Schizophrenia is a severe mental disorder of unknown etiology. Recent reports suggest that a number of environmental factors during prenatal development may be associated with schizophrenia. We tested the hypothesis that environmental lead exposure may be associated with schizophrenia using archived serum samples from a cohort of live births enrolled between 1959 and 1966 in Oakland, California. Cases of schizophrenia spectrum disorder were identified and matched to controls. A biologic marker of lead exposure, δ-aminolevulinic acid (δ-ALA), was determined in second-trimester serum samples of 44 cases and 75 controls. δ-ALA was stratified into high and low categories, yielding 66 subjects in the high category, corresponding to a blood lead level (BPPb) > 15 µg/dL, and 53 in the low category, corresponding to BPPb < 15 µg/dL. Using logistic regression, the odds ratio (OR) for schizophrenia associated with higher δ-ALA was 1.83 (95% confidence interval (CI), 0.87–3.87; p = 0.1). Adjusting for covariates gave an OR of 2.43 (95% CI, 0.69–9.36; p = 0.2). This finding suggests that the effects of prenatal exposure to lead and/or elevated δ-ALA may extend into later life and must be further investigated as risk factors for adult psychiatric diseases. Key words: δ-aminolevulinic acid, developmental, lead, Pb, prenatal, prospective, psychosis, schizophrenia. Environ Health Perspect 112:548–552 (2004). doi:10.1289/ehp.6777 available via http://dx.doi.org/[Online 8 January 2004]
during the early stages of pregnancy and remain stable during the second and third trimesters (Baghurst et al. 1987; Graziano et al. 1990). Therefore, midpregnancy serum samples were sought for this analysis because they were thought to be a point in development when both the exposure and biomarker of interest were likely to be stable. In addition, developmental events during the second trimester of pregnancy have been previously implicated in schizophrenia, such as neuronal migration and synaptogenesis (Beckmann 1999; Bracha et al. 1992).

Case ascertainment, diagnosis, and selection of controls. Screening for potential cases of schizophrenia spectrum disorder was initiated by identifying all possible cases using computerized records from inpatient, outpatient, and pharmacy databases. Possible cases were contacted and assessed by experienced clinical interviewers with master’s level training. Standardized procedures included a structured clinical interview (the Diagnostic Interview for Genetic Studies; Nurnberger et al. 1994) and a consensus diagnosis made by expert clinicians after review of the narrative, psychiatric records, and discussions with the interviewer. A complete description of the methods used has been previously published (Susser et al. 2000).

Cases identified through these methods included 43 subjects with diagnoses of schizophrenia, 17 cases of schizoaffective disorder, 5 cases of schizotypal personality disorder, 1 case of delusional disorder, and 5 cases who met criteria for nonaffective psychoses not otherwise specified. Controls were selected from the remaining subjects without diagnoses of schizophrenia spectrum disorder and were matched to cases on timing of membership in the health plan (such that controls were required to be members of the health plan during the time at which disease status was identified in the matched case), date of birth ± 28 days, sex, date of the first maternal blood draw ± 4 weeks, and equal numbers of maternal serum samples available for study. Forty-four cases and 75 controls (1–2/case) had second-trimester maternal serum available for analysis.

Laboratory protocol. A method published by Endo et al. (1993) and Oishi et al. (1996) to determine plasma levels of δ-ALA was adapted for use as a biologic marker for lead exposure (Tomokuni et al. 1993), and further adapted for use in stored serum samples in this study. Briefly, δ-ALA reacts with acetylacetone and formaldehyde to form 2,6-diactyl-1,5-dimethyl-7-(2-carboxyethyl)-3H-pyrrolizine (Figure 1), a derivative that can be quantified via fluorescence detection at excitation/emission wavelengths of 370 nm and 460 nm, respectively.

Frozen serum samples were identified by coded labels, rendering the analyst blind to case status. Samples were thawed in an ice bath for 1 hr and transferred to Eppendorf tubes. These tubes were placed in a 70°C water bath for 20 min and then centrifuged for 3 hr at 14,000 rpm in a Sorval microcentrifuge (Kendro Laboratory Products, Asheville, TN) at 4°C. For the derivatization reaction, an aliquot of 50 µL of supernatant was removed and added to 16 Kimax glass test tubes (125 mm; Kimble/Kontes, Vineland, NJ) containing 1.5 mL acetylacetone reagent (20% acetylacetone, 20% ethanol in deionized water, vol/vol) and 450 µL 37% formaldehyde. Tubes were loosely capped and held at 100°C for 20 min using a dual aluminum alloy block heater (VWR International, West Chester, PA).

The tubes were then cooled in an ice bath for 10 min and allowed to stand at room temperature in the dark for 1 hr. An aliquot (1 mL) of supernatant was transferred to light-proof Eppendorf tubes and centrifuged for 1 hr. The supernatant was then filtered through 3-cc disposable syringes using acrylic syringe filters with 0.45 µm pores and aspirated through 9.5 mm, 26-gauge needles (Fisher Scientific, Atlanta, GA) to a final volume of 750 µL. The filtrate was transferred to light-resistant 700 µL 8 × 30 mm crimp-top HPLC injection vials with aluminum caps (Alltech Associates, Deerfield, IL).

We used a Perkin-Elmer model LC-250 equipped with an LC-600 autosampler and an LC-40 fluorescence detector (Perkin-Elmer, Norwalk, CT) for analysis. Separation was performed at a flow rate of 1.0 mL/min using an Adsorbosphere HS C18 column (5 µm, 250 × 4.6 mm) attached to a Spherisorb C18 Guard column (5 µm, 17 × 4.6 mm; both from Alltech Associates). A CH-500 integrated heater/controller with aluminum alloy column fittings was used to maintain a temperature of 37 ± 1°C (Eppendorf/Brinkman Instruments, Westbury, NY). δ-ALA was separated using an isocratic mobile phase of methanol/water/glacial acetic acid in proportions of 500:500:10 (vol/vol/vol) that was filtered and degassed using helium.
Intraday variability of 4.7% for this assay was determined by repeating the analytic procedure eight times using a standard solution of δ-ALA in deionized water at a concentration of 25 ng/mL. The interday variability by repeated injection of the same standard solution for 8 consecutive days was 7.8%. Based on the standard curve established using data from intraday analyses, the detection limit for δ-ALA was calculated to be 4.67 ng/mL (Ren et al. 1998). During the primary study, standard concentrations of 50 ng/mL δ-ALA were used for detector calibration before and after every four serum injections.

### Reliability and validity studies

For the purpose of testing the reliability of the laboratory method for measurement of δ-ALA in stored maternal serum and the validity of δ-ALA as an indicator of BPb, we obtained aliquots of sera for 23 subjects (supplied by J.G.) from the Yugoslavia Study of Environmental Lead Exposure and Child Development (Grzianzio et al. 1990). Although these sera had been stored at −20°C for 13–15 years, whole BPb measurements had been made at Columbia University within weeks after collection of the samples. The samples selected for reliability and validity testing were drawn at random from a larger pool of subjects across a range of midpregnancy BPb levels (4.5–41.3 µg/dL).

Based on literature reports of BPb levels for women living in California in the 1960s (Ludwig et al. 1965; Thomas et al. 1967), we estimated that the distribution of BPb levels in our prenatal cohort would range from 3 to 45 µg/dL and that the mean would likely fall between 10 and 20 µg/dL.

Initial assessments of δ-ALA levels from 23 Yugoslavia study subjects indicated that levels were comparable with those expected in freshly drawn sera. A subset of 18 aliquots was available in duplicate to assess the reliability of the laboratory technique for measurement of δ-ALA in stored maternal serum (Figure 2). The intraclass correlation coefficient for the 18 duplicate samples was 0.91, indicating that repeated measures of δ-ALA levels on the same sample were highly correlated. When δ-ALA levels were dichotomized at the median (9.05 ng/mL) such that subjects with levels \( \geq 9.05 \) ng/mL were categorized as “high” and those \(< 9.05 \) ng/mL as “low,” the kappa statistic for duplicate samples was 0.89 with an SE of 0.23, indicating excellent agreement between repeated measures for δ-ALA.

BPb and δ-ALA levels were compared continuously in all 23 samples (Figure 3), and the correlation coefficient was 0.64. When a regression line was drawn, points at the lower levels of BPb and δ-ALA tended to fall outside a 95% confidence interval (CI). We therefore conducted a validity study to establish a method for categorizing δ-ALA as a predictor of BPb. We chose a cutoff point of 15 µg/dL to define our exposure categories, such that subjects with BPb \( \geq 15 \) µg/dL would be classified as “exposed” and those with levels < 15 µg/dL would be defined as “unexposed.”

### Statistical methods

In order to examine the relationship between δ-ALA and schizophrenia spectrum disorders, we used two approaches. The first, a Mantel-Haenszel odds ratio (MH OR), provided a summary measure that estimated the odds of having a diagnosis of schizophrenia spectrum disorder if exposed versus the odds if unexposed, after taking into account the correlation within matched sets. This approach provides a simple and readily interpretable OR that accounts for the matching variables, although it does not adjust for other covariates. Second, conditional logistic regression models were fitted (Neuhaus 1992), including δ-ALA as a predictor of schizophrenia spectrum disorder, while adjusting for covariates (Greenland 2000, Greenland et al. 2000). Parameter estimates for the fitted models were calculated using the STATA statistical package (Stata Corp., College Station, TX).

Potential confounders were assessed on the basis of their known association with both lead exposure and schizophrenia, including maternal and paternal age, education, race/ethnicity, family income, father’s income, maternal smoking, maternal alcohol use, hemoglobin levels, and number of previous pregnancies. After testing each for associations between serum δ-ALA and disease, addition and removal procedures were performed. During construction of the regression models, the utility of all potential covariates was assessed through sequential inclusion and exclusion. A change of ± 10% in the point estimate corresponding to δ-ALA provided justification for including a variable in the model.

### Results

#### Demographics

Thirty-one case–control sets contained 2 controls, and the remaining 13 had 1 control, for a total of 175 subjects.

**Table 1.** Demographic characteristics of parents (%) by case status.

| Case (n=44) | Control (n=75) |
|------------|---------------|
| Father's age at delivery (years) | |
| 15–19 | 0 | 1 |
| 20–29 | 34 | 33 |
| 30–39 | 36 | 37 |
| 40–45 | 25 | 12 |
| Unknown | 5 | 16 |
| Father's race | |
| White/Caucasian | 43 | 43 |
| African American | 41 | 31 |
| Mexican, other | 7 | 12 |
| Unknown | 9 | 15 |
| Father's education | |
| < High school diploma | 20 | 16 |
| High school or vocational | 29 | 33 |
| Some college | 18 | 20 |
| College graduate | 20 | 20 |
| Unknown | 11 | 11 |
| Family annual income | |
| $< 2,500 | 0 | 3 |
| $2,500–5,999 | 32 | 28 |
| $6,000–9,999 | 33 | 31 |
| $10,000–14,999 | 10 | 14 |
| $15,000 | 2 | 1 |
| Unknown | 25 | 21 |
| Mother's age at delivery (years) | |
| 15–19 | 9 | 14 |
| 20–29 | 50 | 44 |
| 30–39 | 38 | 37 |
| 40–45 | 2 | 5 |
| Unknown | 0 | 0 |
| Mother's race | |
| White/Caucasian | 45 | 49 |
| African American | 45 | 37 |
| Mexican, other | 7 | 12 |
| Unknown | 2 | 1 |
| Mother's education | |
| < High school diploma | 18 | 8 |
| High school or vocational | 38 | 43 |
| Some college | 18 | 24 |
| College graduate | 17 | 16 |
| Unknown | 9 | 8 |

**Figure 3.** BPb and δ-ALA levels in midpregnancy sera, Yugoslavia study. Midpregnancy BPb levels in 23 subjects from the Yugoslavia Study of Environmental Lead Exposure and Child Development (Grzianzio et al. 1990) are compared with δ-ALA levels in archived serum samples stored for 13–15 years at −20°C. A regression line and 95% CIs are shown.
and 46 controls) were categorized as unexposed. 66 subjects with levels < 9.05 ng/mL (20 cases from the primary study. Fifty-three subjects point was used to classify exposure in subjects yielding high sensitivity and positive predictive (corresponding to a BPb level of 15 µg/dL) the use of a cutoff point of 9.05 ng/mL

Conditional logistic regression, unadjusted 1.89 (0.86–4.11) 0.109
MH OR, unadjusted 1.83 (0.87–3.87) 0.106

Discussion
Our study represents the first report of a prospective examination of a prenatal chemical exposure as a risk factor for an adult psychiatric disease. Lead was widely distributed throughout urban areas during the era when this cohort was founded. Although BPb levels in the United States have declined, lead exposure continues to be of great concern. Despite bans on both leaded gasoline and lead-based paint that have been in effect for more than two decades, it has been estimated in national samples of children and neonates that 5% still have BPb ≥ 10 µg/dL (Satcher 2000), with regional rates as high as 29% (Vivier et al. 2001).

Internationally, lead exposure continues to be a concern because use of leaded gasoline persists in many parts of the world.

On the basis of our results, we suggest that further study is required to determine whether prenatal exposure to lead and/or elevated levels of serum D-ALA during the second trimester of pregnancy may be associated with an increased risk of schizophrenia spectrum disorder. When our finding is adjusted for covariates, the observed effect approaches statistical significance. These conclusions are subject to several limitations. First, the sample size is modest. Second, although methods for adjusting for potential confounders were used, some confounders may not have been adequately controlled for as a consequence of the matched design and sample size or because of a lack of sufficient information. For instance, data on family history of mental illness are incomplete for this cohort. Third, for similar reasons, we were unable to examine postnatal factors that might modify the effects of prenatal lead exposure. For example, childhood socioeconomic status (SES) may reverse lead-induced neuropsychologic deficits in high-SES children (Tong et al. 2000).

Although D-ALA is a biologic indicator of lead exposure, other factors may affect D-ALA levels. One alternative hypothesis that might explain our finding relates to the fact that ALAD is polymorphic. The most common variant, designated ALAD-1, is differentiated from its counterpart, ALAD-2, by a single locus G-to-C transversion in the coding region (Kelada et al. 2001). The carriers of the ALAD-2 allele have been shown to have higher BPb levels and lower levels of lead in bone, whereas individuals homozygous for ALAD-1 have higher levels of D-ALA in plasma and urine (Kelada et al. 2001). A variety of findings suggest that interactions between ALAD polymorphisms and lead exposure may affect long-term outcomes, including differences in neuropsychologic effects of lead exposure (Bellinger et al. 1994). It is possible that a specific ALAD polymorphism may be a risk factor for schizophrenia, either independently or through interactions with blood lead. DNA samples were not available from the maternal cohort, although genotyping of the subjects may be possible in order to test this hypothesis in the future.

In considering lead exposure during development as a risk factor for adult mental illness, both direct and indirect mechanisms may be postulated. Direct mechanisms could involve physical interactions between lead and the developing nervous system, interfering with growth, differentiation, or structural development. Examples supported by experimental evidence include effects on molecules of neural adhesion (e.g., nerve-cell adhesion molecule, N-cadherin, L1 neural cell adhesion molecule) (Prozialeck et al. 2002) and alterations of synaptic function (e.g., N-methyl-d-aspartate receptor expression) (Toscano et al. 2002). Both have been implicated in the pathogenesis of schizophrenia (Olney et al. 1999; Vawter 2000).

Indirect mechanisms might include effects of lead that are not specific to the central nervous system, such as renal damage (Loghman-Adham 1997), altered transthyretin secretion at the choroid plexus (Zheng et al. 1999), or interactions with nutrient absorption and distribution (Dawson et al. 1999). One specific indirect mechanism that must be considered is the potential toxicity of D-ALA. D-ALA is a known neurotoxin, and elevated levels of D-ALA are associated with psychosis in adults, as characterized by various forms of porphyria (Estrov et al. 2000). In experimental models, D-ALA has been shown to interfere with gamma-aminobutyric acid neurotransmission (Percy et al. 1981), a process that has also been implicated in schizophrenia (Benes 1997). Thus, it is possible that D-ALA itself

Table 2. Estimated ORs relating D-ALA (categorized as ≥ 9.05 and < 9.05 ng/mL) and schizophrenia spectrum disorder using three statistical methods.

| Method                      | Estimated OR (95% CI) | p-Value |
|-----------------------------|-----------------------|---------|
| MH OR, unadjusted           | 1.83 (0.87–3.87)      | 0.106   |
| Conditional logistic regression, unadjusted | 1.85 (0.86–4.11) | 0.109   |
| Conditional logistic regression, adjusted for mother’s age at delivery | 2.43 (0.95–5.56) | 0.051   |
elevates the risk of schizophrenia spectrum disorders, independently or as a consequence of lead exposure.

Over the past century, regulatory standards governing lead exposure during pregnancy and childhood have become less permissive, recognizing detrimental effects at progressively lower concentrations. In parallel with this trend, research has begun to focus on the effects of prenatal lead exposure at increasingly distal points along the life course as cohorts move out of infancy and childhood, through adolescence. The results of our study expand this premise to include an adult psychiatric disease, suggesting that lead-induced prenatal damage to the developing brain may manifest throughout the decades after the initial exposure.

References

Baghurst PA, McMichael AJ, Vimpani GV, Robertson EF, Clark PD, et al. 1987. Determinants of blood lead concentrations of pregnant women living in Port Pirie and surrounding areas. Med J Aust 146:69–73.

Beckmann H. 1999. Developmental malformations in cerebral structures of schizophrenic patients. Eur Arch Psychiatry Neuropsy 249(suppl 4):44–47.

Bellinger D, Hu H, Titlebaum L, Needlman HL. 1994. Attentional functioning of children with elevated blood lead. Environ Health Perspect 102:153–157.

Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL. 1992. The role of stress and dopamine-GABA interactions in the vulnerability for schizophrenia. J Psychiatr Res 26:225–235.

Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Begg MD, Gorman JM, et al. 2000. Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. Schizophr Bull 26:287–295.

Dawson EB, Evans DR, Harris WA, Van Hook JW. 1999. Amniotic fluid B12, calcium, and lead levels associated with neural tube defects. Am J Perinatol 16:373–378.

de Klerk M, Weideman A, Malan C, Shanley BC. 1975. Urinary porphyrins and porphyrin precursors in normal pregnancy. Relationship to urinary total oestrogen excretion. S Afr Med J 49:581–583.

Dietrich KN, Ris MD, Suggop PA, Berger DG, Bornschein RL. 2001. Early exposure to lead and juvenile delinquency. Neurotoxicol Teratol 23:511–518.

Endo Y, Okayama A, Endo D, Horiguchi S, Nakazono N. 1993. Improvement of urinary delta-aminolevulinic acid determination by HPLC-fluorometry using pre-column derivatization [in Japanese]. Sangyo Igaku 35:126–127.

Estrov Y, Scaglia F, Bodamer OA. 2000. Psychiatric symptoms of inherited metabolic diseases. J Inherit Metab Dis 23:2–6.

Factor-Litvak P, Wasserman G, Kline JK, Graziano J. 1999. The Yugoslavias Prospective Study of Environmental Lead Exposure. Environ Health Perspect 107:3–15.

Graziano JH, Popovac D, Factor-Litvak P, Shrouf P, Kline J, Murphy MJ, et al. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environ Health Perspect 89:95–100.

Greenland S. 2000. When should epidemiologic regressions use random coefficients? Biometrics 56:915–921.

Greenland S, Schwartzbaum JA, Finkle WD. 2000. Problems due to small samples and sparse data in conditional logistic regression analysis. Am J Epidemiol 151:531–539.

Jones PB, Bebbington P, Foerster A, Lewis SW, Murray RM, Russell A, et al. 1993. Premordial social underachievement in schizophrenia. Results from the Camberwell Collaborative Psychosis Study. Br J Psychiatry 162:265–271.

Keldana SN, Shelton E, Kaufmann RB, Khoury MJ. 2001. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: a HuGe review. Am J Epidemiol 154:1–13.

Kim R, Hu H, Rotnitzky A, Bellinger D, Needlman H. 1991. A longitudinal study of chronic lead exposure and physical growth. Environ Health Perspect 103:952–957.

Korpela H, Louvonen R, Vrijenhoeck E, Kaupilla A. 1986. Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes. J Obstet Gynaecol 155:1086–1089.

Loghman-Adham M. 1997. Renal effects of environmental lead exposure and occupational lead exposure. Environ Health Perspect 105:928–939.

Ludwig JH, Diggs DR, Hesselberg HE, Maga JA. 1965. Survey of fluorides of pregnant women living in Port Pirie and surrounding areas. Med J Aust 146:69–73.

Murphy MJ, et al. 1990. Determinants of elevated blood lead in the atmosphere of three urban communities: a prospective birth cohort study. Environ Health Perspect 49:105–111.

Pocock SJ, Smith M, Baghurst P. 1994. Environmental lead and children’s intelligence: a systematic review of the epidemiological evidence. Br Med J 309:1189–1193.

Prozialek WC, Gruenwald GB, Dey PM, Reuhl KR, Parrish AR. 2002. Cadherins and NCAM as potential targets in metal toxicity. Toxicol Appl Pharmacol 182:295–305.

Satcher DS. 2000. The Surgeon General on the continuing tragedy of childhood lead poisoning. Public Health Rep 115:579–580.

Sobotta JM, Rahvan N. 1995. Teratogenesis induced by short- and long-term exposure of Xenopus laevis progeny to lead. J Toxicol Environ Health 44:469–484.

Susser ES, Brown A, Matte TD. 1999. Prenatal factors and adult mental and physical health. Can J Psychiatry 44:256–334.

Susser ES, Schaefer CA, Brown AS, Begg MD, Wyatt RJ. 2000. The design of the Prenatal Determinants of Schizophrenia Study. Schizophr Bull 26:257–273.

Thomas HV, Milmore BK, Heidbreder GA, Kogan BA. 1987. Blood lead of persons living near freeways. Arch Environ Health 15:695–702.

Toscano CD, Hashemzadeh-Gargari H, McGlothan JL, Bassett DM, Petitti DB, Westmoreland B, et al. 2004. Early exposure to lead and juvenile delinquency. Environ Health Perspect 102:153–157.

Tong S, McMichael AJ, Baghurst PA. 2000. Interactions between environmental lead exposure and sociodemographic factors on cognitive development. Arch Environ Health 55:330–335.

Toscano CD, Hashemzadeh-Gargari H, McGlothan JL, Keilholz AL, Giullarte TR. 2002. Developmental Pb2+ exposure alters NMDAR subtypes and reduces CREB phosphorylation in the rat brain. Brain Res Dev Brain Res 139:217–226.

Udry JR, Morris NM, Kovercock J. 1985. Androgen effects on women’s gendered behaviour. J Biosci Sci 27:359–368.

Vander PM. 2000. Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. Eur J Pharmacol 405:395–395.

Vivier PM, Hogan JW, Simon P, Leddy T, Dansereau LM, Alario AJ. 2001. A statewide assessment of lead screening histories of preschool children enrolled in a Medicaid managed care program. Pediatrics. 108:E29. Available: http://pediatrics.aappublications.org/cgi/reprint/108/2/e29.pdf [accessed 18 February 2004].

Wasserman GA, Li X, Popovac D, Factor-Litvak P, Kline J, Watermael C, et al. 2000. The Yugoslavias Prospective Lead Study: contributions of prenatal and postnatal lead exposure to early intelligence. Neurotoxicol Teratol 22:811–818.

Weinberger DR. 1996. On the plausibility of “the neurodevelopmental hypothesis” of schizophrenia. Neuropsychopharmacol 14(suppl 3):115–115.

Xenopus laevis progeny to
