for *a priori* defined developmental periods (i.e., birth–age 2 years, age 2–4 years, age 4–7 years).

For each outcome, we 1) developed a core model including all potential determinants of outcome (for continuous variables) and confounders; 2) tested associations between BPb with each outcome, adjusting for variables in the core model; and 3) tested whether associations were consistent between the two towns. If associations were not consistent, we considered whether the disparities were likely to be due to measurement error, bias, or an unmeasured confounder, or were informative about the shape of the dose–response curve. Other analyses added the quadratic BPb term to further describe the shape of the dose–response curve. These analyses determine whether floor or ceiling effects were present.

Results

Blood lead concentrations. In the exposed town, BPb rose continuously until age 6 years, and began to decline thereafter (Fig. 1). A similar pattern was found in the unexposed town, although the rise was much smaller. EP followed a similar pattern in K. Mitrovica. No differences were found between towns for SF and Hgb.

Developmental outcomes. BPb was associated with poorer intellectual functioning at ages 2 (20), 4 (14), and 7 (39) years. Adjusted losses in intellectual function, as BPb increased from 10–30 µg/dl, were 2.5 points at age 2 [95% confidence interval (CI), 0.2–4.8], 4.5 points at age 4 (CI, 2.2–6.8), and 4.3 points at age 7 (CI, 3.4–5.1). At ages 4 and 7, associations were consistently stronger for performance subscales compared to verbal subscales (Table 2). For example, at age 7, the estimated loss in Performance and Verbal IQ, as BPb increases from 10 to 30 µg/dl, were 9.4 (CI, 5.6–13.3) and 7.1 (CI, 3.7–10.5) points, respectively. While associations were generally found for all measures (i.e., each 6-month time point) of BPb (data not shown), the strongest associations were

found for BPb measured concurrent with the developmental assessment.

At all ages, associations were larger after adjustment for potential confounders (Table 2). We explored the nature of this suppression at age 4 by considering the associations between BPb, GCI, and the main confounding variables, namely, maternal education, maternal Raven score, and HOME score (Table 3). In the total sample and within each town, increased maternal education, maternal Raven score, and HOME score were associated with increases in GCI. Different patterns of association in each town, however, were found between these variables and BPb. In K. Mitrovica, increased BPb was associated

with higher maternal education and higher maternal Raven score. In Pristina, increased BPb was associated with decreases in quality of the home.

Hemoglobin was independently associated with the MDI at 2 years of age (20). A decrease of 1 g/dl of Hgb measured at age 1.5 years was associated with a 1.7 point decrease in MDI (CI, 0.3–3.0). By age 4, Hgb was no longer associated with cognitive development (14).

Behavior problems. Reported behavior problems were more prevalent in Pristina (mean CBCL 2/3 score 61.4) than in K. Mitrovica (mean CBCL 2/3 score 46.3). In both towns combined, BPb, measured concurrently with the CBCL 2/3, was associated

Table 2. Associations between blood lead (BPb) concentration and measures of cognitive development in Kosovska Mitrovica and Pristina, Yugoslavia

Cognitive measure	Age (years)	β^a	β^b	CI ^c
Bayley Scales of Infant Development	2			
Mental Development Index		-3.3	-5.3	(-10.1– -0.5)
McCarthy Scales of Children's Abilities	4			
Global Cognitive Index		-7.1	-9.4	(-14.2– -4.6)
Perceptual Performance		-6.6	-7.1	(-10.2– -3.9)
Verbal		-0.8	-2.7	(-5.4– -0.1)
Quantitative		-5.5	-5.9	(-9.6– -2.3)
Memory		-1.0	-3.2	(-5.8– -0.5)
Motor		-2.6	-4.3	(-8.3– -0.3)
Wechsler Intelligence Scales for Children-III	7			
Full Scale IQ		-4.7	-9.0	(-12.4– -5.5)
Performance IQ		-4.5	-9.4	(-13.3– -5.6)
Verbal IQ		-3.7	-7.1	(-10.5– -3.7)

CI, 95% confidence interval; Hgb, hemoglobin.

^aEstimated regression coefficient for log₁₀ BPb concentration, adjusted only for Hgb measured concurrently with the cognitive measure.

^bEstimated regression coefficient for log₁₀ BPb concentration, measured concurrently with the cognitive measure. All estimated regression coefficients adjusted for Home Observation for Measurement of the Environment score, ethnic group (Serbian, Albanian, other), maternal age, birth weight (kg), maternal Raven's Progressive Matrices score, maternal education (years), birth order (ages 2 and 4) or sibship size (age 7), and sex. At ages 2 and 4, we also adjusted for Hgb concentration at the time of cognitive testing.

^cCI for estimated β , adjusted for the full set of covariates^b.

Table 3. Relationships between blood lead (BPb) concentration at age 4, General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities, and measures of the social environment in Kosovska Mitrovica and Pristina, Yugoslavia

Variable	Kosovska Mitrovica		Pristina	
	GCI	BPb	GCI	BPb
HOME Score				
4–21	65.7 ± 12.0	38.7 ± 11.7	74.5 ± 12.9	10.1 ± 3.9
22–26	80.4 ± 13.5	40.2 ± 10.3	79.6 ± 10.3	10.3 ± 3.1
27–31	80.1 ± 17.2	37.7 ± 11.9	90.2 ± 11.3	9.4 ± 2.6
32–35	82.1 ± 14.8	40.9 ± 11.3	91.3 ± 12.8	9.7 ± 3.3
36–49	96.4 ± 15.5	40.6 ± 8.3	99.9 ± 18.2	8.2 ± 2.8
Maternal Education (years)				
None	68.7 ± 15.3	38.5 ± 12.2	68.0 ± 11.5	11.4 ± 5.7
1–8	75.3 ± 14.9	38.2 ± 11.2	79.8 ± 13.0	9.6 ± 2.9
9–12	87.6 ± 16.6	41.9 ± 11.1	93.7 ± 15.1	9.4 ± 3.0
≥13	98.3 ± 11.3	43.0 ± 8.2	97.1 ± 23.7	8.6 ± 4.6
Maternal Raven Score^a				
4–17	69.8 ± 13.7	37.1 ± 11.2	77.1 ± 14.5	9.2 ± 2.4
18–26	78.9 ± 19.4	41.0 ± 10.8	81.4 ± 15.5	10.9 ± 4.7
27–35	78.9 ± 11.3	37.4 ± 12.2	88.3 ± 15.7	8.8 ± 2.7
36–43	83.8 ± 16.4	40.2 ± 8.6	90.4 ± 12.7	9.9 ± 2.5
44–60	91.4 ± 17.7	44.2 ± 11.2	98.5 ± 17.7	9.3 ± 3.4

HOME, Home Observation for the Measurement of the Environment. Values shown are mean ± standard deviation.

^aRaven's Progressive Matrices.

with small increases in scores on the CBCL 2/3 subscales anxious/depressed, withdrawn, sleep problems, somatic problems, aggressive behavior, and destructive behavior (40). For each 10 µg/dl increase in BPb, subscale scores rose between 0.8 (sleep problems, CI, 0.1–1.4) and 2.1 points (withdrawn, CI, 0.8–3.4) (Table 4).

Blood pressure and renal function. At age 5.5 years, mean systolic blood pressure was 100.5 ± 10.7 [mean \pm standard deviation (SD)] and 98.4 ± 10.0 mm Hg in K. Mitrovica and Pristina, respectively. Mean diastolic blood pressure was 59.1 ± 7.5 and 58.4 ± 6.7 mm Hg, respectively.

Combining data from both towns, increases in BPb were associated with small increases in both systolic and diastolic blood pressure. Associations were reduced by approximately 50% after adjustment for potentially confounding variables (Table 4) (21). We estimate that a 10-µg/dl increase in BPb was associated with adjusted increases in systolic and diastolic blood pressure of 0.5 mm Hg (CI, -0.2–1.3) and 0.4 mm Hg (CI, -0.1–0.9), respectively.

The prevalence of proteinuria in children at ages 5.5 and 7 years was 9%. The odds ratio for proteinuria in K. Mitrovica was 2.9 (CI, 1.7–5.1), which increased to 3.5 (CI, 1.7–7.2) after adjustment for potential confounders (N. Mervish, unpublished data). Within K. Mitrovica, where almost all BPbs were greater than 20 µg/dl, the

adjusted odds of proteinuria increased by 1.15 (CI, 1.1–1.2) per unit increase in BPb. Within Pristina, where nearly all BPbs were below 15 µg/dl, proteinuria was unrelated to BPb.

Measures of growth. No associations were found between BPb (measured during midpregnancy or in the umbilical cord) and prenatal growth, including intrauterine growth retardation (birth weight, adjusted for length of gestation), length of gestation (days) (Table 4), or preterm delivery (i.e., delivery before 36 completed weeks of gestation) (19).

At age 4 years, mean height was nearly identical in Pristina (100.9 ± 4.6 cm; mean \pm SD) and K. Mitrovica (101.0 ± 4.9 cm) (41). Associations between BPb and height were found in Pristina but not in K. Mitrovica. In Pristina, where BPbs ranged from approximately 1 µg/dl to 14 µg/dl, height decreased by 7.3 cm (CI, 1.8–12.8) per log unit increase in BPb.

Hematopoiesis. Associations between Pb exposure and EPO were examined in women at midpregnancy and delivery (25) and in children at ages 4.5, 6.5 (26), and 9.5 years (27). Pregnant women with the highest and women with the lowest BPbs within four strata of Hgb (9–9.9, 10–10.9, 11–11.9, 12–12.9 g/dl) were selected for the first study. Independent of Hgb, a strong predictor of EPO, BPb was inversely related to serum EPO at both midpregnancy and

delivery. In contrast, among children, BPb was positively related to serum EPO at ages 4.5 and 6.5 years, and not associated with serum EPO at age 9.5 years. Adjusted for Hgb, EPO increased by 0.072 mIU (CI, 0.034–0.11) and 0.043 mIU (CI, 0.0041–0.082) per microgram per deciliter BPb at ages 4.5 and 6.5, respectively.

Discussion

Our data indicate that prenatal and childhood exposure to environmental Pb is associated with a wide range of health outcomes, including decrements in intelligence (14,20,30), increases in blood pressure (21), higher risks of proteinuria, increases in behavior problems (40), and perturbed hematopoiesis (25–27). Although no association between Pb exposure and prenatal growth was found (19), the data suggest decrements with height at age 4, but only when BPbs were relatively low (41). We also found associations between Hgb and intelligence (14,20). In general, these results are consistent with prior observations. Our results, and those of others, imply that the deficits attributable to lead are small and modest in relation to other variables associated with each of the outcomes. Methodological issues relevant to the interpretation of these results include the use of BPb as a biomarker of exposure, confounding in the form of suppression, and variations in the associations between towns.

BPb as a biomarker of exposure. For all outcomes we examined associations with BPb measured at earlier time points, including at birth. Often (e.g., intelligence, blood pressure, height) we found the strongest associations when BPb was measured close to or concurrently with the outcome. This first appears counterintuitive since these outcomes reflect processes that began earlier. We initially expected BPb at earlier ages to show the strongest associations with outcomes at ages 4 and older.

BPb, although considered by many to reflect recent exposure, represents exposure from both exogenous (i.e., environmental) and endogenous (i.e., bone and tissue Pb) sources. We hypothesize that as a child ages, the relative contribution from the latter becomes more important. Indeed, at ages 1, 2, 3, and 4 the correlation coefficients between current BPb and prior cumulative exposure, assessed by the area under the BPb versus time curve, were 0.72, 0.81, 0.79, and 0.89 in K. Mitrovica and 0.42, 0.52, 0.59, and 0.63 in Pristina, respectively. Together with evidence from toxicokinetic models (42), these observations indicate that BPb reflects an equilibrium between body burden and environmental sources. We suspect that BPb measured concurrently with the outcome may better

Table 4. Associations between blood lead (BPb) concentration and measures of behavior problems, blood pressure, and growth in Kosovska Mitrovica and Pristina, Yugoslavia

Measure	Age	β^a	β^b	CI ^c
CBCL 2/3	3			
Anxious/depressed		1.48 ^{d,e}	1.45 ^{e,f}	(0.039–2.86)
Withdrawn		3.26	3.07	(1.21–4.93)
Sleep problems		1.25	1.09	(0.15–2.03)
Somatic problems		1.76	1.71	(0.34–3.08)
Aggressive		2.03	1.85	(-0.27–3.97)
Destructive		2.15	2.07	(0.87–3.27)
Blood pressure^g	5.5			
Systolic		0.090	0.054 ^h	(-0.024–0.13)
Diastolic		0.045	0.042 ⁱ	(-0.010–0.090)
Physical growth				
Intrauterine growth retardation	Birth	-0.06	32.1 ^j	(-34.4–98.5)
Length of gestation (days)	Birth		-0.5 ^j	(-2.9–1.9)
Height	4	-8.9	-7.3 ^k	(-12.8–-1.8)

Abbreviations: CI, 95% confidence interval; CBCL 2/3, Child Behavior Checklist 2/3; HOME, Home Observation for the Measurement of the Environment.

^aEstimated unadjusted regression coefficient for BPb concentration.

^bEstimated adjusted regression coefficient for BPb concentration, measured concurrently with the outcome.

^cFor estimated β adjusted for covariates.

^dEstimated β adjusted for the effect of town.

^eFor the CBCL 2/3 measures, all regression coefficients are for the natural log of BPb.

^fAll CBCL 2/3 measures adjusted for town, sex, ethnic group (Albanian, Serbian, other), maternal education (years), HOME score, and home type (house, apartment, farm).

^gMean of last two of six blood pressure measures.

^hRegression coefficient for BPb adjusted for height, body mass index, sex, ethnic group (Albanian, Serbian, other), and birth order.

ⁱRegression coefficient for BPb adjusted for waist circumference, ethnic group (Albanian, Serbian, other), and birth order.

^jRegression coefficient for BPb expressed as µmol/l.

^kRegression coefficient for base 10 logarithmic BPb in Pristina adjusted for maternal height, HOME score, ethnic group (Serbian, Albanian/other), maternal age, and sex.

tap true biologic levels of exposure to target organs.

Confounding by socioeconomic variables. Recent meta-analyses of literature on lead and intelligence (6–8) point out that despite consistency between studies, methodological issues may hinder causal interpretations. Adjustment for confounders presents such a problem. In samples of low SES (12,15,43,44), adjustment for parental education, intelligence, and quality of the childrearing environment diminishes (12,43,44) or negates (15) the association between BPb and cognition. Associations are also diminished in one study of mixed SES (13,45). In contrast, in a sample of middle to upper SES children (11,46,47), it was only after adjustment for SES that an association between BPb and cognition was detected. We observed a similar phenomenon in our data; that is, adjusted estimates were stronger than unadjusted estimates (14,20,39).

Examination of the relationships between potential confounders, BPb, and developmental outcome may, in part, explain these disparate observations. In U.S. populations of relatively low SES, exposure to Pb is associated with lower maternal education, lower maternal IQ, lower quality of the childrearing environment, and other indicators of poverty (12,15,43,44). Within these narrow ranges of SES, higher ratings on these variables predict better childhood IQ scores (43). Thus, the unadjusted association between Pb and intelligence overestimates the true association. In Boston, where the study population consisted of children from middle to upper SES families (46), exposure to Pb was associated with higher maternal education and IQ and better childrearing environments. Better social circumstances suppressed the association between Pb and intelligence such that no association was evident until these variables were controlled (11,46,47).

We expected no associations between Pb and SES in our Yugoslavia sites. However, prior to data collection, new housing within 2 km and downwind of the smelter was built in K. Mitrovica. Occupants had higher education, IQ, and HOME scores. Thus, our unadjusted estimates of association between Pb and cognition were biased downward.

Town-specific associations. For some outcomes we observed associations with BPb in only one town. These inconsistencies may 1) be an artifact due to systematic measurement error between towns, 2) reflect real differences between towns that cannot be or were not measured and were not controlled in the regression models, or 3) reflect differences in the sequelae of high

and low Pb exposure. Systematic measurement error was minimized, for example, by using automated instruments for blood pressure measures, and evaluated in the statistical analysis, including performing analyses separately for each town.

An example of systematic measurement error concerns our early analysis of BPb and blood pressure during pregnancy (48). At similar levels of BPb, blood pressures were consistently higher in K. Mitrovica than in Pristina. To minimize such systematic error in the pediatric study, automated monitors were used. Using these monitors, data collected over several years suggest that mean blood pressures remained relatively constant, demonstrating that systematic error in the pediatric study was highly unlikely (21).

Because scores on the CBCL 2/3 were higher in Pristina than in K. Mitrovica, we evaluated the possibility of systematic error in reporting. The difference between towns was observed among both more (i.e., >7 years of school) and less educated mothers and was not explained by data collection methods. The magnitude of the association between BPb and behavior problems did not differ by town nor was there a significant interaction between town, BPb, and behavior (40). We considered whether other factors could be responsible for this town difference in mean scores. Knowledge of the social situations in the towns led us to speculate that scores were related to presence of a major university in Pristina. Mothers living in this university town may be more sophisticated in their reporting of behavior problems. Indeed, the magnitude of the association was anticipated from earlier studies (49–52), some of which incompletely controlled for potential covariates (49,50) or used convenience samples of either cases or controls (51,52).

Dose-response relationships. Ruling out important sources of bias, we considered whether our results clarify the shape of the dose-response relationships. Collectively, the prospective studies of cognition found associations that persisted beyond the preschool period (39,44,45,47). Depending on the study population, associations were found for a relatively narrow range of low BPbs (47) and/or for a wide range of BPbs (44,45). Despite these differences in absolute exposure level, the magnitude of the association was similar across studies. Thus, in Boston, where the mean BPb at age 2 was 6.5 µg/dl, the estimated loss of IQ points at age 4 was approximately 3 points as BPb increased from 10 to 20 µg/dl (11). In our data (14) and in Port Pirie (13), with wide ranges of BPb, estimated loss of IQ points at age 4 was 3–4 points for the same increase. In the Cincinnati study (12), which represents a low SES sample,

associations of similar magnitude were found. The constancy of these findings suggests a causal association between Pb and intelligence, which is linear across a wide range of BPb. Indeed, a recent meta-analysis (8) concluded that the available evidence shows no evidence of a Pb threshold.

At the study's inception, anecdotal reports and a few ecologic and etiologic studies suggested that Pb exposure was associated with increased risk of spontaneous abortion (53–55), intrauterine growth retardation (53,55,56), premature birth (46,57), and stunted physical growth (53–55). Our results, although limited to pregnancies progressing more than 28 weeks and less than 44 weeks, are consistent with no associations between Pb exposure and any measure of fetal growth (17,19,48).

Cross-sectional studies using the National Health and Nutrition Examination Survey (NHANES)-II (58) and the Hispanic Health and Nutrition Examination Survey (HHANES) (59) found associations between increased BPb and reductions in height. In Yugoslavia, we found similar associations; however, they were restricted to children with BPb levels less than 16 µg/dl (41). Because most BPb levels in the NHANES-II and HHANES studies were less than 20 µg/dl (58,59), our results are consistent with these population-based U.S. studies.

Prospective data from Cleveland (60) and Boston (61) found no associations with height, although a positive association was found for body mass index in the latter. In Cleveland, the cohort was selected such that half the mothers admitted alcohol use during pregnancy, an exposure that impacts physical growth (62). Although cumulative exposure in the Boston cohort was measured 13 years after an initial assessment of dentine Pb using bone Pb measures, the lack of association may be due to inadequate control for parental size and other predictors of childhood height (61).

Together with previously reported nephrotoxic effects in children (63) and adults in the general population (64,65), our results suggested that exposure to environmental levels of Pb are harmful to both the developed and developing kidney. In animal models, Pb hinders energy production in the mitochondria and inhibits resorption of low-molecular weight proteins from the renal filtrate, resulting in proteinuria (66). Low level Pb exposure is also associated with decrements in creatinine clearance (64) and with increased urinary excretion of *N*-acetyl-β-D-glucosaminidase, a marker of renal tubular damage (63).

Further support that relatively low level Pb exposure is associated with renal dysfunction comes from our findings regarding

EPO production (25–27). In pregnant women exposed to relatively high levels of Pb for most of their lives, EPO production was impaired (25). More recent data in children suggest hyperproduction of EPO (26); we hypothesize that at older ages mean EPO will become lower, reflecting increasing damage to the kidney (27). These data suggest subtle renal dysfunction at the low end of the dose–response curve; however, the shape of that curve is still unclear.

In pregnant women, we observed an association between BPb and blood pressure across a wide range of BPb levels (48). Consistent with associations in other studies in adults (67–70), these results were small and imprecise and left open the possibility of residual confounding. Our data in children (21) are less likely to have such residual confounding because smoking and alcohol use are unlikely in children. Support for a Pb–blood pressure association derives from epidemiological data in occupational settings (71–74) and animal studies (73,75). While the effect was not significant in our cohort, the consistency of the blood pressure findings with other studies, along with evidence from experimental studies, lead us to believe that continued Pb exposure may contribute to the incidence of hypertension.

The Yugoslavia cohort study, currently in its fourteenth year, distinguishes itself by the broad range of outcomes studied and the wide range of exposure. We have found associations between Pb exposure and intelligence, physical growth, preschool behavior problems, renal function, blood pressure, and hematopoiesis. Although all associations are small, collectively they support the notion that Pb is a toxicant with numerous adverse effects. From a public health standpoint, efforts to reduce undue exposure should continue to be a priority.

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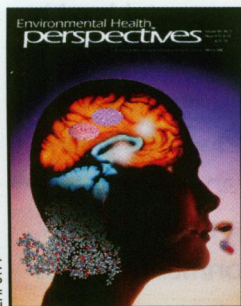
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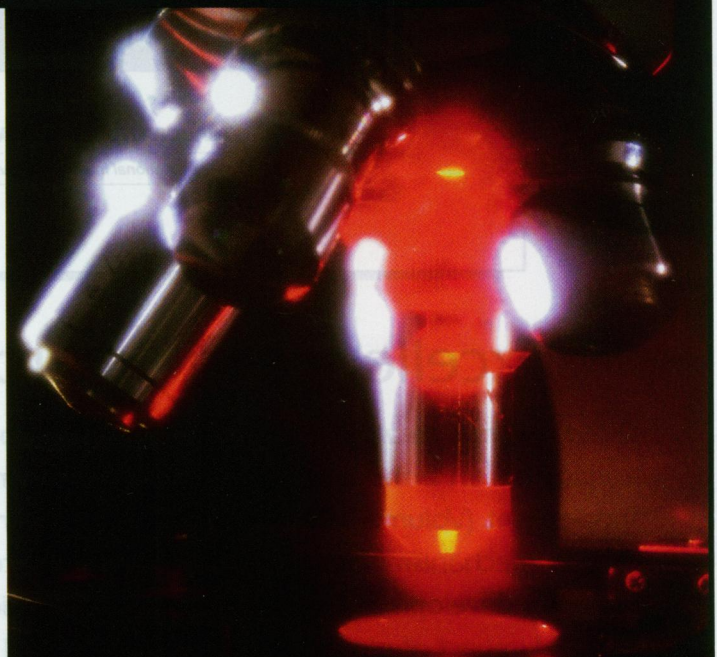
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