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MULTISITE LEAD AND CADMIUM EXPOSURE STUDY WITH BIOLOGICAL MARKERS INCORPORATED

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ABSTRACT

A multisite study design was utilized to evaluate populations near four National Priority List (NPL) sites for possible health effects related to chronic low-level lead and cadmium exposure. Biomedical tests were also incorporated in the study's design to detect subtle pathophysiological changes in four organ-systems: Renal, Hematopoietic, Immune, and Hepatobiliary.

Multivariate analysis was used to evaluate relationships between body fluid levels of lead and cadmium, area of residence, behavior, socio-economic factors, and concentration of these contaminants in four environmental media [Yard soil, house dust, drinking water, and interior paint]. Area of residence, environmental media concentrations of lead, socioeconomic, and behavioral factors were significantly associated with blood lead in children 6 to 71 months of age. These factors accounted for 26% of the mean blood lead variance observed in study participants.

Among children [6 to 71 months and 6-14 years] old, the mean blood lead level in target areas was higher [4.26 µg/dl and 3.45 µg/dl] than the comparison areas. Up to 93% of participants in all study areas had blood lead levels below 10 µg/dl. The mean blood lead level in smelting area study sites was higher than in mining area study sites. About 93% of mining area participants and 85% of smelting area participants had blood lead levels below 10 µg/dl.

The mean urine cadmium excretion was significantly higher in target areas than comparison areas. Ninety five percent of all participants had urine cadmium excretion below one µg/g creatinine. Mean urine cadmium excretion was significantly associated with area of residence, socio-economic, and behavioral factors in study participants 15 years of age and older. Cadmium concentration measured in yard soil, house dust, and tap water was not significantly associated with mean urine cadmium. There was no evidence of kidney dysfunction related to lead and cadmium exposure at levels observed in this study.

Of the five biomarkers of hematopoietic function evaluated, blood lead was significantly associated with and predictive of hematocrit (HCT) in adults 15 to 75 years old ($p=0.027$, $r^2=0.28$, $n=381$). Less than one percent (0.3%) decrease in HCT was predicted for each µg/dl increase in blood lead.

Of the six biomarkers of immune system function evaluated, mean IgG level was significantly lower in target area participants, (p<0.01). Mean blood lead level was associated with increase mean serum IgA in young children six to 71 months old. In this age group an eight percent IgA increase was predicted for each µg/dl increase in blood lead using area of residence as the independent variable ($p=0.005$, $r^2=0.26$, $n = 934$).

INTRODUCTION

In 1991, ATSDR initiated a multisite study of human exposure to lead and other heavy metals at smelting and mixed mining/smelting sites in four states (Illinois, Kansas, Missouri, Pennsylvania). Four concurrent investigations of exposure and adverse health effects at the sites were conducted with ATSDR financial support and technical guidance. Study planning and data collection were coordinated among the four investigative teams and three regional offices of the U.S. Environmental Protection Agency (EPA) to enhance the ability to pool data from the four study sites. The multisite study evaluated biological indicators of exposure and the association of such indicators with environmental concentrations of metals and biological markers (biomarkers) of adverse health effects in target populations living near the sites and comparison populations living farther away.

Background

Multisite Studies

In response to Senate Committee Report Number 101-128 (i), in 1990 the Agency for Toxic Substances and Disease Registry convened a workshop to evaluate the feasibility and value of performing multisite epidemiological studies at Superfund sites (*1*). A review panel comprised of experts in environmental epidemiology and occupational and community medicine concluded that there were distinct advantages in conducting multisite studies. The potential advantages of a multisite study design included: 1) increased sample size and greater statistical power which enhance the validity of study findings, and 2) added diversity in the sample improved the likelihood that results were real and not due to random variations. The panel members also concluded that in some situations, multisite studies may provide the only methodology to determine the range of health effects resulting from environmental exposures to hazardous waste sites.

The panel members cited many examples of health studies in which data from multiple sources were collected and analyzed to achieve study objectives that might not otherwise have been possible if only one source of data had been used were used. As in the present study, multisite studies require the use of a variety of techniques and logistical considerations to reduce bias and ensure data quality. The National Cancer Institutes' national bladder cancer study reported in 1984 was cited as an example of a multisite case control study that was successfully completed (*1,2*). However, panel members noted that it was inappropriate to use pooled data from multiple sources without the necessary advanced planning, coordination, and appropriate consideration to data quality issues.

In the present study, similar site and population characteristics, as well as the presence of common contaminants at each of the four sites, made it feasible to consider a multisite study design. Consequently, sample size was increased and data comparability was ensured by using standardized data collection instruments, uniform specimen collection, uniform specimen collection, handling procedures, and laboratory analytical techniques for specimens obtained at each study site. The larger sample size increased the statistical power to detect differences between those participants presumed to be exposed and those presumed unexposed to contaminants at the study sites.

Exposure to Lead

Lead poisoning in young children is a common health problem. Increasing information and public awareness about the toxicity of lead and other heavy metals at progressively lower levels has resulted in heightened concern among public health officials about the potential impact of lead exposure in this population. In young children less than six years old, for example, increases in blood lead levels may be associated with exposure to soils containing 500 ppm or more (*3*). The blood lead level at which the risk of toxicity exists continues to be revised downward. The Centers for Disease Control and Prevention (CDC), in a recent report stated that a blood lead level below 10 micrograms per deciliter ($\mu g/dl$) is not considered to be indicative of lead poisoning (*4*). For example, blood lead levels as low as 10 $\mu g/dl$ are potentially harmful to neurobehavioral development in fetuses of pregnant women and young children (*5,6*). Maternal and cord blood lead levels of 10-15 $\mu g/dl$ appear to be associated with reduced gestational age and reduced weight at birth (7). It is not clear what impact blood lead levels below 10 $\mu g/dl$ have on central nervous system function; it may be that even these levels are associated with adverse effects and continued research may help clarify this issue.

Lead has been shown to produce injury to the kidney following chronic high level exposure (*6,7*). However, the magnitude of risk following low-level environmental exposure to lead remains uncertain. It also is not certain what effect combined exposure to lead and cadmium may have on kidney function. Moreover, lead toxicity following various levels of exposure is also evident in red blood cells (*8,9*).

Exposure to Cadmium

Cadmium is a naturally occurring metal contaminant of phosphate and a by-product of zinc and lead smelting. Working in or living close to a major source of airborne cadmium emission such as zinc, lead, or copper smelters and/or consumption of vegetables grown in soil with a high cadmium concentration may result in higher than average exposure. Cadmium has a long biological half-life (10-30 years). At birth the total body burden of cadmium is almost absent (less than 1 μg), but by the age of 50 the body burden may have reached 20-30 mg (*10*). Normal urinary excretion of cadmium is generally below 2 μg/day and increases with age, probably paralleling an increasing renal and total body burden (*11*).

Long-term low-level exposure to cadmium is a public health concern. Emphysema, anemia, and liver damage have been observed in workers chronically exposed to cadmium (*11*). Although cadmium may concentrate in other vital organs, the kidney is the primary organ affected by cadmium exposure. Because of life-long accumulation and possibly irreversible renal tubular dysfunction caused by such exposure, sensitive biological markers of cadmium exposure or its effects are needed in order to detect early pathophysiological changes associated with cadmium exposure.

Environmental Contamination

The extent to which lead and cadmium present in different environmental media may threaten human health has not been fully established. However, recent studies concerned with environmental pathways of human exposure have demonstrated that elevated concentrations of lead and other heavy metals found in soil may pose a human health risk following direct contact with soil (*7,12*). It is not clear to what extent socioeconomics and individual behavior may also influence the magnitude of health risk posed by heavy metals in soil and other environmental media. Earlier reports concluded that lead in soil and house dust may be responsible for blood lead levels in children increasing when the concentration in these media exceeds 500 to 1,000 parts per million (ppm) (*3,4,13*).

The US Environmental Protection Agency (EPA) is responsible for assessing and remediating hazardous waste sites where the presence of lead and other contaminants may threaten environmental safety and the health of people. To better assess the exposure potential and effects of environmental lead in children, the EPA has developed the Integrated Exposure Uptake/Biokinetic (IEUBK) model. Matched data sets of environmental and blood lead levels collected as part of the present multisite study will be used to evaluate the utility of the IEUBK model.

Adverse Health Effects

In 1988, the CDC/ATSDR Subcommittee on Biological Markers of Organ Damage and Dysfunction recognized medical test batteries for three organ-systems: immune system, hepatobiliary system, and urinary system *(14)*. For each organ-system, the subcommittee classified and recommended laboratory tests that it considered to be suitable biomarkers and classified the tests in the three following categories: (a) basic screening panel, (b) tests for specialized studies, and (c) tests requiring more research and development. This initial classification of organ-specific test batteries was used as a basis to select biomedical tests that were used in the present study. Certain hematopoietic measures were also included because of the potential impact of lead on the blood-forming organ.

Biomarkers that measure subtle physiological change in organ-system function can aid researchers in evaluating the impact of chronic low-level exposure on organ-systems that may cause adverse health effects. Clinically obvious toxicity endpoints associated with high-level lead and cadmium exposure (e.g., anemia, peripheral neuropathy, and renal dysfunction) are well established *(9,15,16,17)*. Subtle health effects such as slowed nerve conduction, impaired neurobehavioral development, impaired hemoglobin synthesis, renal tubular cell injury, and impaired uric acid absorption may also result from or be potentiated by chronic low-level exposure to lead and cadmium *(18,19,20,21,22)*. It has been suggested that such effects may represent intermediate phases of toxicity along an exposure-disease continuum that may lead to more pronounced symptoms or disease.

Study Sites

Population-based cross-sectional studies were conducted in communities adjacent to the following four National Priority List (NPL) sites:

Illinois: NL Industries/Taracorp, Granite City, Madison County
Kansas: Cherokee County Subsite, Galena, Cherokee County
Missouri: Oronogo-Duenueg, Joplin, Jasper County
Pennsylvania: Palmerton Zinc Pile, Palmerton, Carbon County

Concentrations of lead and cadmium in residential soil elevated above background level were the common characteristics at each site that determined the feasibility of conducting a multisite study.

Illinois

ATSDR awarded a grant to the Illinois Department of Health to conduct an environmental health study at the NL Industries/Taracorp Site (23). This site was located in a mixed industrial and residential area within the city limits of Granite City, Illinois. The NL Industries/Taracorp site was a former secondary lead smelter that ceased operation in 1983. Smelting operations at the site were believed to have contributed to off-site, residential soil contamination during an eighty year period in which airborne lead emission may have occurred. In addition, surface run-off and fugitive dust emissions from contaminated surface soil and slag piles on-site were also suspected. The population potentially affected by site-related contamination was estimated to be about 45,000.

<u>Soil</u>- On-site soil samples collected in 1987 contained lead in concentrations that ranged from 1,500 to 48,000 ppm. Samples from slag piles and other surface waste contained concentrations of lead up to 300,000 ppm. Cadmium soil concentrations were not reported. Off-site soil samples collected from residential yards and gardens by Illinois Department of Health, IEPA, and USEPA in the early 1980s revealed lead concentrations that ranged from 27 to 5.400 ppm (mean 1,087 ppm, median 675 ppm, and n=48). In 1988, IDPH conducted additional soil sampling as a part of an area-wide lead study. Soil lead concentrations ranged from 106 to 9,493 ppm (mean = 1,030 ppm, median = 905, n=40).

For the most part, sampling revealed that soil lead levels were highest around the perimeter of the site with concentrations decreasing as distance from the site increased.

<u>Surface water</u>- The two main surface water bodies, the Mississippi River and Horseshoe Lake, were monitored frequently and showed no evidence of site related heavy metal contamination.

<u>Groundwater</u>- Analysis of water samples from site monitoring wells did not detect elevated levels of lead or other contaminants in ground water downgradient to the site. Lead concentration in groundwater has not exceeded 20 ppb.

<u>Air</u>- Ambient air monitoring since 1983 showed air lead levels taken from monitors closest to the site were well below the 1.5 $\mu g/m^3$ NAAQS standard for lead. The highest quarterly average recorded was 7.3 $\mu g/m^3$ during the final months of 1981 (1981 yearly average of 3.03 $\mu g/m^3$). Because of persistent air standard violations, Taracorp was denied a state license to

operate its smelter in 1983.

Kansas

ATSDR awarded a grant to the Kansas Department of Health to conduct an environmental health study at the Cherokee County Subsite in Galena (*24,25*). This site was located approximately 7 miles from the Joplin, Missouri site with which it shares a contiguous border. It is one of several sites that make up the tri-state mining district. The size of the residential population potentially affected was about 4,000. Soil contamination in the target area resulted from air and soil deposits associated with lead mining and secondary smelting operations during the late 1800s that continued into the mid 1960s. Off-site, soil contamination in nearby residential areas probably resulted from airborne lead emission related to smelter operations, surface run-off, and fugitive dust emissions from contaminated on-site surface soil and slag piles.

Soil - On-site soil samples collected in 1989 by the USEPA contained lead in concentrations ranging from 500 ppm in surface soil to 3,800 parts per million (ppm) in surface mine waste and tailings. Soil cadmium was found in concentrations ranging from 12 ppm in surface soil to 60 ppm in surface mine waste.

Surface water- Maximum surface water samples collected in 1989 contained lead concentrations of 290 ppb in open pit mine ponds to 67 ppm in other local creeks and rivers. Cadmium concentrations ranged from 200 parts per billion (ppb) in open pit mine ponds to 140 ppb in nearby creeks and rivers.

Groundwater - Samples taken at 50 monitoring wells and 22 private wells in 1985 exhibited lead concentration as high as 390 ppb. Cadmium concentration in private wells had a high of 180 ppb. Groundwater monitoring for heavy metal contamination of the Galena municipal drinking water supply was negative for lead and cadmium.

Air - Ambient air monitoring conducted by EPA in 1983 and 1985 did not detect appreciable concentrations of heavy metals absorbed to particulate found in ambient air near tailing piles or at other areas considered to be a potential source for heavy metal contaminated dust.

Missouri

ATSDR awarded a grant to the Missouri State Health Department to conduct an environmental health study at the Jasper County Oronogo-Duenweg Superfund Site (*26,27*). This site consisted of approximately 20 square miles of waste and mining products in an unfenced general site area where lead and zinc were extensively mined from the 1850's to the mid 1960s. Shallow ground water, surface water, sediment, and surface soil were contaminated with heavy metals including zinc, lead, cadmium, and nickel. Surrounding towns in the area used the deep aquifer and surface water. However, approximately 1,500 people were reported to use private wells from the shallow aquifer at the time of the ATSDR supported study. Human exposure to heavy metals may have occurred by ingesting contaminated ground water, soil, sediment, and inhalation of contaminated air. Levels of lead found in private wells did not appear to pose a significant public health threat alone, but may have been a significant contributor to overall lead exposure by area residents.

__Soil__ - Ten random soil samples collected from the site area in 1986 by EPA contained lead in concentrations ranging from 73 to 7,300 ppm with a mean of 2,501 ppm. Cadmium soil concentration ranged from 5.9 to 250 ppm with a mean of 80 ppm. By comparison a U.S. Geologic Soil Survey estimated that average Missouri soil lead concentration range from 2.1 to 930 ppm, mean 44 ppm and cadmium at less than 1 ppm.

__Surface water__- The main surface water runoff contained maximum dissolved lead and cadmium concentrations of 400 and 1,400 ppb, respectively. As a source for excess lead and cadmium exposure, surface water was considered less of a risk because of limited exposure associated with dermal absorption. No evidence of food chain contamination was reported. Reported consumption of fish obtained from local surface waters was not in quantities believed to be sufficient to pose a significant health concern.

__Groundwater__- Analysis of water samples for lead measurement that were obtained from site monitoring wells detected 6 ppb lead and did not exceed 20 ppb in subsequent sampling.

__Air__ Ambient air monitoring data were not available at the time of the study.

Pennsylvania

ATSDR provided technical assistance to the Pennsylvania Department of Health to conduct an environmental health study in Palmerton (*28,29,30*). The town (1990 population 5,393) lay in a narrow east-west linear valley bounded by parallel ridges rising up to 1,000 feet above the valley floor: Stony Ridge to the north and Blue Mountain to the south. The East Plant was located on the south bank of Aquashicola Creek near its confluence with the Lehigh River. The West Plant was located on the north bank of the Lehigh River. As such, the topographical character of the area surrounding the plants was favorable for soil contamination secondary to waste site runoff.

New Jersey Zinc (NJZ) began smelting operations in Palmerton (West Plant) in 1898. In 1915, after construction of the East Plant, NJZ began roasting sphalerite, a zinc sulphide containing small amounts of cadmium and lead. The primary products from these smelting operations, occupying 267 acres, were metallic zinc and zinc oxide. Before smelting activities ceased in December 1980, daily emissions from this source were estimated to be 6,000-9,000 kg/day zinc and 70-90 kg/day cadmium. At the time of the ATSDR supported study, activities for refining dust into base metals were being conducted in the East Plant, while the West Plant remains closed to all activities. In addition to the widespread deposition of heavy metals through past airborne emissions, there was potential for contamination of groundwater and surface water via leaching and runoff from a large site of waste deposition adjacent to the East Plant. This site, which lay at the base of Blue Mountain just south and east of Palmerton, consisted of some 33 million tons of processed waste or slag piled 200 feet high and covering an area approximately 2.5 miles long and up to 1,000 feet wide.

Soils- Sampling conducted by the U.S. Department of Agriculture (DOA) in 1980, found that soil concentrations of zinc, cadmium, and lead in the Palmerton area generally exceeded typical background levels in the U.S. (zinc <100 ppm, cadmium ≤0.5 ppm, lead <25 ppm).

In 1985 and 1986 off-site contamination of soils in and around the town of Palmerton confirmed that zinc and cadmium concentrations were elevated and that they decreased with increasing distance from the plants. Nearly all of the Palmerton residential soils tested contained below 500 ppm lead. Soil samples containing higher concentrations of lead were generally confined to areas not subject to human use.

Surface Water - In May 1979, a hazard evaluation study was conducted by the EPA National Enforcement Investigations Center (NEIC) to assess heavy metal pollution in Aquashicola Creek and the groundwater in Palmerton area wells *(28)*. Seven-day average concentrations of zinc and cadmium were found to be 0.86 ppm and 0.04 ppm respectively at a location in Aquashicola Creek just downstream from the East Plant. These downstream metal concentrations were about 30 times higher than the concentrations observed at a reference location upstream from the plant. The report concluded that the observed concentrations of heavy metals were sufficiently high to cause fish growth retardation and mortality, and that the contaminated section of the creek was not safe for trout stocking or for potable water (the drinking water standard for cadmium was exceeded by a factor of 4-5).

Ground water - Drinking water supplies for the Palmerton area were not found to have elevated levels of cadmium or lead.

Air - In 1983, Hartwell measured ambient air concentrations of cadmium and lead at four different locations *(30)*. Average cadmium concentrations recorded by Hartwell exceeded normal rural background levels and showed an inverse relationship with distance from the smelter. Average lead concentrations ranged from 0.128-0.563 $\mu g/m^3$. These concentrations were well below the National Primary Air Quality Standard for lead of 1.5 $\mu g/m^3$ annual average. The air lead concentrations showed no relationship to distance from the smelter. In September and October 1990, ambient air sampling was conducted on an area of Blue Mountain undergoing a pilot soil remediation program. The nearest residences were approximately 1,500 feet from the sampling locations. Median air lead concentrations ranged from 0.088 $\mu g/m^3$ to 0.349 $\mu g/m^3$. Median air cadmium concentrations ranged from 0.003 to 0.011 $\mu g/m^3$ *(31)*.

OBJECTIVES

(1) Analyze blood and urine specimens for biological evidence of lead and cadmium exposure among residents of communities adjacent to four NPL sites (target areas) and among residents of comparison areas.

(2) Evaluate the exposure contribution of contaminated soil and house dust among target and comparison area residents, by measuring and controlling for other possible environmental contaminant sources and factors known to influence lead and cadmium exposure.

(3) Evaluate the extent to which behavioral, occupational, and socio-economic factors influenced exposure to lead and cadmium in target and comparison populations.

(4) Estimate exposure among target populations near sites where the predominate environmental source of lead and cadmium resulted from mixed mining/smelting activities compared to populations near sites where the predominant source of contaminants resulted from smelting activities.

(5) Characterize the distribution of selected biomedical

test batteries to evaluate four organ-systems in target and comparison populations and compare results with standard reference ranges.

(6) Evaluate the extent to which blood lead and urine cadmium levels were associated with organ-system dysfunction, as indicated by biomedical test battery values.

METHODS

Rationale for Study Design

Similar site and population characteristics, as well as the presence of common contaminants at each of the four sites, made it feasible to utilize a multisite cross-sectional study design. Standardized data collection instruments, uniform procedure for specimen collection, handling, and analyses were specified in advance to ensure comparability of data.

Data Collection

ATSDR staff participated in training census survey workers and interviewers responsible for administering questionnaires, and observed all aspects of the studies to ensure that data were collected consistently. Data collection at each study site occurred in two phases. In order to capture peak exposure potential for children under six years old, all data collection was performed between June and September 1991.

The first phase was a door-to-door census survey of households located in the target and comparison areas. At least four attemps were made to contact occupants of each household. Length of residence, age, and sex were recorded for all individuals in each household. All persons who satisfied a 60-90 day residency requirement were eligible to participate. Of those eligible participants, random selection was used to choose particpants for the second phase of data collection. The 60-90 day residency requirement was used to establish a minimum duration of exposure to local environmental contaminants and was based on the half-life of circulating red blood cells.

The second phase consisted of collecting interview data, blood and urine specimens, and residential environmental samples. Blood and urine specimens were analyzed to measure biological evidence of exposure to lead and cadmium. Reference ranges for biomedical tests that were used in this study are displayed in Tables 1-6. Environmental samples of drinking water, yard soil, house dust, and interior house paint were collected from a representative sub-sample of study participants' households for analysis.

Study Area

Target areas were located adjacent to four NPL sites and known or suspected to have elevated residential soil levels of lead and cadmium. Comparison areas were similar to target areas with respect to demographic and socio-economic characteristics, but were located at least three miles from the NPL sites and there was no other known point sources of the contaminants being investigated or evidence of elevated lead or cadmium concentration based on past and current soil sampling.

For data analyses and other comparisons, study sites were further characterized on the basis of historical smelting or mining activities at the NPL sites, that was believed to be associated with residential lead and cadmium deposition. The purpose of this comparison was to evaluate exposure differences that may be related to variation in lead forms emitted in connection with different manufacturing processes. Thus, target area participants from the Illinois and Pennsylvania study sites were classified as smelting area residents while those from Kansas and Missouri were classified as mixed mining/smelting area residents (Table 7).

Census Survey

A census was conducted in the target and comparison communities to obtain a complete listings of eligible participants, from which representative samples were drawn. Census data identified eligible participants by age, sex, and length of residence in target and comparison areas.

Advance notification to household residents of the upcoming census was accomplished by either leaflets left at the homes or through the mail. An example of a census survey form developed by ATSDR used to collect census information is displayed in (Attachment 2). Households not returning the mailed census forms were visited by a censustaker. Four attempts, on different days and at different times of the day, were made to contact residents of each household in the census areas. A notice was also left at each residence where no one was home. This notice informed residents that a census taker came by and provided a phone number for contacting the census taker.

Participant Selection

Residents between the age of 6 months to 75 years who had lived in the study areas for at least 60 to 90 days were eligible to participate in the study. The residency requirement was established to ensure that measures of exposure reflected a high probability for local exposure. Within each age group (i.e., 6-71 months, 6-14 years, 15 years and older), eligible participants were randomly selected. Young children were oversampled because of their increased sensitivity to the effects of lead, and they were also more likely to be exposed from environmental sources because of common hand-to-mouth activity in this group.

A total of 2,208 people were selected from eligible participant pools among the four sites (Illinois 10,000, Missouri 8,000, Pennsylvania 7,000, and Kansas 1,857). Although the presence of elevated soil lead and cadmium concentrations at each site was a major consideration in the decision to include each site in the study, it was also noted that residents of the different study locations were comparable with respect to age, sex, and socioeconomic characteristics (Tables 8 and 9).

Calculation for study power and sample size required for the multisite study (i.e., combinded data from the four studies) was based on urine excretion of alanine aminopetidase (AAP), a renal tubular enzyme associated with tubular damage. This enzyme has been found to be a sensitive indicator of renal damage in workers exposed to lead and cadmium. Of the renal tubular enzymes used in this study, AAP is more sensitive to renal effects associated with cadmium exposure. Since cadmium exposure was anticipated to be low in the focus population (children <6 years old) UAAP was selected as the variable most likely to capture subtle renal injury that may result from concurrent exposure to lead and cadmium. Sample size and power calculations are based on being able to detect a 10% change in UAAP, at alpha = 0.05%, control fixed and target varying. It was determined that a sample size of 300 target and 300 comparison area participants was sufficient to obtain power of 83%

Participants from target and comparison study areas were selected according to a protocol mutually agreed upon by principal investigators for each of the four studies. A suitable comparison population was not identified near the Illinois study site; therefore all participants in the Illinois study were target area residents.

Participant Consent

Consent of each participant was confirmed at the beginning of each interview by completion of a participant consent form approved by the CDC and Human Subjects Review Committee of each of the respective state health departments. Permission to collect blood and urine specimens for heavy metal exposure measurement and biomedical testing was obtained from each participant.

Interviews

Residents who consented to participate in the study were interviewed by trained interviewers. A core questionnaire was developed by ATSDR in collaboration with the state health departments and standardized for use in the present study. A copy of the questionnaire was not included in this report because disclosure of its contents would limit its usefulness in future studies. The questionnaire was used to collect information about demographic, residential, hobby, occupational, and behavioral characteristics that could influence exposure to heavy metals, or the health of participants as indicated by biomedical test values. For children under 16 years of age, a parent or legal guardian was required to participate in the interview process. All interviews of adults were answered by the participating adult.

Only core questionnaire responses were used in the present multisite analysis. However, the investigative team at each of the four study sites appended additional questions as needed to deal with variables of local importance.

Biological Specimens

Venous blood and urine specimens were collected at central locations convenient to participants. Specimen collection procedures used at each study site were standardized by protocols specified by each laboratory performing specimen analyses. For consistency in specimen collection, storage, handling, and transport, on-site laboratory support for each study was provided by a single contractor, Midwestern Research Institute (MRI). To ensure consistency of laboratory analyses and quality control/quality assurance standards, as well as minimize interlaboratory variability, tests in each major category were analyzed at the same laboratory using identical analytical techniques. For example, urine cadmium, blood lead, urine renal tubular enzyme analyses (i.e., AAP, NAGA, and GTT) and immune function tests were conducted at the Centers for Disease Control and Prevention (CDC) laboratory. Liver enzymes and electrolyte analyses were performed at Roche Laboratory, whose services were obtained by a sub-contract with Midwestern Research Institute

(MRI). Complete blood counts and urine analyses were done within eight hours after collection to ensure specimen integrity. These procedures were performed at local hospital laboratories arranged by MRI. Because these test procedures were well standardized, comparability of results obtained by different hospital laboratories was expected to be satisfactory.

Laboratory Variability

Specimen collection technique, volume requirement, limits of detection, and quality control measures were standardized across the four study sites to minimize the impact of laboratory variability on test results.

Blood lead and urine cadmium analyses were performed using the Zeeman graphite furnace atomic absorption method. Quality control was established by duplicate analysis of whole blood pools, where target values were established by thermal ionization isotopic dilution mass spectroscopy (*32,33*). Means and ranges for controls were all within previously established 95% confidence limits.

Other specific measures taken in order to assure specimen integrity as well as reproducibility of test results are described in the following sections.

Collection Apparatus

To minimize specimen contamination, all collection materials were screened for lead and cadmium before transporting to study sites. These materials were kept in plastic bags until used. Screened collection materials [vacutainers, syringes, collection cups, and needles] were provided by the Center for Environmental Health Laboratory Sciences (CEHLS) at CDC and Midwest Research Institute (MRI).

Collection Method

MRI provided on-site laboratory support services at each study site. Trained phlebotomists were on-site to collect venous blood specimens. Phlebotomists with specialized skills in pediatric phlebotomy were utilized for specimen collection at each study site.

Blood specimens that were obtained from young children, age 6 through 71 months, were collected with a syringe-butterfly or vacutainer-butterfly apparatus. A minimum of seven milliters of venous blood was necessary to accomplish all laboratory analyses administered during the study.

All urine specimens were "spot" (untimed specimens collected on site. Verbal and written instructions for urine collection procedures were given to each participant. Parents or guardians received additional instructions for assisting young children and infants who were not toilet trained.

Specimen Handling and Transport

To minimize the possibility of loss specimens or incorrect assignment of laboratory values, each sample was labeled with a unique identification number. Every item containing a participant's data-including census form, questionnaire, and consent form was labelled and identified using the same unique number that identified biological and environmental specimens. All participant information was maintained as a unit, checked for consistency, and transported to the designated laboratory(s) daily. The study group status [target versus comparison] of each participant was not displayed and therefore unknown to laboratory personnel performing the analysis.

Blood and urine specimens were shipped by overnight mail to CDC for analysis of cadmium, AAP, GGT, and NAG. Blood specimens for the panel of immune tests were shipped daily by overnight mail to CDC. Blood specimens for liver enzymes and serum chemistries were batched and shipped daily by courier to Roche Laboratory for analysis. Complete blood counts (CBCs) and urine analysis were performed locally at certified hospital laboratories. Urine specimens were divided into four 50ml aliquots on-site, labeled, stored, and shipped to CDC for analysis.

Environmental Sampling

A sub-sample of study participants' homes was systematically selected for environmental sampling in target and comparison areas. Households with children between the ages of 6 through 71 months old were intentionally oversampled because of the high priority place on examining this group. The following environmental media specimens were collected from within and around participants' residences: (1) drinking water, (2) yard soil, (3) house dust, and (4) house paint (interior). All samples were collected in contaminant-free containers using a protocol developed by USEPA Regions V and VII for use in this study (Appendix C). A similar protocol was developed by Region III USEPA and used for environmental sample collection. Samples were transported to a designated EPA contract laboratory for analysis. Investigative teams at each study site were responsible for accurate sample collection, labeling, and transport. Each sample was labeled so as to clearly identify the type of sample and the residence from which it was obtained. Samples collected from each household were transported in containers of sufficient size so that transport of individual household samples were transported as a unit. This precautionary measure was taken in an attempt to decrease the opportunity for delayed arrival of individual samples at the laboratory or the possibility of being lost. All environmental sampling and laboratory analyses were performed to meet EPA quality assurance and quality control requirements.

Privacy and Notification

Privacy Act of 1974

Under the federal privacy Act of 1974 (5 U.S.C. Section 552a [e]), employees of federal agencies are responsible for protecting data collected on identifiable persons or organizations when the supplier of that data has not given consent to the agency to make the data public. The responsibility for protection extends to unauthorized visual observation, accidental loss, or theft. This implies that confidential records should be kept out of sight of unauthorized persons, stored in locked cabinets or locked rooms when not being used, copied only when absolutely necessary, and stored in sealed containers when transferred to archives. Statistics derived from such confidential data should be reported without inadvertent disclosure about particular study subjects.

This report, and all reports made available to the public, do not contain laboratory results or findings that identifies any individual or person and only report aggregate data. All such records continue to be maintained in compliance with the Privacy Act of 1974.

Individual Notification

Tests results from each of the four study sites were received from each laboratory and reviewed by the investigative team. Test values were transmitted in writing to adult participants and to parents/guardians of minor participants by the respective state health department involved in the studies, along with explanations of test results and follow-up recommendations as indicated.

Findings of Immediate Significance

Test results indicating immediate clinical significance to a person's health received notification immediately. Individuals were notified by the state health department upon receipt of tests results from ATSDR. Repeat testing was offered to participants who had blood lead levels of 15 μg/dl and above for confirmation and to alert state health departments of the possible need to implement community surveillance and lead exposure prevention strategies. Unadjusted urine cadmium values of 2 μg/L and above were confirmed by blood cadmium measurements to rule out possible specimen contamination.

Data Analysis Methods

The Statistical Analysis System (SAS), Release 6.07 (SAS Institute, Cary, North Carolina) was used for data management and statistical analyses.

Data Entry

Interview/questionnaire data were double-entered by each of the investigative teams. Data were visually inspected for completeness and consistency before entry. Following data entry, internal consistency computer programs, as well as validity and range checks were used to identify possible coding and data entry errors.

As ATSDR staff received data from CDC, MRI, EPA, and states, all values were inspected to determine the presence of outliers. Suspected outliers were validated by inspecting original data forms and changes made, if necessary. Discrete or categorical variables were checked to determine whether the responses for each variable were valid and within the appropriate ranges.

Data Management

Data from all four states were entered in separate computer files as follows: (1) biological exposure measurements, (i.e., blood lead, and urine cadmium), (2) concentrations of lead and cadmium measured in four environmental media, (3) biomedical test results, and (4) interview/questionnaire responses. Data were submitted on a tape standard label, (6250 BI) in ASCII format. ATSDR received all original data sets except questionnaire data which were collected and entered by the four investigative teams. A merged datafile was created for each study site containing the four data components and given to each investigative team for site-specific data analyses and quality checks.

The datafile created for the present multisite analysis consisted of individually merged datafiles from the four study sites, and contained: interview data, exposure variables, biomedical test results, and environmental data for each study participant. The four merged datafiles were concatenated to create a single multisite data set. The concatenated data set was used for all subsequent analyses.

Data Transformation

Laboratory values for all biological analytes less than the detectable limit were redefined and entered as one-half the value of the detection limit. All laboratory values were transformed to natural logarithm, to approximate normal distribution for statistical analysis. Because spot urine specimens were collected rather than timed specimens, cadmium levels were adjusted to account for urine concentration differences observed in study participants. This was a particular concern because of the large number of children in the study and that urine creatinine excretion is generally lower in children and women due to less muscle mass *(34,35)*. Urine cadmium values were examined across a wide range of urine dilutions and creatinine measures. It appeared that urine specimens with creatinine less than 25 mg/dl resulted in erratic and unreliable cadmium values when adjusted for creatinine. Specimens with creatinine values below 25 mg/dl were considered too dilute for accurate adjustment and therefore, only specimens with creatinine levels equal or greater than 25 mg/dl were used for statistical analysis of urine cadmium results. A previous study that evaluated renal tubular enzyme excretion in connection with cadmium exposure suggested that creatinine adjusted spot urine specimens with creatinine levels less than 50 mg/dl may not have been reliable (Muller 1980). Thus, for renal tubular enzyme analysis, creatinine adjustment were performed only for those specimens with creatinine values of 50 mg/dl and higher. Adjusted cadmium excretion was obtained by applying one of the following formulas:

$$Urine\ Cadmium\ (\mu g/g\ Creatinine) = \frac{UrCd\ ng/ml}{UrCr\ mg/dl} X\ 100$$

$$Enzyme\ Creatinine\ Ratio(\mu g/L) = \frac{Urine\ Enzyme\ Value\ (\mu g/L)}{UrCr\ mg/dl} X\ 100$$

where:

UrCdCr = Urine cadmium, adjusted to urine creatinine
UrCd = Urine cadmium, unadjusted

UrCr = Urine creatinine

Data Analyses

The present study was designed to evaluate differences in exposure to lead and cadmium among populations residing within target and comparison study areas. Study sites were also classified as either smelting [Illinois and Pennsylvania] or mixed mining/smelting [Kansas and Missouri] on the basis of the predominant activity associated with lead and cadmium deposition, to evaluate differences in exposure potential that might be related to the form of lead deposited. In all cases data were analyzed to determine whether persons had blood lead or urine cadmium levels significantly different based on area of residence (i.e., target versus comparison and smelting versus mixed mining/smelting sites).

Upper and lower reference ranges were computed for male and female participants who resided in each study area of interest for all biomedical tests and compared to test values obtained from the reference population. For biomedical tests not having well established ranges of normal, these values were computed and defined as less or greater than 5% and 95%, respectively of values observed in the comparison areas. In addition to computed upper and lower limits, mean biomedical test values for each age group within each study area were used to evaluate differences observed.

Although the present study oversampled children 6 to 71 months of age, data analysis was performed in a similar manner for each age group in three phases. First, the distribution of selected variables were characterized for each study area [target versus comparison]. Second, the distributions were compared to determine whether blood lead and urine cadmium levels differed significantly among participants residing at smelting versus mixed mining/smelting sites. Third, additional data from study area participants were examined to determine associations between other independent variables and blood lead or urine cadmium. Linear and logistic regression models were constructed to determine the association between lead and cadmium exposure measures while controlling for the effect of multiple potentially confounding variables.

Univariate Analysis

Univariate analysis was used to provide a summary of descriptive statistics for each study group. More specifically, it was used to characterize distributions of biological exposure measures (i.e, blood lead and urine cadmium), environmental media concentrations (i.e, soil, dust, water, and paint), biomedical test results, and questionnaire responses including demographic and behavioral factors in each study group. Descriptive statistics used to characterize continuous variables were mean, standard deviation, and ranges. Frequencies and percentiles were used to characterize categorical variables.

The Student's t-test was applied to log-transformed biological and environmental data to determine whether differences between mean values among study area groups were statistically significant. Independent variables of primary interest in this study were age and area of residence.

Odds ratios and 95% confidence intervals were used to determine the association between blood lead and urine cadmium levels in each study group and other independent and potentially confounding variables. Stratified analyses were performed to examine variables that could be effect modifiers or confounders.

Bivariate Analysis

Bivariate analysis was performed to evaluate the relationship between two variables or compare two study groups. Bivariate analyses include the two-sample t-test, odd ratios, correlations between two continuous variables, and simple linear regression.

The two sample t-test was used to determine whether participants in each study group had significantly different mean blood lead, urine cadmium, and biomedical test values.

Correlations—Correlation coefficients were calculated to evaluate the strength of associations between exposure variables [blood lead and urine cadmium], and environmental media concentrations of these contaminants. Strong associations indicated by a correlation coefficient value of 0.20 or greater were further examined by including the variables in multivariate models.

Simple linear regression was used to examine the relationship between area of residence and biological analytes of interest, environmental media concentrations, and questionnaire variables. This technique was used to predict the value of a dependent variable (e.g., blood lead or urine cadmium) with an independent variable, such as age, smoking, or other variables of interest.

Odd ratios - Odd ratios were calculated to estimate the relative risk for having elevated blood lead and urine cadmium levels in each study group. No actual disease outcome measures were used; the outcome variable was defined as elevated blood or urine levels of lead or cadmium, respectively. The odds ratio was defined as the odds of a positive outcome in (exposed) participant with certain characteristics relative to those who lacked the characteristics evaluated (unexposed).

Multivariate Analysis

Multivariate analysis was used to show the relationships between three or more variables, and included a covariance model (ANOCOV), regression analysis, and logistic regression for dichotomized outcome variables.

ANOCOV model - Based upon results of the two-sample test, and correlation coefficients, an ANOCOV model was derived as an extension of the two-sample t-test. The ANOCOV model compared mean values of variables, while adjusting for other variables (covariables) such as age, and occupation. The ANOCOV model consisted of three components: (1) response or dependent variable, (2) main effect, which in this study was the area of residence being compared, and (3) covariables, which were factors known to be associated with or possibly have influence on the response variable such as age, sex, occupation, hobbies, etc.

Multiple regression - For each continuous outcome variable [exposure and biomedical values], a regression model was fitted to best describe or predict the outcome variable from two or more predictors (i.e., independent variables). The search for the "best" model was based upon the correlation coefficients, and to a large degree upon the stepwise procedure used for model building. The stepwise backward approach began with a full model [10-15 variables], and at each subsequent step, variables contributing least to the overall model fit were removed. The procedure retained variables in the model for which the p-value for the regression coefficients was ≤ 0.05. Colinearity between two or more environmental measures indicated by a correlation coefficient of .20 and $p \leq .05$ were evaluated for there effect on the final model. In such instances, separate models were constructed which contained each variable in question and a determination was made concerning the relative effect that each variable had on the final model.

Logistic regression - For dichotomous outcome variables, logistic regression was used to adjust for the effect of risk factors and potential confounders. This was an extension of the bivariate analysis of the odds of a defined outcome. The search for the "best" model involved stepwise backward approach similar to that used for linear regression. This model was also used to evaluate whether the odd ratio was statistically significant.

Biomarker Analysis

Biomedical tests [biomarkers] were used to assess the relationship between exposure variables [blood lead, urine cadmium, and area of residence] and biomarkers of three priority health conditions (immune function disorder, kidney dysfunction, liver dysfunction and the hematopoietic system). Because the exposure pattern and level of exposure were not remarkably different among participants residing in historical smelting or mixed mining/smelting areas, study groups were not characterized in this manner during the analysis of biomedical test results.

Univariate and bivariate analysis constituted the majority of analyses performed and reported. The frequency distribution, mean, standard deviation, minimum, and maximum medical test values were used to describe the distribution of continuous variables among target and comparison area participants. The Student's t-test and Kolmogorov-Smirnov (K-S) tests were used for between-group comparisons of continuous data. Because less stringent assumptions about underlying data distribution are required when nonparametric methods are used, the K-S test ($p \leq 0.05$) was selected as the criterion to assess statistical significance for between-group comparison of mean biomedical test values.

Multivariable regression models were constructed to examine the association between independent variables of exposure [area of residence, blood lead and urine cadmium] and dependent variables [biomedical tests) while controlling for covariates of interest [age, sex, smoking, and drinking alcohol]. More than 200 models and statistical tests were performed. Only models that showed statistically significant associations for the entire model and the variable of interest, and that had an adjusted r^2 equal to or greater than 0.20 were used for interpretation. Models with adjusted r^2 less than 0.20 were considered poor and were not considered further. Clinical significance was further assessed by evaluating the size of the effect on the dependent variable [biomedical test].

The following biomedical test variables were analyzed for each organ-system:

<u>Kidney Dysfunction</u>: Alanine aminopeptidase (UAAP); Gamma glutamyl transferase (UGGT); N-acetyl-β-D-glucosaminidase (UNAG); creatinine; and blood urea nitrogen (BUN).

<u>Hematopoietic Function</u>: Hematocrit (HCT); Hemoglobin (HGB); Mean corpuscular volume (MCV); Mean corpuscular hemoglobin (MCH); and Mean corpuscular hemoglobin concentration (MCHC).

Immune Function Disorders: Immunoglobulins (IgA, IgG, IgM), Total lymphocyte count and flow cytometric analysis for immunophenotyping (CD4, CD4/CD8 ratio).

Liver dysfunction: Gamma-glutamyl transferase (GGT); Aspartate transaminase (SGOT); Albumin; and Total protein.

RESULTS

SECTION I

Demographics

Demographic characteristics of target and comparison populations were similar with about twice as many participants residing in target areas as those who resided in the comparison areas (Tables 8 and 9). The proportions of males and females, level of education, and age of housing were similar. Young children under six years old were over represented. Fifty one percent (51.8% and 36.3%) of participants were children less than six years old from the target and comparison areas, respectively. Participants in all study groups were predominantly Caucasian. More households (31.9%) in the target areas, had an annual income less than $15,000, than households in the comparison areas (16.9%).

SECTION II

Environmental Lead Concentration

Of the 2,208 study participants, 1,465 (66%) had household environmental samples taken to measure concentrations of lead in: yard soil, house dust, interior paint, and drinking water to assess potential exposure (Table 9). From households where environmental samples were taken about 38% (552) of participants were found to have yard soil lead concentration greater than 500 mg/kg and 43% (544) of the households sampled had house dust lead concentrations greater than 500 mg/kg.

The mean concentration of lead in soil and water was higher among households in the comparison areas. By contrast, target area households had a higher mean lead concentration in house dust and interior paint (Table 9 and Figure 1). Notably, of the 544 participants living in households where interior lead paint concentration measured by XRF was above 0.6 mg/cm^2, 81% (441) were from the Illinois study site where 377 households were tested.

The mean concentration of lead in interior paint measured by

XRF was almost twice as high in mixed mining/smelting area households compared to mean concentrations found in smelting area households (Figure 2).

Correlation coefficients showed statistically significant associations between blood lead and lead concentration in soil and dust, but not paint and water (Table 11).

Blood Lead Levels

Mean blood lead values differed among age groups and areas of residence (Table 12). The mean blood lead was higher among young children in each study group and was higher among participants in every age group in smelting areas (Figures 3 and 4).

The highest blood lead values measured were 41.9 μg/dl and 37.9 μg/dl in the target and comparison areas, respectively. About ninety percent (90.1%) of target and 93.1% of comparison area participants had blood lead levels below 10 μg/dl (Table 13). Only about 2% to 3% of participants in each study area had blood lead values above 15 μg/dl.

When study areas were evaluated on the basis of lead deposition historically related to either smelting or mixed mining/smelting activities, the distribution of blood lead levels among participants were similar to that found among participants who resided in target areas that were defined by their proximity to respective study sites. About eighty nine percent (88.9%) of smelting area participants and 92.5% of mining/smelting area participants had blood lead values less than 10 μg/dl (Table 14).

Children 6 Months Through 71 Months

Target versus Comparison areas

Univariate—Eighty four percent (84.1%) of children in the target areas had blood lead levels below 10 μg/dl, contrasted with 91.8% in comparison areas.

Bivariate—The mean blood lead level among children living in the target areas (5.37 μg/dl) was significantly higher than among children living in the comparison areas (3.97 μg/dl), $p < 0.01$. Mean blood lead was also higher among children who lived in households where: household income was less than $15,000, head of household had less than a high school education, or the house was built before 1950. Children who lived in houses with air conditioning had lower mean blood lead (4.71 μg/dl) compared to those in houses without air conditioning (7.24 $\mu g/dl$), ($p<0.01$).

Children who were reported to put their mouths on furniture had higher mean blood lead levels than children who were not so reported. Children who were reported to carry food outside also had higher blood levels (5.93 $\mu g/dl$ compared to children who did not carry food outside (4.62 $\mu g/dl$), (Table 15).

Multivariate—After controlling for other variables in the model, residing in one of the target areas was a statistically significant predictor of blood lead in young children ($p<0.01$). Other variables that were significant predictors of blood lead were living in a household where: the household income was less than $15,000, the head of the household had a high school education or less, someone smoked tobacco in the house, the child was male, ($p<0.01$). Living in a house that was constructed after 1950 or a house that has air conditioning was associated with a lower mean blood lead (Table 16).

Lead measurements for four environmental media were added to the reduced model (Table 17). Of the four media sampled, yard soil and house dust concentration of lead, both presumed to be site-related, were positive predictors of blood lead, ($p<0.01$). In order to maximize use of all paint data, X-ray fluorescence (XRF) values of zero (i.e., below the detection limit) that yielded an infinite value when the log was taken, were assigned a value of 0.001 mg/cm^2. Interior paint lead, which was not considered to be site-related, was not retained in the model. Similarly, tap water lead concentration was not a predictor for blood lead level and was not retained in the model.

Multicolinearity in the final model, indicated by the high correlation coefficient for soil lead and dust lead (i.e., $r = .46$, $p <0.01$), was evaluated by constructing separate models containing the soil lead or dust lead variable. Parameters for the models were ($r^2 = 0.195$, $n = 731$, $p = 0.0001$) and ($r^2 = 0.197$, $n = 734$, $p = 0.0001$) for soil and dust lead, respectively.

In the logistic model for young children (Table 18) blood lead was dichotomized at 10 $\mu g/dl$. Area of residence was the independent exposure variable of interest. Covariates significantly associated with the dependent variable at ($p\leq0.05$) were retained in the model if they contributed significantly to the overall explanation of the data.

As in the linear regression model, the logistic model showed that living in a target area (OR = 2.69, 95% CI = 1.41-5.12) or living in the Pennsylvania target area (OR = 2.29, 95% CI = 1.36-3.87) was significantly associated with a blood lead level ≥

10 μg/dl. ORs were slightly elevated for the following variables: male, age 1-2 years, and living in a house with someone who smokes tobacco. A significant negative correlation (i.e., blood lead ≤ 10 μg/dl) was shown for participants living in houses constructed after 1950 or in houses with air conditioning.

The final regression model (Table 19) was constructed by adding dichotomized environmental variables to the reduced logistic model after removing area of residence as an independent variable. The following cutpoints were used to dichotomize environmental variables: yard soil and house dust lead concentrations equal to or greater than 500 mg/kg, tap water equal to or greater than 15 μg/L; and interior paint equal to or greater than 0.6 mg/cm^2. Of the environmental variables entered in the model, only yard soil equal to or greater than 500 mg/kg was moderately associated with blood lead level above 10 μg/dl.

Smelting versus mixed mining/smelting (All Target)

Univariate—The distributions of blood lead levels among young children (Table 20) residing in smelting and mixed mining/smelting areas were similar. The mean blood lead level observed in young children was higher in smelting areas (5.70 μg/dl) than in the mixed areas (4.81 μg/dl). Although the observed difference was statistically significant ($p<0.01$), the clinical significance was considered negligible.

Bivariate—Activities and behaviors (Table 20) found to be significantly associated with mean blood lead in this age group were similar to those noted in the target versus comparison area analysis.

Multivariate—In the linear model (Table 21) type of study site [smelting versus mixed mining/smelting] was not a significant predictor of blood lead, ($p=0.239$). Significant predictors of blood lead included: household income less than $15,000, head of household with less than high school education, lack of air conditioning in the house, male sex, whether food was carried outside, and whether the house was constructed before 1950.

When environmental media measurements were added to the model and type of study site was removed, all of the previous variables were retained as significant predictors of blood lead (Table 22). Of the environmental measurement variables entered in the model, soil and dust were statistically significant predictors of blood lead. In the resulting model, almost nine percent (8.8%) of additional blood lead variability was accounted for, as the adjusted r^2 increased from .174 to .262.

In the logistic model that was constructed from the reduced linear model, the dependent variable, blood lead, was dichotomized at 10 μg/dl (Table 23). In this model the type of study site [smelting versus mixed mining/smelting] was the exposure variable. The covariates that were significantly associated with the dependent variable ($p \leq 0.05$) were retained in the model as shown.

Similar to the linear regression model, type of study site was not significantly associated with blood lead equal to or greater than 10 μg/dl (OR = 1.07, 95% CI = 1.41-5.12). ORs were statistically significant for the following variables: head of household with a high school education or less, lack of air conditioning in the house, male sex, whether food was carried outside, and living in a house built after 1950 (Table 23).

An additional regression model was constructed by adding environmental media measurements for lead to the reduced logistic model after removing type of study site (Table 24). Environmental variables were dichotomized at the following values: yard soil and house dust 500 mg/kg; drinking water 15 μg/L; and interior paint 0.6 mg/cm^2. Of the environmental variables entered in the model, only yard soil greater than 500 mg/kg was associated with increased blood lead greater than 10 μg/dl (OR = 1.80, 95% CI = 1.35-3.23).

Children 6 Years To 14 Years

Target versus Comparison

Univariate—The percentages of children with blood lead levels less than 10 μg/dl living in target and comparison areas were 97.1% and 94.2%, respectively. The highest blood lead levels were 25.9 μg/dl, and 21.9 μg/dl among target and comparison area participants, respectively.

Bivariate—The mean blood lead level in the target areas (3.49 μg/dl) was higher than in the comparison areas (2.88 μg/dl) ($p<0.01$). Two individual behavior variables were associated with elevated blood lead levels. Children who reported sucking thumb riding dirt bikes or having a family member who road dirt bikes in the vicinity of the site had higher blood lead levels than children who did not report these behaviors. Other variables associated with elevated blood lead ($p<0.01$) included: household income less than $15,000, lack of air conditioning in the house, male sex, and living in a house constructed before 1950 (Table 25).

Multivariate—Linear regression models were constructed using log blood lead level as the dependent variable (Table 26). In this age group, residing in the target area was not a significant predictor for blood lead level, ($p=0.467$). Variables significantly associated with higher blood lead, ($p<0.01$) included: household income less than $15,000, male sex, living in a household with someone who smoked tobacco, and sucking thumb. Air conditioning in the house; increasing age (continuous variable); and living in a house constructed after 1950 were associated with a lower mean blood lead level.

In the linear regression model that was constructed, environmental measurement variables were added and area of residence was excluded (Table 27). Variables that were significant in predicting mean blood lead levels included: household income less than $15,000; age as a continuous variable; living with someone who smoked in the household; soil and dust lead concentrations. Variables that were retained in this model accounted for about 17 % of the variance of blood lead observed in this group ($r^2=.171$). Lead concentration in house dust was associated with lower blood lead levels in this age group.

Of the 642 participants in this age group for whom a blood lead was obtained, only 27 [14 in the target areas and 9 in the comparison areas] had blood lead levels 10 μg/dl or higher. For this reason, contingency tables and ORs were not calculated and logistic modeling was not indicated.

Smelting versus mixed mining/smelting (All Target)

Univariate—The distributions of blood lead levels less than 10 μg/dl among participants in both types of study sites were similar. The highest blood lead levels recorded were 25.9 μg/dl in the smelting areas and 18.9 μg/dl in the mixed areas.

Bivariate—The mean blood lead level in the smelting areas (3.71 μg/dl) was higher than in the mixed areas (3.13 μg/dl) in this age group, ($p<0.01$). Variables associated with elevated blood lead ($p<0.01$) included: annual household income less than $15,000, male sex, living in a house constructed before 1950, and lack of air conditioning in the house (Table 28).

Multivariate—A linear regression model was constructed with blood lead as the dependent variable and type of study site as the exposure variable (Table 29). In this model, type of study site was not a significant predictor for blood lead after controlling for other confounders. When environmental media measurements of lead concentration were added to the model, only yard soil and house dust were retained as significant predictors of blood lead (Table 30). About one percent more of the blood lead variability was accounted by this model as indicated by an increase of the adjusted r^2 from .171 to .180. Contingency tables and ORs were not calculated because of the small number of participants (14) in this age group with blood lead levels equal to or greater than 10 µg/dl.

Adults Age 15 Years to 75 Years

Target versus Comparison

Univariate—Fewer than 2% of adult participants exceeded the blood lead reference value for non-occupational exposure [10-25 µg/dl] established by the 1980 WHO Study Group for non-occupational exposure. Less than one percent (0.7%) of adult participants residing in the target area and (1.4%) in the comparison area exceeded this value. The highest blood lead levels were 33.9 µg/dl in the target areas, and 37.9 µg/dl in the comparison areas. The geometric mean blood lead level among target area participants (3.06 µg/dl) was lower than among comparison area participants (3.63 µg/dl, $p<0.01$).

Bivariate—Variables that were significantly associated with blood lead level ($p<0.01$) included: male sex; living in a house with someone who smokes tobacco; having air conditioning in the household; and living in a house constructed before 1950 (Table 31).

Multivariate—A linear regression model was constructed to evaluate the relationships between area of residence [target versus comparison] and blood lead level while adjusting for other factors that might also influence blood lead levels (Table 32). Variables associated with blood lead in adults included: residing in target areas at the Illinois or Pennsylvania study sites, being male, and being a current smoker of tobacco. In this age group, residing in the target area was not significantly associated with blood lead levels ($p=0.9$).

When area of residence was deleted and environmental measurements were added to the model (Table 33), the adjusted r^2 decreased from 0.388 to 0.299. Similar to previous models, interaction and confounding was assessed and non-significant terms ($p > 0.05$) were removed. Significant predictors of blood lead in this age group were: male sex and age, $p<0.01$. Of the environmental variables evaluated, house dust was the only statistically significant environmental predictor of blood lead.

Contingency tables and ORs were not calculated and logistic regression was not done because of the small number (25) of participants with blood lead levels greater than 10 µg/dl. Of these, 15 resided in the target areas and 10 in the comparison areas.

Smelting versus Mining/smelting (All Tqrget)

Univariate—Ninety four percent (94%) of smelting area participants, and 97.8% of mixed mining/smelting area participants had blood lead levels below 25 µg/dl. The highest blood lead level was 33.9 µg/dl. The mean blood lead among smelting area participants was 3.25 µg/dl compared to 2.64 µg/dl mixed area participants ($p=0.017$).

Bivariate—Variables that were significantly associated with blood lead ($p<0.01$) included: male sex; living in a house that was constructed before 1950; living in a house with someone who smoked tobacco; and lack of air conditioning in the house (Table 34).

Multivariate—The linear regression model (Table 35) illustrated that residing in smelter areas was a significant predictor of blood lead, $p=0.014$. Other variables found to be significant predictors of blood lead were: living in a house that lacked air conditioning, male sex, and currently smokes tobacco. When environmental media measurements were added to the model and type of study site was excluded (Table 36), an additional one percent of blood lead variability was accounted for [adjusted $r^2=0.279$ compared to $r^2=0.284$]. Of the environmental measurements added to the model, dust lead concentration was the only significant predictor of blood lead, $p=0.016$.

SECTION III

Environmental Cadmium Concentration

There was a statistically significant difference in mean soil cadmium concentration between target and comparison areas, 6.9 mg/kg, and 3.25 mg/kg, respectively, ($p=0.15$), (Table 37). Dust concentration of cadmium was also significantly different in the target (9.87 mg/kg) and comparison areas (5.00 mg/kg) ($p<0.01$).

The mean cadmium concentrations for soil, dust, and drinking water among different type of study areas were significantly different, ($p<0.01$). Notably, the cadmium concentration in drinking water in smelting areas (1.84 µg/L) was about six times higher than that measured in mixed areas (0.30 µg/L), ($p<0.01$). However, when correlation coefficients were calculated using adjusted urine cadmium excretion and cadmium concentrations measured in soil, water, and dust (Table 38), there was only a weak correlation with drinking water.

Urine Cadmium Analysis

None of the study participants exceeded the World Health Organization (WHO) occupational reference value for urine cadmium excretion of 5 μg/g creatinine (Tables 39 and 40). Of 1,333 urine specimens obtained from target area participants, 593 (44.5%) were below detection limits compared to 227 of 409 (55.5%), of the specimens from comparison area participants. The percentage of urine cadmium specimens below detection limits was less among smelting area participants (34%) than among mixed mining/smelting area participants (62%) (Table 41 and Figure 7).

Children Age 6 Months Through 71 Months

Target versus Comparison Areas

Univariate—The percentages of children under six years of age with urine cadmium excretion less than or equal to 1 μg/g creatinine who lived in target and comparison areas were about the same, 94.5% and 98.7%, respectively. The highest level observed in the target areas was 4.4 μg/g creatinine, compared to 1.4 μg/g creatinine in a young child in the comparison areas.

After inspecting urine cadmium values over a wide range of urine dilutions, unadjusted and creatinine-adjusted, it was apparent that creatinine-adjusted urine cadmium measured in dilute urine specimens [urine creatinine < 25 mg/dl] produced inconsistent and unreliable results. For this reason, urine specimens with creatinine levels less than 25 mg/dl were excluded from further data analysis.

Bivariate—Mean cadmium excretion among target area participants was twice as high (0.19 μg/g creatinine), compared to (0.10 μg/g creatinine) comparison area participants, ($p<0.01$). Being a resident in one of the target areas was associated with urine cadmium above the detection limit (OR = 3.4, 95% CI = 2.3-5.0). Of the behavioral variables for which ORs were calculated, two were significantly associated with detectable urine cadmium: carrying food outside (OR = 1.60, 95% CI = 1.2-2.1) and residing in a household with someone who smoked tobacco (OR = 1.61, 95% CI = 1.2-2.1) (Table 42).

Multivariate—Linear regression models were not created because of the high percentage of urine cadmium levels below the detection limit (< 0.1 μg/L), 44.5% and 55.5% among target and comparison area participants, respectively. A logistic regression model was used to further assess the association between urine cadmium excretion and area of residence while controlling for confounders. Urine cadmium was dichotomized at the detection limit [0.1 μg/L] and used as the outcome variable in the model because of the large percent of urine specimens below the limit of detection. To minimize the impact of the large number of dilute urine specimens in this group, a term for urine creatinine level ≥ 25 mg/dl was included in the model (Table 43). None of the behavioral variables was retained. Study participants residing in target areas at smelting sites were more likely to have detectable urine cadmium than those who lived in target areas at mixed mining/smelting sites. However, this observation may not be clinically relevant because more than 96% of all participants at target sites had urine cadmium excretion at 1 μg/g creatinine or less in this age group.

When environmental measurements were modeled using logistic regression, cadmium concentration in tap water, dichotomized at 5 μg/L, did not show a significant association (p=0.075) (Tables 43 and 44). Although this cut point represented the action level for cadmium in drinking water determined by USEPA, the confidence interval (95% CI = 0.67-95.7) indicated a wide range of variability.

Smelting versus mining/smelting (All Target)

Univariate—About forty eight percent (47.8%) of children in the smelting areas had urine cadmium levels below the limit of detection (i.e., < 0.1 μg/g creatinine) compared to 65.3% of children from the mixed mining area. The mean cadmium excretion (0.31 μg/g creatinine) was about three times higher among smelting area participants than among mixed area participants (0.09 μg/g creatinine), (p<0.01).

Being a resident in one of the smelting area sites [Illinois] was associated with urine cadmium above the detection limit (OR = 7.4, 95% CI = 5.3-10.5). Of the behavioral variables for which ORs were calculated, living in a household with someone who smoked tobacco and carrying food outside were significantly associated with detectable urine cadmium (Table 45).

Children Age 6 Years To 14 Years

Target versus Comparison Areas

Univariate—Among older children, 98.6% had urine cadmium excretion less than or equal to 1 μg/g creatinine in the target areas, compared to 100% in the comparison areas. The highest urine cadmium excretion observed in the target areas was 2.2 μg/g creatinine, compared to 1.0 μg/g creatinine in the comparison areas.

Bivariate—The geometric mean urine cadmium excretion (Table 46) was higher in the target areas (0.13 μg/g creatinine) than among comparison area participants (0.08 μg/g creatinine), $p<0.01$). Variables that were significantly associated ($p<0.01$) with detectable urine cadmium included: living in the target area; household income less than $15,000; dirt bike riding; eating home grown vegatables; and living in a house constructed before 1950 (Table 47).

Living in one of the target areas was the only variable associated with urine cadmium above the detection limit (OR = 3.29 95% CI = 2.3-4.8).

Multivariate—A logistic regression model was constructed that included area of residence and other variables found to be associated with cadmium excretion from the bivariate analysis. The dependent variable for the model was urine cadmium dichotomized at the detection limit (0.1 μg/L). Residing in one of the target areas was not significantly associated with detectable urine cadmium (OR = 1.20, 95% CI = 0.76-1.88). A stronger association with detectable urine cadmium was observed at the Pennsylvania study site (OR = 2.09, 95% CI 1.35-3.24), and the strongest association was observed at the Illinois study site (OR = 39.31, 95% CI = 21.4-71.9), $p<0.01$ (Table 47).

When environmental variables were entered into a logistic model, none were significantly associated with an increased odds for detecting cadmium in the urine. This observation suggested that excessive cadmium exposure related to environmental sources examined in this study was not a high probability in this age group.

Smelting versus mixed mining/smelting (All Target)

Univariate—The percentage of older children with cadmium excretion less than or equal to 1 μg/g creatinine was similar at both types of study sites, 98.8% in the smelting, and 100% mixed. The highest cadmium excretion observed in the smelting areas was 2.2 μg/g creatinine compared to 0.6 μg/g creatinine in the mixed areas. The geometric mean cadmium excretion observed in smelting areas was more than twice as high (0.19 μg/g creatinine), as in mixed areas (0.07 μg/g creatinine) ($p<0.01$).

Bivariate—Variables that were significantly associated with higher urine cadmium excretion in this age group included: annual household income greater than $15,000, and living in a house that was constructed before 1950 (Table 48).

Multivariate—Multivariate linear modeling of questionnaire and environmental data did not show any statistically significant associations. A logistic regression model (Table 49) was used to further evaluate urine cadmium excretion observed in this age group. Urine cadmium was dichotomized at the detection limit and used as the outcome variable in the model. In this model, none of the behavioral variables was found to be statistically significant. Living in one of the smelter areas was the only variable associated with detectable urine cadmium (OR = 9.61, 95% CI = 6.23-14.8).

Adults Age 15 Years To 75 Years

Target versus Comparison Areas

Univariate— Among adults the mean urine cadmium excretion was higher in the target areas than in the comparison areas, 0.28 and 0.18 μg/g creatinine, respectively, ($p<0.01$). None of the adults had urine cadmium excretion above the WHO occupational reference value (5 μg/g creatinine) *(36)*. Ninety percent (90%) of adults in the target areas had urine cadmium excretion ≤ 1 μg/g creatinine compared to 95.5% in the comparison areas. The highest excretion measured in was 3.0 μg/g creatinine in the target areas, and 2.8 μg/g creatinine in the comparison areas.

Bivariate—Variables that were found to be significant at $p\leq0.05$ level included: area of residence, household income; living in a house constructed before 1950; and living in a household with someone who smoked tobacco (Table 50). Each variable was included initially in the multivariate analysis.

Multivariate—The linear regression model that was constructed (Table 51) accounted for approximately 33% of the urine cadmium variance observed in this age group. Of the variables entered in the model, living in one of the target areas or in the Illinois study area, being a current smoker of tobacco, age, and household income less than $15,000 were significantly associated with urine cadmium excretion, ($p\leq 0.05$).

The logistic regression model (Table 52) was constructed using 0.5 μg/g creatinine as a cutpoint for urine cadmium excretion. This value represented about half the average urine cadmium excretion expected in populations without occupationally related cadmium exposure *(37,38)*. Significant predictors of urine cadmium excretion included: living in one of the target areas (OR = 2.66, 95% CI = 0.76-1.97); living in the Illinois study area (OR = 2.79, 95% CI =17.9-59.8); being a current smoker of tobacco (OR = 2.14; 95% 1.17-2.91); and age as a continuous variable, ($p<0.01$).

Smelting versus mixed mining/smelting (All Target)

Univariate—Ninety two percent (92.1%) of smelting area participants had urine cadmium concentration of 1 μg/g creatinine or less compared to 97.7% in mixed mining/smelting area participants. The highest urine cadmium excretion measured was 3.0 μg/g creatinine in the smelting areas and 2.2 μg/g creatinine in the mixed areas. The mean urine cadmium excretion was significantly higher in the smelting areas (0.33 μg/g creatinine) than in the mixed areas, (0.18 μg/g creatinine, $p<0.01$).

Bivariate—Variables that were significantly associated with higher urine cadmium excretion in adults included: household income greater than $15,000, house constructed before 1950, and being a current smoker of tobacco (Table 53).

Multivariate—Linear models were constructed using urine cadmium excretion as the dependent variable. Variables of interest and those found to be significant at the $p\leq0.05$ from the preceding analysis were initially included as independent variables in the model. Variables of age and sex were also included. Although living in one of the smelter areas and being a current smoker of tobacco were significant, p<0,01, only 10 % of the variance in urine cadmium excretion was accounted for in the model (Table 54). When environmental measurements were added, and type of study site was excluded no variables showed a positive association with urine cadmium excretion. House dust cadmium showed an inverse association, $p=0.027$ (Table 55).

SECTION IV

Biomedical Tests Analysis

Biomarkers of Kidney Dysfunction

Univariate/Bivariate—The medical test battery results were generally within the 95th percentiles in target and comparison area participants across all age groups (Tables 55-58).

Creatinine—Ninety nine percent of participants in target and comparison areas had creatinine values less than 0.8 mg/dl. This value was slightly less than half the upper limit of normal (1.5 mg/dl). Although the mean serum creatinine among children age 6-71 months old was higher in target areas ($p=0.015$) than in comparison areas (Table 55), this relationship did not persist when further evaluated using multiple linear regression, controlling for age and sex.

Blood Urea Nitrogen (BUN)—Mean BUN was generally higher in the comparison areas and among young children 6-71 months of age and adults (Table 56). However, 99% and 95% of target and comparison area participants, respectively had values of 23 mg/dl or lower, [upper limit of normal 26 mg/dl].

The difference in mean urine Alanine aminopeptidase (UAAP) and N-Acetyl-β-D-Glucosaminidase (UNAG) among participants in target and comparison areas across age groups was not statistically significant (57-58).

The mean urine Gamma glutamyl transferase (UGGT) in adult participants 15-75 years of age was higher ($p=0.034$) in the target areas than in the comparison areas, (Table 59). However, when this relationship was evaluated further using multivariate regression models and controlling for age, sex, smoking and alcohol use, neither area of residence, blood lead level, or urine cadmium excretion were predictive for UGTT.

Multivariate—Models were constructed using area of residence, blood lead, urine cadmium as independent variables and serum creatinine, BUN, UAAP, UGTT, and UNAG as dependent variables. No significant associations were demonstrated. Multivariate models were constructed using each exposure (independent) variable and each kidney biomarker (dependent) variable; but no statistically significant associations were demonstrated.

Biomarkers of Hematopoietic Dysfunction

More than 95% of the hematopoietic indicators that were evaluated in participants living in target and comparison areas were within the established normal ranges. However, significantly different mean values among age groups were observed (Tables 61-65). Five records were excluded from the analysis because the data could not be verified.

Hematocrit (HCT)—Univariate/Bivariate—Ninety five percent (95%) of participants in the target and 99% in the comparison areas had hematocrit values within the normal ranges. The mean hematocrit was significantly higher overall among comparison area participants and among children 6 to 14 years of age, $p<0.01$. There was no difference observed in other age groups between target and comparison areas.

Multivariate—Multivariate models were constructed to evaluate the relationship between exposure variables [blood lead, urine cadmium, and area of residence] and the dependent variable

HCT. In each model, age and sex differences were controlled for in children. Additionally, smoke-years and drink-years were controlled for in models constructed to evaluate adults. Associations were not consistent across age groups for any of the exposure variables that were examined.

Among children 6-14 years old living in one of the target areas was weakly associated with a lower HCT ($p=0.058$, $r^2=0.22$ $n=651$). A 0.1% decrease in HCT was predicted for each $\mu g/dl$ increase in blood lead. An association between urine cadmium excretion was also demonstrated in this group. In the model constructed to evaluate this relationship, a 4.6% decrease in HCT was predicted for each $\mu g/g$ creatinine increase in urine cadmium ($p=0.03$, $r^2=0.25$, $N=456$). The model constructed to evaluate the relationship between cadmium and HCT in adults 15 years and older produced opposite results. More than a two percent (2.9%) increase in HCT for each $\mu g/dl$ increase in blood lead level ($p=0.033$, $R_2=0.31$, $n = 282$). In the model where blood lead was the independent variable, a 0.3% increase in HCT for each $\mu g/dl$ increase in blood lead was predicted ($p=0.027$, $r^2=0.28$, $n=381$) in this age group.

Hemoglobin (HGB)—Univariate/Bivariate—Ninety percent (90%) of target area participants' hemoglobin values were within the normal range compared to 95% in the comparison areas. The mean hemoglobin was significantly lower among target area participants and specifically in children 6-14 years of age (Table 62). However, this initial relationship was not confirmed upon further evaluation using linear regression to control for difference in age and sex.

Mean Corpuscular Volume (MCV) and (MCHC)—Univariate/ Bivariate—Ninety percent (90%) of target and comparison area participants had MCV values within the normal range. Children 6-14 years old who lived in the target areas (Table 63) had a mean MCV slightly higher than children in the comparison areas, K-S test $p<0.03$. Bivariate analysis produced similar results for MCH and MCHC (Tables 64 and 65).

When the difference in MCV initially observed in children 6-14 years old was examined further using multivariate models and controlled for difference in age and sex, this relationship did not persist. None of the models demonstrated significant associations between area of residence, blood lead level, urine cadmium concentration, MCH, or MCV in any other age group.

Multivariate—Multiple linear regression models were constructed using as independent exposure variables for each age group: area of residence, blood lead, and urine cadmium for five hematopoietic outcome variables to further evaluate the relationship of outcome and exposure variables. Where appropriate age, sex, smoking, and alcohol use were held constant. The resulting associations were inconsistent across age groups. For example, the model constructed to evaluate area of residence and MCHC in 15 years old and older target area participants (p<0.003, N=382, adjusted r^2=0.30), predicted a 1.6% increase in MCHC for each µg/dl in blood lead increase. When a similar model was constructed where blood lead was the independent variable, a 0.2% decrease in MCHC was predicted for each µg/dl increase in blood lead (p=0.003, N=381, adjusted r^2=0.28). Age, sex, pack-years smoked, and drink-years were included in both models and adjusted for.

Biomarkers of Immune Dysfunction

Immunoglobulins—Univariate/Bivariate—The mean IgG level was lower overall in target area participants, but 99% of participants in the target and comparison areas were within the normal range (Table 66). The mean IgA level was lower overall in target area participants, but it was higher among target area children 6-71 months of age (Table 67) (KS test p=0.029). There was no difference in mean IgM levels between target and comparison area participants (Table 68).

Multivariate—The multivariate models that were developed to further evaluate the relationship between blood lead, urine cadmium excretion, and area of residence and immunoglobulin levels showed a significant association between blood lead and serum IgA levels among children 6-71 months of age p=0.005, (adjusted r^2=0.26, n=930). The model also predicted a one percent increase in IgA for each µg/dl increase in blood lead. Similar results were obtained when area of residence was the independent variable of interest and IgA was the dependent variable (p=0.058, adjusted r^2=0.26, n=934). While the model predicted an 8% increase in IgA for each µg/dl increase in blood lead, the level of statistical significance was borderline. No other exposure variable was significantly associated with this parameter of immune function in any of the other age groups.

Lymphocyte Count And Phenotypes—Univariate/Bivariate—The 5th and 95th percentile total lymphocyte counts in target area participants were 1,700 and 6,600 cells per cubic millimeter (mm^{3}), compared to 1,674 mm^3 and 6,200 mm^3 in comparison area participants. The mean total lymphocyte count in target area participants was higher than in comparison area participants overall p<.01, and in two age groups (6-14 years p<0.01 and 15-75 years p=0.019), (Table 69).

The mean CD4 count in target area participants (Table 70) was higher overall ($p<0.01$), and in adults age 15-75 years old, ($p<0.04$) than in comparison area participants. The CD4/CD8 ratio was not significantly different in target and comparison area participants in any age group (Table 71).

Multivariate—Multivariate models were developed to further evaluate the relationship between exposure variables (i.e., area of residence, blood lead, and urine cadmium) total lymphocyte count and lymphocyte phenotypes. None of the models showed either of the exposure variables [lead or cadmium] to be predictive of, or significantly associated with biomarkers of immune function evaluated in the present study.

Biomarkers of Liver Dysfunction—Univariate/Bivariate—Over 95% of test values in target and comparison participants were within the range of normal. GGT was slightly higher (Table 72) in children age 6 to 71 months in target areas than in comparison areas ($p<0.01$). There was no significant difference demonstrated in other age groups. Associations that were observed initially between area of residence and increased GGT and SGOT (Table 73) did not persist upon further analysis that controlled for age and sex. Target area children 6-71 months old (Table 74) had lower mean serum albumin than comparison area children ($p=0.04$). No such difference was observed in the two other age groups (Tables 74 and 75).

DISCUSSION

Rationale

Each of the four studies encompassed by the present multisite investigation report was undertaken to evaluate lead and cadmium exposure in residents living near a National Priority List (NPL) site. The presence of elevated lead and cadmium concentrations in residential soil, the comparable pathways of exposure, and the desire for a sufficient size study population were primary considerations in determining to combined data from the four sites for multisite analysis.

Clinically overt effects have been documented in workers following chronic exposure to high concentrations of lead and cadmium *(6,9,13,16,17)*. However, it is uncertain whether subtle organ-system defects related to low-level environmental exposure to these contaminants can, with sustained exposure, progress to more severe organ dysfunction and adverse health effects *(9,20,21)*. The primary public health concern examined in the present study was whether nonoccupational residential exposure to lead and cadmium contaminated media (soil, indoor house dust, tapwater, and interior house paint) significantly influenced levels of these contaminants in blood and urine, and whether concomitant changes in selected organ-system function could be detected.

Limitation of Study Design

Several issues that were unique to the present study, limit the investigators' interpretations of study results. These included: variation in sample size at individual study sites; disparity in socio-economic status at individual study sites between target and comparison area participants; lack of a comparison population for each study site; incomplete characterization of lead sources and species; the proportion of paint XRF measurements (10%) reported at less than the detection limit; the lack of identical strategies used in selecting households for environmental sampling among individual studies; and lack of knowledge base needed to accurately interpret biomedical test results.

Biomarker Interpretation

Although biochemical, molecular, genetic, and immunologic indicators are increasingly being used to evaluate health risks associated with environmental exposure and represents an advancing area of interest in epidemiologic research, the biomedical tests used in this study have not been validated for use in this capacity (*13,39,40,41*). Without detailed medical histories and review of systems, none of the biomedical test results can be considered specific for disease, pathological condition, or disease etiology. Instead, the biomedical tests as they were used in this study assisted investigators to identify early patterns of organ-system dysfunction and characterize the overall health status of study participants. Predictably, many of the biomedical tests showed considerable overlap between exposed and unexposed populations. Therefore, attributing abnormal biomedical tests results to environmental lead and/or cadmium exposure cannot be precise although these associations, when observed, were examined in the study.

Existing gaps in information concerning the practical and appropriate use of biomedical tests in environmental epidemiologic investigations provided much of the motivation for the multisite approach (*42,43,44*). The goal was to be able to advise communities located near hazardous waste sites whether or not their test results demonstrate differences in function in major organ systems. The ability to interpret results in this context was critical and required normative data to evaluate health outcome variables (*13,40,41,45)*). Such data were available for many of the medical tests used in this study. For other tests, investigations of comparable size would be necessary to assemble the requisite knowledge base.

The ability to accurately interpret relationships between exposure biomarkers [blood lead and urine cadmium] and biomarkers of effect [biomedical test batteries] is limited. Although body fluid measurement of lead and cadmium document exposure to these contaminants and reduced the potential for exposure misclassification, it does not reflect total body burden. For example, blood lead, and to a lesser extent urine cadmium measurements, do not accurately reflect concentrations in target tissues (eg., bone, liver, and kidney). Measures of blood lead and urine cadmium obtained at a single point in time indicate exposure that may have occurred over an indefinite time period. For this reason, precise interpretation of associations between abnormal biomedical test results, specific sources of lead and cadmium, and body fluid measures of these contaminants was not possible.

Although test values for presumably nonexposed comparison populations were used in this study, it was not clear whether this was adequate for distinguishing reliable endpoint values to define effects and non-effects of exposure. For example, the magnitude of variability in individual immune responses that may occur unrelated to exposure is uncertain, and it is conceivable that any response observed may be influenced by numerous known confounders and others factors that may not yet be recognized (*41,46*). The ability to interpret biological marker results from a health/risk context will probably be possible only after carefully conducted longitudinal epidemiologic studies, where exposure estimation is combined with standardized laboratory procedures that accurately measure biological indicators of exposure and effect endpoints. Studies that include multiple data sources, as described here, can be performed following careful planning and consideration of logistic issues. Such studies represents a practical approach to identifying and addressing many important issues necessary for more reliable use and interpretation of biological markers in epidemiologic investigations.

Potential Sources of Bias

There were minor variations in household selection criteria for environmental sampling. Stratified sampling was used to select households for environmental sampling at the Kansas and Missouri study sites. Both strata consisted of randomly selected households where at least one occupant was less than six years of age. The second strata consisted of households in which one occupant was less than six years of age and also had blood lead values above 10 $\mu g/dl$. Random sampling was used at the Illinois study site among households where at least one occupant was less than six years of age. Random sampling was used to select households among eligible participants at the Pennsylvania site. However, the impact on study conclusions were probably negligible because all inferences were based on objective measures of contaminants found in blood, urine, or environmental media related to a pool of participants that were each randomly selected to participate in the studies initially.

Study Strengths

Multisite Design

The advantages of using a multisite study design to conduct this study included: achieving a large sample size, increased diversity among study groups, preservation of time and resources developing and using a single study protocol, the same laboratories, and field support. Use of a standardized questionnaire combined with uniform biological and systematic environmental specimen collection and handling procedures helped to reduce inter-study variability, informational bias, and increased data comparability.

Comparison Population

Comparison communities were used as reference for background level estimates for all of the tests performed in the study. Comparison areas selected were suitable as a reference for target areas based on similar measures of socio-economic status, age distribution, race, and age of housing stock.

Contaminant Measurements

Another important strength of this study included the use of measured contaminants in body fluids and four environmental media to estimate exposure risk and document the magnitude of current exposure. Environmental sampling of participants' residences was conducted to approximate exposure potential to heavy metals rather than using distance from each site as a proxy measure of exposure. Using concentrations of lead and cadmium in various media provided a more reliable estimate of current exposure potential. In addition, assessment of possible effect on the kidney, the major target organ system, was correlated with lead and cadmium concentrations found in body fluids and environmental media.

This was ATSDR's first field implementation of biomarkers in an epidemiologic health investigation, and possibly the largest such study where lymphocyte phenotyping using flow cytometry methods were employed. The study provides population-based evidence that concurrent biological measures of exposure may be an important adjunct when assessing low-level exposure in populations at risk. It also provides evidence that current biological measures of exposure, in addition to environmental measures of contaminants, may be useful in designing and implementing cost effective strategies to prevent excess exposure to lead and cadmium contaminated soil, dust, and other environmental media.

Laboratory Variability

Measures were implemented to control interlaboratory variability and maintain specimen integrity throughout collection and laboratory analysis. The Centers for Disease Control and Prevention laboratory performed all metal analyses and biomedical tests requiring specialized procedures. More routine biomedical analysis was performed by MRI. All laboratory specimen collection and analysis followed strict standards to ensure accuracy of results. Blood and urine specimens were analyzed using graphite furnace atomic absorption methods to accurately measure lead and cadmium. Quality control methods included the use of collection tubes pre-screened for lead and cadmium, testing field blanks, running duplicate quality control specimens, and repeating tests on values of urine cadmium of higher than two $\mu g/g$ creatinine and confirming serum cadmium to rule out contamination and repeat of blood lead values higher than 10 $\mu g/dl$.

Interpretation

Exposure to Lead

Blood lead levels measured in humans may be influenced by multiple exposure sources. The primary sources of lead considered in this study were: yard soil, house dust, tap water, and interior paint. The contribution of lead uptake following exposure to environmental sources to overall blood lead level has been estimated by other investigators and has been modeled considerably by USEPA. Soil and house dust lead concentration are considered to have an important influence on blood lead levels, particularly in young children who put hands, toys, and other soiled objects in their mouths. Variables affecting lead uptake following contact with contaminated soil and other environmental media include: soil lead concentration, particle size, chemical form of lead, as well as the presence of zinc, calcium, and other trace elements *(11,47)*. The estimated contribution to blood lead levels by different pathways and sources varied among study participants depending on how area of residence was defined [i.e., Target vs Comparison determined by proximity to point source or Smelting vs mining based on historical manufacturing processes associated with lead and deposition].

The present study combined data from four study sites to evaluate the association of different environmental lead sources with mean blood lead levels. Regression analysis was used to evaluate the relationship between blood lead and several independent variables including area of residence, behavioral and socio-economic characteristics, and concentration of lead present in four environmental media. The mean blood lead in young children [age 6-71 months] residing in the target areas was higher than in comparison areas. Although the mean for both areas was well below 10 µg/dl, mean blood lead was positively correlated with several behavioral and demographic variables. Twenty three percent of the variance in blood lead level was accounted for by: area of residence, annual income less than $15,000, education less than high school, presence/absence of air conditioning, male sex, between 1-2 years of age, and residing in a household where there is a smoker, and house built before 1950. An additional two percent of the variance was accounted for when environmental concentrations of lead in soil and interior house dust were considered.

When children 6-71 months old were considered based on the primary manufacturing activity associated with lead emission in the study area [i.e., snmelting versus mixed mining/smelting], similar results were obtained. Behavioral and demographic factors accounted for about 17.6 % of the variance in blood lead level. When environmental factors were considered, and area of residence was excluded, an additional 8.6 % of variance was accounted for. This suggested that mean blood lead level was influenced somewhat more by environmental lead sources in young children when area of residence was defined by historical lead emission related to smelting and mining/smelting activities rather than by proximity to the lead source [target versus comparison] as used in the current study. However, the implication of this observation is less certain because differences in lead species, conditions of painted surfaces, and the presence of ground cover on residential soil were not evaluated (47). Although about 26% of blood lead variance was accounted for during data analyses, factors that seemed to influence blood lead levels in young children did not change substantially whether or not study areas were classified by proximity to a lead source [target versus comparison] or by differences in manufacturing activities associated with lead emission [smelting versus mining/smelting].

Among older children, 6 to 14 years of age in target and comparison areas, similar exposure and behavioral variables accounted for about 24% of variance in blood lead level. When environmental measures were considered, only soil and dust lead variables were retained in the model, but decreased the variance of blood level accounted for to 17%. This suggest that factors other than the contaminated environmental media evaluated during this study had more influence on mean blood lead level in this age group. Results obtained for adults were similar.

Among children age 6 to 14 years in smelting and mining/smelting areas, 17% of variance in blood lead level was accounted for by the behavioral and environmental factors evaluated. When environmental media concentrations of lead were considered, 18% of the variance in blood lead level was accounted for. This suggested that environmental lead concentration in the four environmental media evaluated in this study was a minor influence on blood lead levels in this age group. The mean blood lead was influenced to a similar degree without regard to whether area of residence was defined by proximity to the lead source [target versus comparison study area] or by historical smelting or mining/smelting activities. This observation suggest that the definition of smelting versus mining/smelting based on historical sources of lead deposition may be an imprecise characterization of the sites studied.

Behaviors such as smoking appeared to be more important predictors of blood lead in adults than lead concentrations found in the environmental media examined in this study.

Exposure to Cadmium

Foods are an important source of cadmium for the general population *(37,48)*. The average dietary intake of cadmium is about 30 µg/day. Typical levels of cadmium present in food, water, and ambient air do not represent a health threat for the general population. However, because cadmium accumulates in the kidney, liver, and other tissues, long-term exposure poses a concern. Cadmium is eliminated from the body primarily in urine. With normal renal function, urinary cadmium most closely reflects body burden while blood concentration reflect recent exposure. The contribution of environmental sources on total body burden is not entirely clear.

Because cadmium bio-accumulates over long-term exposure, it was not surprising to find a high percentage of urine specimens

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were below the detection limit (< 0.1 µg/g creatinine) for cadmium (target area 54% and comparison area 79%) among young children 6 to 71 months old.

The mean urine cadmium level in young children (age 6-71 months) was significantly higher in target area residents than in comparison area residents. Mean urine cadmium in young children was not significantly associated with any personal behavioral variables. The OR in young children age 6-71 months old residing at the Illinois study site having detectable cadmium in urine was about 18. This observation cannot be explained by substantially different cadmium levels measured in environmental media or urine cadmium excretion levels among participants at the Illinois study site.

Among older children, 6 to 14 years old, several behavioral and economic variables were statistically significant ($p<0.01$) while none of the environmental measures for cadmium showed significance. Thus the influence of environmental cadmium on urine cadmium concentration in this age group appeared to be less important than personal behaviors and soci-economic factors.

In adults, 15 years and older; age, smoking, and area of residence were found to be important predictors of urine cadmium. These variables accounted for 32% of cadmium variance in this group.

Organ-System Dysfunction

Biomarkers of Kidney Dysfunction—Kidney dysfunction characterized by proteinuria and enzymuria associated with increased urine cadmium excretion have been demonstrated in several studies *(9,17,21,22,36)*. However, the mean level of urine cadmium excretion where such observations were made occurred at urine cadmium excretion levels more than ten times greater than that measured in the present study. Further, in most of the studies where kidney dysfunction was associated with elevated urine cadmium, duration of exposure appeared to be an important factor *(9,17,49)*. On average, the duration of exposure for study participants was 10-15 years in a variety of occupational settings. In the present study, no association was demonstrated between urine cadmium and kidney dysfunction, as indicated by renal biomedical tests that were used. The present study was comprised largely (48%) 1017 of young children less than 6 years of age and less than 50% of these young participants had urine cadmium levels above the detection limit. The duration and level of exposure for many participants in this study may not have been sufficient to detect early changes of kidney dysfunction that have been demonstrated in other studies *(50)*.

Alternatively, the biomarkers used to evaluate kidney dysfunction may not be sensitive enough for detection of early renal impairment at the urine cadmium levels experienced by the population under study. While the former proposition is more likely, the latter is also reasonable given that sensitivity, specificity, predictive values, and reference ranges for the tubular enzymes that were used in this study to indicate early renal dysfunction has not been firmly established in pediatric populations.

Biomarkers of Hematopoietic Dysfunction—Previous studies have demonstrated correlations between blood lead levels, iron levels, and changes in red cell morphology that are linked to inhibition of heme biosynthesis and decreased life span of circulating red blood cells in addition to changes in certain immune function parameters *(3,6,8,36,51,52)*. Multivariate models constructed to evaluate the relationship between exposure variables [blood lead, urine cadmium and area of residence] and hematopoietic were not consistent in the associations shown except for blood lead level, area of residence and hematocrit (HCT). In children 6-14 years old blood lead was associated with an increase in HCT by (<0.1%) per μg/dl of blood lead increase. In adults however, the predicted HCT change associated with blood lead was a decrease in HCT by 0.3% for each μg/dl blood lead increase. The observation in children 6-14 years old represents a departure from previous reports on the toxic effects of lead and hematopoietic parameters and brings into question the reliability of the results obtained in the present study *(53)*.

The decrease in HCT predicted for adults is consistent with other reports on the effects of lead or cadmium toxicity. However, in other studies the impact produced by lead and cadmium on HCT and other RBC parameters is more commonly observed at considerably higher mean blood lead levels than that observed in this study *(6,8,53,54)*. Moreover, the HCT is a broad indicator of the total red cell mass and changes in HCT can occur with a variety of events that are unrelated to toxicity. For example, the HCT in a blood specimen may be artificially reduced in blood samples collected from an individual who has been standing erect or elevated in individuals who are dehydrated *(55)*. In the absence of corollary changes in MCV and other RBC morphologic indices, the observed changes in HCT may not reflect true changes in hematopoietic function. Finally, the models that were constructed accounted for only 28% of HCT variance in adults.

A similar pattern of association was observed when multivariate models were constructed to examine the relationship between urine cadmium excretion and HCT in children 6-14 and adults 15 years of age and older. For example, a 4.6% decrease in HCT was predicted for each $\mu g/g$ creatinine urine cadmium in children 6-14 years old while in adults, a 2.9% increase in HCT was predicted and a corollary (0.08)% increase in MCV. Whether this observation indicates that lead and cadmium, separately or collectively, at levels as low as those observed in the current study can produce such effect is not certain. Because of the limited understanding of many factors that may influence the HCT and other outcome parameters examined in this study, precise interpretation of this and other observations cannot be certain.

Biomarkers of Immune Function—Adverse affects on the immune system have been documented in animal and human studies following exposure to lead and cadmium. Typical immunotoxic effects associated with high level lead and cadmium exposure include an increased number of B lymphocytes, increase in serum IgA, decreases in secretory IgA, and serum IgM and IgG (*56,57,58*). Reduction in the absolute number of circulating T cells (CD3) and T helper cells (CD4) has also been reported. The present study demonstrated a moderately strong association between blood lead and serum IgA in children 6-71 months of age ($p=0.005$, adjusted $r^2=0.26$, $n=930$); the model also predicted a 1% increase in serum IgA for each $\mu g/dl$ change in blood lead. Although the association between area of residence and serum IgA was of borderline statistical significance ($p=0.058$, $r^2=0.26$, $n=934$) the linear regression model predicted an 8% increase in IgA for each 1 $\mu g/dl$ increase in blood lead. This observation agreed with findings reported elsewhere, but the clinical implication and mechanisms involved were uncertain (*58*).

IgA is an antibody found in serum and more important, is the primary antibody present on mucosal surfaces, in tears, saliva, and nasal and gastrointestinal secretions (*59*). A significant decrease in IgA over a prolong period could potentiate an increase frequency and severity of typically self-limiting respiratory and gastrointestinal illnesses produced by commonly occurring viruses and bacteria. A recent study of lead workers exposed to high levels of lead over a prolong period found a higher frequency of upper respiratory illness associated with decreased levels of secretory IgA in a recent study (*58*). In this study there was no association between serum IgA and secretory IgA. The relationship between serum and secretory IgA levels in other health and disease conditions is not consistant (*60*).

Several factors may act to confound and complicate interpretation of immune biomarker results. In this study, interpretation may be confounded by the occurrence of an outbreak of several cases of upper respiratory illness during data collection at the Illinois site. Since data were not collected about recent or ongoing illness, it was not possible to gauge the influence of these events relative to immune results obtained.

Biomarkers of Hepatobiliary Dysfunction—No significant associations were observed indicating organ toxicity at the generally low levels of exposure observed in this study. This finding was consistent with other reports of human populations exposed to lead and cadmium (*6,19*).

CONCLUSIONS

1. Among children [6 to 71 months and 6 to 14 years] old, the mean blood lead level in target areas was higher [4.26 µg/dl and 3.45 µg/dl] than in comparison areas. Up to 93% of participants in both study areas had blood lead levels below 10 µg/dl.

2. The mean blood lead level among participants at smelting area study sites was higher than mining area study sites. About 93% of mining area participants and 85% of smelting area participants had blood lead levels below 10 µg/dl.

3. Socioeconomic factors including: head of household's level of education, household income, and being a male between 1-2 years old were variables associated with blood lead ≥10 µg/dl. The presence of air conditioning in the home and living in a home built after 1950 was negatively associated.

4. Of the four environmental factors evaluated, only yard soil [containing lead concentration greater than 500 mg/kg] was associated with mean blood lead above 10 µg/dl in young children six months to 71 months old when study area was defined by proximity to the site (i.e., target area). These children were more than twice as likely likely to experience a mean blood above 10 µg/dl as children not expsosed to mean soil lead concentrations ≥500 mg/kg.

5. Yard soil and house dust containing lead at a concentration greater than 500 mg/kg were predictive for blood lead above 10 µg/dl among children six to 71 months of age who resided in historically smelting or mixed smelting/mining areas as defined in this study. These children were more than 1.8 times or 80% more likely to experience a mean blood lead ≥ 10 µg/dl compared to children not expsosed to soil lead concentrations 500 mg/kg.

6. Ninety five percent of all study participants in target and comparison study areas had urine cadmium excretion levels below 1 µg/g creatinine. The difference in mean urine cadmium excretion [0.18 versus 0.11 µg/g creatinine] was

higher in target areas than in comparison areas, respectively. None of the environmental media for which the concentration of cadmium was measured were important predictors of urine cadmium excretion.

7. There was no evidence of excess cadmium exposure or related kidney dysfunction among study participants resulting from contact with environmental media examined in this study (soil, dust, and water).

8. This investigation demonstrated that a multisite study design is a practical approach for conducting environmental epidemiologic studies and is a complement to ongoing biomarker research.

RECOMMENDATIONS

1. Parents should be counseled about sources and pathways of exposure to environmental lead and cadmium as well as behaviors that may contribute to childhood exposure.

2. Where the potential for multi-media exposure exist, each likely source and its associated pathway should be thoroughly evaluated. Exposure prevention strategies should carefully consider the magnitude of risk for human exposure in order to determine and implement the most cost-effective method of exposure prevention.

3. Strategies for human exposure prevention should be site specific and incorporate current biological surveillance data including body fluid measures of specific contaminants, when possible. Biological surveillance data may be useful in distinguishing populations at greatest risk and assessing the magnitude of such risk.

4. Ongoing research must be directed towards establishing sensitivity, specificity, and predictive values for biological markers if they are to be used effectively in field epidemiologic investigations and accurately interpreted in a health-risk/exposure context.

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TABLES

Table 1.—Hepatobiliary Function Tests and Reference Values, Multisite Lead and Cadmium Study, 1991.

Test	Roche Laboratory Reference Range*	Expected Coefficient of Variability (%)†	Comparison Reference Range§
AST (SGOT)	0-6 mo 0-120 IU/L 7-12 mo 0-110 IU/L 1-5 yr 0-75 IU/L 6-10 yr 0-60 IU/L > 10 yr 0-50 IU/L	5.41	<2 yr 20-55 IU/L 2-5 yr 20-55 IU/L 5-8 yr 20-50 IU/L 8-12 yr 15-40 IU/l 12-14 yr 15-35 IU/L 14-16 yr 15-30 IU/L Adults Male 8-46 IU/L Female 7-34 IU/L
ALT (SGPT)	0-50 IU/L	8.33	Infants 0-54 IU/L <2 yr 3-37 IU/L 2-8 yr 3-30 IU/L 8-16 yr 3-28 IU/L Adult Male 7-46 IU/L Female 4-35 IU/L
GGT	Male 0-65 IU/L Female 0-45 IU/L	6.45	M 3-12 mo 5-65 IU/L F 3-12 mo 5-35 IU/L 1-15 yr 0-23 IU/L M Adult 9-69 IU/L F Adult 3-33 IU/L
Albumin	3.5-5.5 g/dl	2.78	3.5-5.5 g/dl
Total Protein	Newborn 4.6-7.2 g/dl <2 yr 5.7-8.2 g/dl ≥2 yr 6.0-8.5 g/dl	3.23	6.0-8.0 g/dl

*Provided by Roche Biomedical Laboratories (MRI)
†Roche Biomedical Laboratories (MRI)
§Source: Interpretation of Diagnostic Tests, Fourth Edition

Table 2.—Renal Function Indices and Reference Values, Multisite Lead and Cadmium Study, 1991.

Test	Roche Laboratory Reference Range*	Expected Coefficient of Variability (%)†	Comparison Reference Range§
Creatinine	0.5-1.5 mg/dl	4.76	0.6-1.3 mg/dl 1 yr ≤0.6 mg/dl 2-3 yr ≤0.7 mg/dl 4-7 yr ≤0.8 mg/dl 8-10 yr ≤0.9 mg/dl 11-12 yr ≤1.0 mg/dl 13-17 yr ≤1.2 mg/dl 18-20 yr ≤1.3 mg/dl
BUN	7-26 mg/dl	7.14	5.0-25 mg/dl
BUN:CREAT Ratio	N/A	N/A	10:1
Specific Gravity	1.000-1.035	N/A	1.003-1.030
pH	5.0-8.0	N/A	4.6-8.0
Serum Electrolytes			
Sodium	135-148 mEq/L	1.43	136-148 mEq/L
Potassium	3.5-5.5 mEq/L	2.44	3.5-5.5 mEq/L
Chloride	94-109 mEq/L	1.98	100-106 mEq/L

*Provided by Roche Biomedical Laboratories (MRI)
†Source: Interpretation of Diagnostic Tests, Fourth Edition
§Creatinine values for females are 0.1 mg/dl lower than males

Table 3.—Hematopoietic System (CBC, Differential and Indices), Multisite Lead and Cadmium Study, 1991.

Test	Roche Reference Range	Comparison Range
WBC	4.8-10.8/mm^3	7.4-11.9/mm^3
RBC	7 mos-12 yrs 3.90-5.4X10^6/mm^3 Adults F 4.3-5.9 M 3.5-5.5	6 mos-15 yrs 4.6-4.8X10^6/mm^3 Adults F 4.8 ± 0.6 M 5.4 ± 0.8
Hemoglobin (Hbg)	12-16 gm/dl	11.8-16 gm/dl
Hematocrit (HCT)	37-47 (%)	F 35.5-42 ±5.0 M 35.5-47 ±5.0
MCV	81-99	77-87
MCH	27-31 (pg)	26-29 (pg)
MCHC	32-36 (%)	33-34 (%)
Platelet Count	150-450X 10^3/mm^3	140-350 X 10^3/mm^3
White Blood Cells Percent		
Granulocytes	42.2-75.2	34.2-62.2 ±15
Lymphocytes	20.5-51.1	34-61 ±10
Monocytes	1.7-9.3	4-4.8
Eosinophils	0.0-10	2.5-2.7
Basophils	0.0-2.5	0.4-0.5
White Blood Cell Count X (10)^3/mm^3		
Granulocyte	1.5-10	2.5-7.6
Lymphocyte	0.6-6.0	2.5-7.3
Monocyte	0-1.0	0.3-0.58
Eosinophil	0-0.7	0.2-0.3
Basophil	0-0.3	0.04-0.05

Table 4.—Renal Tubular Enzymes, Multisite Lead and Cadmium Study, 1991.

Urine Analyses	CDC Expected Range	Method Reference
Alanine aminopeptidase (AAP)	1.80-8.91 μg/L	Jung K and Scholz D, An Optimized Assay of Alanine Aminopeptidase Activity in Urine, Clin Chem 26: 1251-1254, 1980.
Gamma glutamyltransferase (GGT)	5.19-83.51 μg/L	S.V.R. GGT Test Reagents, Behring Diagnostic, Lab, Jolla, CA.
N-acetyl-β-D-glucosaminidase (NAG)	0.17-3.50 μg/L	Leadback and Walker, Studies on Glucosaminidase. 4. The Flourimetric Assay of N-acetyl-β-D-glucosaminidase, Biochem J 78: 151-156, 1961.
	Literature Expected Range	
Creatinine (CREAT)	F 0.6-1.5 g/24hr M 0.6-2.5 g/24hr	Dupont ACA Chemistry Manual, Wilmington, Delaware, 1985.

1. Urinary enzyme activity was calculated as a ratio to creatinine to correct for dilution using the equation below. Previous studies indicate that tubular enzymes excretion correction using creatinine values less than 50 mg/dl may not reliable and are not included when target and comparison populations were compared.

2. Renal Tubular Enzymes Range 5th to 95th percentile determined from CDC employees reporting no kidney or urinary tract disease within six months prior to sampling as follows: AAP n = 78; GGT n = 63; NAG n = 88.

$$Enzyme\ Creatinine\ Ratio(\mu\ g/L) = \frac{Urine\ Enzyme\ Value\ (\mu\ g/L)}{UCREA\ mg/dl} X\ 100$$

Table 5.—Blood and Urine Cadmium and FEP Reference Values, Multisite Lead and Cadmium Study, 1991.

Test	Reference Range	Literature Reference
Blood Cadmium	Non Smokers 0.4-1.0 μg/L Smokers 1.4-4.0 μg/L Occupation Exposure 10.0-100 μg/L	ATSDR, Toxicological Profile For Cadmium, March 1989.
Urine Cadmium	Adults Normal 2.6-<2.8 μg/g CREAT Cumulative Frequency of 95.8% in 1,000 samples from an apparently normal population.	Kowal Norman E., Zirkes Mona, Urinary Cadmium and $Beta_2$-Microglobulin: Normal Values And Concentration Adjustment, Journal of Toxicology and Environmental Health, 11:607-624, 1983.
	Occupation Related 1.32-13.88 μg/g CREAT Average 0.35 μg/g CREAT	ATSDR, Toxicological Profile For Cadmium, March 1989.
Erythrocyte Protoporphyrin	≥35 μg/dl	CDC, Preventing Lead Poisoning in Young children, October, 1991

Urine cadmium excretion was adjusted as a ratio of creatinine to account for difference in urine concentration using the formula below. Creatinine ratios calculated for urine values of creatinine less than 25 mg/dl produced spurious results especially in young children and were considered unreliable and were not included when study populations were compared.

$$\textit{Urine Cadmium } (\mu g/g \textit{ Creatinine}) = \frac{UrCd\ ng/ml}{UrCr\ mg/dl} \times 100$$

Table 6.—Age-Adjusted Normal Ranges (mg/dl) for IgG, IgA, and IgM, Multisite Lead and Cadmium Study, 1991.

Age	IgG Range	IgA Range	IgM Range
4-6 Months	80-500	3-42	23-96 mg/dl
7-24 Months	270-1000	8-85	27-190 mg/dl
2-5 Years	470-1500	16-140	43-200 mg/dl
6-8 Years	690-1500	57-200	53-190 mg/dl
9-11 Years	770-1600	52-260	48-290 mg/dl
12-16 Years	700-1600	52-190	47-310 mg/dl
Adult	560-1800	85-390	45-250 mg/dl

Reference ranges were taken from data obtained at Fitzsimmons Army Medical Hospital. All values were rounded to two significant figures to reflect the limits of analytical precision and accuracy. Results were calibrated with respect to U.S. National Reference Preparation #12-0575C, established in 1982 and related to the WHO international reference material. These reference ranges cannot account for normal population differences that may exist between Multisite study cohorts and the control population used for the Fitzsimmons Army Medical Hospital.

Table 7.—Multisite Lead and Cadmium Exposure Study Summary, Multisite Lead and Cadmium Study, 1991.

Study Sites	Galena Kansas	Joplin Missouri	Granite City Illinois	Palmerton Pennsylvania
Contaminant Media	Lead/Cadmium Soil/Dust	Lead/Cadmium Soil/Dust	Lead/Cadmium Soil/Dust	Lead/Cadmium Soil/Dust
Area Population Eligible Participants Required Residency	4,000 1857 90 days	46,000 8067 90 days	46,210 10,000 90 days	45,000 7,000 180 days
Sample Size Target Comparison	 167 283*	 412 283*	 842 N/A	 280 224
Participant Age Groups	6-71 months 6-14 years 15+ years	6-71 months 6-14 years 15+ years	6-71 months 6-14 years 15+ years	6-71 months 6-14 years 15+ years
Environmental Samples	Yard Soil House Dust Water Interior Paint			
Analytes	Urine Cadmium Blood Lead			

*Comparison population for Missouri and Kansas are the same

Residential Soil (mg/kg)

Interior House Dust (mg/kg)

Drinking Water (μg/L)

Interior House Paint Measured by XRF (mg/cm^2)

Table 8.—Demographic Characteristics By Site. Multisite Lead and Cadmium Study, 1991.

Category	Galena Kansas	Joplin Missouri		Granite City Illinois	Palmerton Pennsylvania		Total	
Sex	Target Area	Target Area	Comparison Area	Target Area	Target Area	Comparison Area	Target Area	Comparison Area
Male	75 44.9%	207 50.2%	140 49.5%	425 50.5%	138 49.3%	114 50.9	845 49.7%	254 50.1%
Female	92 55.1%	205 49.8%	143 50.5%	417 49.5%	142 50.7%	110 49.1%	856 50.3%	253 49.9%
Age								
<6	67 40.1%	241 58.5%	137 48.4%	487 57.8%	86 30.7%	57 25.4%	881 51.8%	194 38.3%
6-14	64 38.3%	115 27.9%	93 32.9%	227 27.0%	94 33.6%	73 32.6%	500 29.4%	166 32.7%
15-44	36 21.6%	54 13.1%	53 18.7%	110 13.1%	52 18.6%	52 23.2%	252 14.8%	105 20.7%
45+	0 0.0%	2 0.5%	0 0.0%	18 2.1	48 17.1%	42 18.8%	68 4.0%	42 8.3
Race								
Black	1 0.6%	3 0.7%	3 1.0%	138 16.4%	0 0.0%	0 0.0%	142 8.3%	3 0.6%
White	158 95.6%	396 96.1%	273 96.5%	687 81.6%	277 98.9%	223 99.5%	1518 89.2%	496 97.8%
Other	8 4.8%	13 3.2%	7 2.5%	17 2.0%	3 1.1%	1 0.5%	41 2.5%	8 1.6%
Ethnicity								
Hispanic	6 3.6%	20 4.9%	11 3.9%	38 4.5%	16 5.7%	2 0.9%	80 4.7%	13 2.6%
Non-Hispanic	161 96.4%	391 95.1%	270 96.1%	804 95.5%	264 94.3%	221 99.1%	1619 95.3%	491 97.4%

Table 9.—Socio-economic characteristics Education, Income, and Age of Houses By Site. Multisite Lead and Cadmium Health Study, 1991.

	Galena Kansas	Joplin Missouri		Granite City Illinois	Palmerton Pennsylvania		Total	
Category	Target	Target	Comparison	Target	Target	Comparison	Target	Comparison
Education								
<High School	134 21.1%	55 13.8%	20 7.1%	146 17.8%	30 11.3%	19 9.1%	265 15.7%	39 7.7%
High School Graduate	77 47.8%	159 39.3%	116 41.1%	359 43.8%	132 49.8%	132 63.2%	727 43.1%	248 49.2%
College/ Tech School	41 25.5%	157 39.3%	115 40.8%	279 34.1%	86 32.5%	47 22.5%	605 35.9%	175 34.7%
Graduate School	9 5.6%	28 7.0%	31 11.0%	35 4.3%	17 6.4%	11 5.2%	89 5.3%	42 8.3%
Income								
<15,000	58 50.9%	95 23.9%	51 18.9%	323 39.3%	33 12.6%	31 14.5%	509 31.9%	82 16.9%
15 -24,999	24 21.0%	113 28.3%	74 27.4%	193 23.5%	52 19.9%	46 21.5%	382 22.4%	120 24.8%
25 - 34,999	32 28.1%	95 23.9%	74 27.4%	160 19.5%	70 26.7%	80 37.4%	357 22.4%	154 31.8%
>35,000	0 0%	95 23.9%	71 26.3%	146 17.7%	107 40.8%	57 26.6%	348 21.8%	128 26.4%
Year House Constructed								
<1940	63 46.0%	118 45.6%	39 30.0%	312 64.6%	121 65.4%	141 83.9%	614 58.3%	180 60.4%
1940 -1979	34 24.8%	53 21.4%	51 39.3%	155 32.1%	52 28.1%	21 12.5%	294 29.7%	72 24.2%
>1980	40 29.2%	77 31.0%	40 30.8%	16 3.3%	12 6.5%	6 3.6%	145 13.8%	46 15.4%

Note: Because of rounding percent values may not equal 100%

Table 10.—Geometric Means for Environmental Media Concentration of Lead by Study Areas (i.e., Target, Comparison, Smelting and Mining). Multisite Lead and Cadmium Health Study, 1991.

Category	Target Area				Comparison Area			
	Mean	N[¶]	Min	Max	Mean	N[¶]	Min	Max
Soil Lead[*]	519	1259	20	10,600	1220	197	18	7,760
Dust Lead[*]	1182	1289	5.2	71,000	1146	198	2.3	48,800
Water Lead[†]	2.9	1274	0.3	96	10.3	202	1.1	166
Paint Lead[‡]	1.6	1136	0.00011	16.5	0.33	121	0.001	1.54
	Smelting Area				Mining Area			
Soil Lead[*]	510	1002	20	10,600	554	257	32	4,830
Dust Lead[*]	1314	1012	5.2	71,000	585	257	14	10,886
Water Lead[†]	3.04	1017	0.3	96	2.58	257	2.0	46
Paint Lead[‡]	1.58	896	0.00011	16.5	1.29	240	0.3	7

[*]Unit for soil and dust concentrations of lead and cadmium = mg/kg.
[†]Unit for water concentration of lead and cadmium = μg/L.
[‡]Unit for XRF lead paint concentration = mg/cm^2.
[¶]Number of participants associated with mean concentrations.

Table 11.—Correlation Coefficients For Blood Lead And Environmental Media Lead Concentration. Multisite Health Study 1991.

VARIABLES	BLOOD LEAD	SOIL LEAD	WATER LEAD	DUST LEAD
BLOOD LEAD				
SOIL LEAD				
r*	0.23			
p-value	<0.01			
Sample size	1416			
WATER LEAD				
r*	0.09	0.19		
p-value	<0.01	<0.01		
Sample size	1436	1444		
DUST LEAD				
r*	0.21	0.46	0.02	
p-value	<0.01	<0.01	0.50	
Sample size	1425	1447	1457	
LEAD PAINT				
r*	0.05	0.10	-0.13	0.24
p-value	0.01	<0.01	<0.01	<0.01
Sample size	1222	1237	1246	1248

* Pearson correlation coefficient.

Table 12.—Natural log and Geometric Means for Blood Lead for Study Populations by Area of Residence (i.e., Target, Comparison, Smelting and Mining). Multisite Lead and Cadmium Health Study, 1991.

Category	Target Area			Comparison Area			T-test*
	Mean	St.d	N	Mean	St.d	N	P-value
All Age Groups							
Blood Lead	1.45 (4.26)	0.71	1645	1.24 (3.45)	0.74	493	<0.01
<6 Years							
Blood Lead	1.68 (5.36)	0.67	833	1.38 (3.97)	0.66	184	<0.01
6-14 Years							
Blood Lead	1.25 (3.49)	0.61	495	1.04 (2.88)	0.75	163	<0.01
15 Years +							
Blood Lead	1.12 (3.06)	0.72	317	1.29 (3.63)	0.76	146	0.026
	Smelting Area			Mining Area			
All Age Groups							
Blood Lead	1.50 (4.48)	0.70	1102	1.33 (3.78)	0.70	543	<0.01
<6 Years							
Blood Lead	1.74 (5.69)	0.65	555	1.57 (4.81)	0.69	278	<0.01
6-14 Years							
Blood Lead	1.31 (3.70)	0.60	321	1.14 (3.13)	0.61	174	<0.01
15 Years +							
Blood Lead	1.18 (3.25)	0.74	226	0.97 (2.64)	0.65	91	<0.017

*t-test based on Natural Logarithm

Table 13.—Frequency distribution of blood lead for target and comparison populations. Multisite Lead and Cadmium Health Study, 1991.

Target Area			Comparison Area	
Blood Lead μg/dl	Number	Cum. %	Number	Cum. %
1.0-1.9	193	11.9	88	18.4
2.0-2.9	248	27.3	102	39.8
3.0-3.9	256	43.1	79	56.4
4.0-4.9	236	57.7	56	68.1
5.0-5.9	163	67.8	39	76.3
6.0-6.9	120	75.2	22	80.9
7.0-7.9	86	80.6	24	86.0
8.0-8.9	71	85.0	21	90.4
9.0-9.9	83	90.1	13	93.1
10.0-10.9	40	92.6	5	94.1
11.0-11.9	28	94.3	4	95.0
12.0-12.9	11	95.0	5	96.0
13.0-13.9	15	95.9	3	96.6
14.0-14.9	11	96.6	4	97.5
15.0-15.9	5	96.9	3	98.1
16.0-16.9	7	97.3	1	98.3
17.0-17.9	6	97.7		
18.0-18.9	6	98.1	4	99.2
19.0-19.9	4	98.3	0	
20.0-20.9	6	98.7	1	99.4
21.0-21.9	5	99.0	1	99.6
22.0-22.9	6	99.4	0	
23.0-23.9	0		0	
24.0-25.9	4		0	
25.0-25.9	0	99.6	0	
26.0-29.9	0		1	99.8
30.0-31.9	1	99.7	0	
32.0-33.9	2	99.8	0	
34.0-35.9	1	99.9	0	
36.0-37.9			1	100.0
38.0-39.9	1	99.9		
41.0-41.9	1	100.0		
Total	1616		477	

Table 14.—Frequency distribution of blood lead for populations by contamination source, Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

Smelting Area			Mining/smelting Area*	
Blood Lead µg/dl	Number	Cum. %	Number	Cum. %
1.0-1.9	110	10.2	83	15.5
2.0-2.9	149	23.9	99	34.1
3.0-3.9	176	40.2	80	49.1
4.0-4.9	149	54.0	87	65.4
5.0-5.9	107	63.9	56	75.8
6.0-6.9	87	71.9	33	82.0
7.0-7.9	67	78.1	19	85.6
8.0-8.9	58	83.5	13	88.0
9.0-9.9	59	88.9	24	92.5
10.0-10.9	31	91.8	9	94.2
11.0-11.9	21	93.7	7	95.5
12.0-12.9	10	94.6	1	95.7
13.0-13.9	11	95.7	4	96.4
14.0-14.9	7	96.3	4	97.2
15.0-15.9	3	96.6	2	97.6
16.0-16.9	7	97.2	0	
17.0-17.9	4	97.6	2	97.9
18.0-18.9	4	98.0	2	98.3
19.0-19.9	3	98.2	1	98.5
20.0-20.9	5	99.7	1	99.8
21.0-21.9	4	99.1	1	98.9
22.0-22.9	4	99.4	2	99.3
24.0-25.9	2	99.6	2	99.6
31.0-31.9	1	99.7		
32.0-33.9	0		1	99.8
38.0-39.9			1	100.0
35.0-35.9	1	99.9		
41.0-41.9	1	100.0		
Total	1082		534	

*Smelting and mining/smelting activity represents predominant source of contamination.

Table 15.—Comparison of natural logs of the mean and standard deviations of blood lead among children less than 6 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	p value
Area				
Target	1.68 (5.37)	0.67	833	
Comparison	1.38 (3.97)	0.66	184	<0.01
Income				
< 15,000	1.85 (6.36)	0.62	311	
> 15,000	1.53 (4.62)	0.67	658	<0.01
Education				
High School or Less	1.69 (5.42)	0.63	456	
College or Technical School	1.44 (4.22)	0.68	387	<0.01
Air Condition				
Yes	1.55 (4.71)	0.66	830	
No	1.98 (7.24)	0.68	187	<0.01
Sex				
Male	1.71 (5.53)	0.66	517	
Female	1.54 (4.66)	0.68	500	<0.01
House Year Built				
≤1950	1.69 (5.42)	0.63	456	
≥1980	1.44 (4.22)	0.68	387	<0.01
Food Outside				
Yes	1.78 (5.93)	0.67	417	
No	1.53 (4.62)	0.66	600	<0.01

Table 15.—continued Comparison of natural logs of the mean and standard deviations of blood lead among children less than 6 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	p value
Sucks Thumb				
Yes	1.57 (4.81)	0.70	220	
No	1.68 (5.37)	0.67	797	0.19
Favorite Toy				
Yes	1.61 (5.00)	0.62	426	
No	1.64 (5.16)	0.71	591	0.43
Eat Non Food Items				
Yes	1.65 (5.21)	0.67	513	
No	1.61 (5.00)	0.68	504	0.33
Mouth Furniture				
Yes	1.69 (5.42)	0.67	361	
No	1.60 (4.95)	0.68	656	0.03
Swallow				
Yes	1.61 (5.00)	0.69	84	
No	1.63 (5.10)	0.67	933	0.72
Eat Vegetables				
Yes	1.57 (4.81)	0.73	159	
No	1.64 (5.16)	0.66	858	0.23
Eat Root				
Yes	1.51 (4.53)	0.79	127	
No	1.64 (5.16)	0.66	890	0.312
Food Original Can				
Yes	1.53 (4.62)	0.79	456	
No	1.65 (4.22)	0.66	890	0.12

Model 1.

Table 16—Linear regression model for blood lead* among children age 6 to 71 months. Multisite Lead and Cadmium Study, 1991.

Variable*	BETA	Standard Error	P-Value
Intercept	1.297	0.081	<0.01
Target	0.250	0.056	<0.01
Illinois	0.053	0.048	0.263
Pennsylvania	0.337	0.065	<0.01
Income (<15,000)	0.167	0.045	<0.01
Education (High School or Less)	0.145	0.041	<0.01
Air Condition	-0.253	0.052	<0.01
Male	0.152	0.038	<0.01
Age (1-2 yrs)	0.089	0.041	0.028
House Smoke	0.161	0.041	<0.01
Home Built (>1950)	-0.243	0.045	<0.01

Model F-ratio = 28.70, $p = 0.0001$, n = 962, adj. r^2 = .232.

* Natural logarithm.

† All variables coded as 1 (yes) or 0 (no), except age, which is used as a categorical variable.

Model 2.

Table 17.—Linear regression model for lead among children age 6 to 71 months with Environmental Factors. Multisite Lead and Cadmium Study, 1991

Variable*	BETA	Standard Error	P-Value
Intercept	0.020	0.170	0.907
Income (< 15,000)	0.209	0.050	<0.01
Education (High School or Less)	0.144	0.048	<0.01
Air Condition	-0.177	0.064	<0.01
Male	0.109	0.046	0.018
Soil Lead	0.156	0.027	<0.01
Dust Lead	0.119	0.021	<0.01

Model F-ratio = 37.18, $p = 0.0001$, $n = 680$, adj. $r^2 = 0.250$.

* All variables coded as 1 (yes) or 0 (no).
† Natural logarithm of blood, soil, and dust lead measurements.

Model 3.

Table 18.—Logistic regression model for blood lead* (< 10 $\mu g/dl$ or ≥ 10 $\mu g/dl$) in children age 6 to 71 months. Multisite Lead and Cadmium Study, 1991.

VARIABLE†	BETA	S.E.§	O.R.¶	95% C.I.**	P-Value
Intercept	-2.638	0.428	-----	-------	<0.01
Target	0.990	0.328	2.69	1.41 - 5.12	<0.01
Pennsylvania	0.830	0.267	2.29	1.36 - 3.87	<0.01
Air Condition	-1.171	0.208	0.31	0.21 - 0.47	<0.01
Male	0.519	0.195	1.68	1.15 - 2.46	<0.01
Age (1-2 yrs)	0.505	0.199	1.66	1.12 - 2.45	0.011
House Smoke	0.679	0.214	1.97	1.30 - 3.00	<0.01
Home Built (>1950)	-1.019	0.278	0.36	0.21 - 0.62	<0.01

Model: p = 0.0001, n = 1017.

* Natural logarithm.
† All variables coded as 1 (yes) or 0 (no)
§ Standard error.
¶ Odds ratio.
** 95% confidence interval.

Model 4

Table 19.—Logistic regression model for blood lead* (< 10 μg/dl or ≥ 10 μg/dl) in children age 6 to 71 months with Environmental Factors. Multisite Lead and Cadmium Study, 1991

VARIABLE†	BETA	S.E.§	O.R.¶	95% C.I.**	P-Value
Intercept	-1.994	0.356	-----	-------	<0.01
Income (<15,000)	0.424	0.222	1.53	0.99 - 2.36	0.056
Air Condition	-0.982	0.237	0.37	0.23 - 0.60	<0.01
Male	0.569	0.216	1.77	1.16 - 2.70	<0.01
Age (1-2 yrs)	0.453	0.218	1.57	1.02 - 2.42	0.038
House Smoke	0.464	0.237	1.59	1.00 - 2.53	0.051
Home Built (>1950)	-0.757	0.315	0.47	0.25 - 0.87	0.016
Soil Lead (>500 ppm)	0.736	0.223	2.08	1.35 - 3.23	<0.01

Model: p = 0.0001, n = 735.

* Blood lead dichotomized into values < 10 μg/dl and ≥ 10 μg/dl.
† All variables coded as 1 (yes) or 0 (no).
§ Standard error.
¶ Odds ratio.
** 95% confidence interval.

Table 20.—Comparison of natural logs of the mean and standard deviations of blood lead among children less than 6 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Area				
Smelting	1.74 (5.70)	0.65	555	
Mining/smelting	1.57 (4.81)	0.69	278	<0.01
Income				
< 15,000	1.87 (6.49)	0.63	250	
> 15.000	1.49 (4.44)	0.66	447	<0.01
Education				
High School or Less	1.67 (5.31)	0.63	276	
College or Technical School	1.41 (4.10)	0.67	334	<0.01
Home Uses Air Conditioning				
Yes	1.53 (4.62)	0.66	605	
No	2.00 (7.39)	0.63	132	<0.01
Sex				
Male	1.70 (5.47)	0.66	375	
Female	1.53 (4.62)	0.69	362	<0.01
House Year Built				
Before 1950	1.67 (5.31)	0.66	316	
1950 -1991	1.34 (3.82)	0.67	215	<0.01
Food Outside				
Yes	1.77 (5.87)	0.65	308	
No	1.51 (4.53)	0.68	429	<0.01

Table 20.—continued Comparison of natural logs of the mean and standard deviations of blood lead among children less than 6 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Sucks Thumb				
Yes	1.56 (4.76)	0.73	154	
No	1.63 (5.10)	0.67	583	0.256
Favorite Toy				
Yes	1.61 (5.00)	0.62	293	
No	1.62 (5.05)	0.72	444	0.80
Eat Non Food Items				
Yes	1.65 (5.21)	0.67	385	
No	1.58 (4.85)	0.69	352	0.138
Mouth Furniture				
Yes	1.68 (5.36)	0.67	273	
No	1.58 (4.85)	0.68	464	0.039
Swallow				
Yes	1.64 (5.16)	0.69	63	
No	1.61 (5.00)	0.68	674	0.774
Eat Vegetables				
Yes	1.52 (4.57)	0.76	119	
No	1.64 (5.15)	0.66	618	0.120
Eat Root				
Yes	1.43 (4.18)	0.87	31	
No	1.63 (5.10)	0.67	706	0.227
Food Original Can				
Yes	1.43 (4.18)	0.79	91	
No	1.64 (5.16)	0.66	646	0.019

Table 21.— Linear regression model for lead* among children age 6 to 71 months by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

Variable	BETA	standard Error	P-Value
Intercept	1.606	0.078	<0.01
Smelter	0.057	0.048	0.239
Income (<15,000)	0.145	0.048	<0.01
Education (High School or Less)	0.208	0.046	<0.01
Air Condition	-0.286	0.059	<0.01
Male	0.151	0.043	<0.01
Take Food Outside	0.222	0.044	<0.01
Home Built (>1950)	-0.233	0.051	<0.01

Model F-ratio = 23.79, $p = 0.0001$, $n = 787$, adj $r^2 = .176$. Multisite, 1991.

*Natural Logarithm.

Model 16

Table 22.—Linear regression model for lead* among children age 6 to 71 months with Environmental Factors by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

Variable†	BETA	Standard Error	P-Value
Intercept	-0.049	0.189	0.795
Income (< 15,000)	0.204	0.052	<0.01
Education (High School or Less)	0.157	0.050	<0.01
Air Condition	-0.176	0.066	<0.01
Male	0.098	0.047	0.039
Take Food Outside	0.236	0.048	<0.01
Soil Lead	0.140	0.030	<0.01
Dust Lead	0.130	0.021	<0.01

Model F-ratio = 31.318, $p = 0.0001$, $n = 623$, adj. $r^2 = 0.262$.

*Natural Logarithm.

Table 23.—Logistic regression model for lead (<10 $\mu g/dl$ vs ≥ 10 $\mu g/dl$) among children age 6 to 71 months by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

Variable	BETA	S.E.	O.R.	95% C.I.	P-Value
Intercept	-1.720	0.344	-----	-------	<0.01
Smelter	0.070	0.234	1.07	1.41 - 5.12	0.764
Education (High School or Less)	0.604	0.229	1.83	1.36 - 3.87	<0.01
Air Condition	-1.145	0.227	0.32	0.21 - 0.47	<0.01
Male	0.505	0.208	1.66	1.10 - 2.49	0.015
Take Food Outside	0.785	0.205	2.19	1.15 - 2.46	<0.01
Home Built (>1950)	-1.114	0.311	0.328	0.21 - 0.62	<0.01

Model: p = 0.0001, n = 826. Multisite, 1991.

Model 18

Table 24.—Logistic regression model for lead (<10 $\mu g/dl$ vs ≥ 10 $\mu g/dl$) among children age 6 to 71 months by contamination source Smelting versus Mining/smelting with Environmental Factors. Multisite Lead and Cadmium Study, 1991.

Variable	BETA	S.E.	O.R.	95% C.I.	P-Value
Intercept	-1.469	0.330	-----	-------	<0.01
Education High School or Less	0.500	0.239	1.65	1.03 - 2.63	0.037
Air Condition	-1.015	0.245	0.36	0.23 - 0.60	<0.01
Male	0.467	0.220	1.59	1.16 - 2.70	0.034
Home Built (>1950)	-0.909	0.322	0.40	0.25 - 0.87	<0.01
Soil Lead (>500 ppm)	0.588	0.222	1.80	1.35 - 3.23	<0.01

Model: p = 0.0001, n = 691. Multisite, 1991.

Table 25.—Comparison of natural logs of the mean and standard deviations of blood lead among children 6 to 14 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Area				
Target	1.25 (3.49)	0.61	495	
Comparison	1.04 (2.82)	0.75	163	<0.01
Income				
<15,000	1.45 (4.26)	0.66	160	
>15,000	1.11 (3.03)	0.64	460	<0.01
Education				
High School or Less	1.18 (3.25)	0.61	292	
College or Technical School	1.09 (2.97)	0.66	257	.097
Air Condition				
Yes	1.14 (3.12)	0.64	520	
No	1.42 (4.13)	0.65	138	<0.01
Sex				
Male	1.28 (3.59)	0.60	350	
Female	1.11 (3.03)	0.70	308	<0.01
Age of House				
≤1950	1.34 (3.81)	0.60	304	
≥1980	0.94 (2.55)	0.64	212	<0.01
Person Rides Bike				
Yes	1.24 (3.45)	0.07	92	0.539
No	1.19 (3.28)	0.03	566	

Table 25.—continued Comparison of natural logs of the mean and standard deviations of blood lead among children 6 to 14 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/dl	s.d.	N	p-value
Sucks Thumb				
Yes	1.39 (4.01)	0.67	67	
No	1.18 (3.25)	0.65	591	0.017
H Bike				
Yes	1.32 (3.74)	0.64	104	
No	1.18 (3.25)	0.65	554	0.047
Eat Vegetables Home Grown				
Yes	1.12 (3.06)	0.63	129	
No	1.22 (3.39)	0.64	529	0.141
Food Original Can				
Yes	1.14 (313)	0.69	72	
No	1.21 (3.35)	0.65	586	0.459

Model 5.

Table 26.—Linear regression model for blood lead* among children age 6 through 14 years by area of residence. Multisite Lead and Cadmium Study, 1991.

Variable†	BETA	Standard Error	P-Value
Intercept	1.546	0.213	<0.01
Target	-0.062	0.086	0.467
Illinois	0.093	0.077	0.231
Pennsylvania	0.217	0.088	0.014
Income (<15,000)	0.312	0.066	<0.01
Air Condition	-0.196	0.073	<0.01
Male	0.187	0.056	<0.01
Age	-0.045	0.012	<0.01
House Smoke	0.161	0.059	<0.01
Home Built (>1950)	-0.213	0.089	<0.01
Suck Thumb	0.203	0.073	<0.01

Model F-ratio = 11.47, p=0.0001, n = 385, adj. r^2 = .235. Multisite, 1991.

* Natural logarithm.
† All variables coded as 1 (yes) or 0 (no).

Model 6.

Table 27.—Linear regression model for lead* among children age 6 through 14 years by area of residence with Environmental Factors. Multisite Lead and Cadmium Study, 1991.

Variable†	BETA	Standard Error	P-Value
Intercept	0.013	0.246	0.956
Income (<15,000)	0.286	0.074	<0.01
Age	-0.033	0.013	<0.012
House Smoke	0.154	0.066	0.024
Soil Lead	0.125	0.035	<0.01
Dust Lead	-0.081	0.028	<0.01

Model F-ratio = 13.23, $p = 0.0001$, $n = 325$, adj. $r^2 = .171$.

* Natural logarithm
† All variables coded 1 (yes) or 0 (no)

Table 28.—Comparison of natural logs of the mean and standard deviations of blood lead among children 6 to 14 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Area				
Smelting	1.31 (3.70)	0.60	321	
Mining/smelting	1.14 (3.12)	0.61	174	<0.01
Income				
<15,000	1.42 (4.14)	0.67	126	
>15,000	1.06 (2.89)	0.64	315	<0.01
Education				
High School	1.16 (3.19)	0.67	205	
College or Technical	1.04 (2.83)	0.59	190	0.065
House uses Air Condition				
Yes	1.12 (3.06)	0.64	391	
No	1.41 (4.09)	0.67	83	<0.01
Sex				
Male	1.26 (3.52)	0.69	258	
Female	1.08 (2.94)	0.62	216	<0.01
Age of House				
Before 1950	1.28 (3.59)	0.60	213	
1950 - 1991	0.93 (2.53)	0.67	153	<0.01
Participant Rides Bike				
Yes	1.18 (3.25)	0.67	63	
No	1.17 (3.22)	0.66	411	0.901

Table 28.—continued Comparison of natural logs of the mean and standard deviations of blood lead among children 6 to 14 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/dl	s.d.	N	P-Value
Sucks Thumb				
Yes	1.33 (3.78)	0.74	48	
No	1.15 (3.16)	0.65	426	0.129
Household Member Rides Bike				
Yes	1.27 (3.56)	0.65	71	
No	1.15 (3.16)	0.66	403	0.202
Eat Vegetables Home Grown				
Yes	1.07 (2.92)	0.64	84	
No	1.20 (3.32)	0.66	390	0.098
Food Original Can				
Yes	1.03 (2.08)	0.74	47	
No	1.19 (3.29)	0.65	427	0.174

Model 19

Table 29.—Linear regression model for lead* among children age 6 through 14 years by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

VARIABLE	BETA	STANDARD ERROR	P-Value
Intercept	1.769	0.146	<0.01
Smelter	0.048	0.059	0.415
Income (<15,000)	0.247	0.061	<0.01
Air Condition	-0.162	0.075	0.031
Male	0.202	0.053	<0.01
Age	-0.060	0.010	<0.01
Home Built (>1950)	-0.150	0.061	0.015

Model F - ratio = 15.63, p = 0.0001, n = 461, adj r^2 = .171.

* Natural Logarithm.

Model 20

Table 30.—Linear regression model for lead* among children age 6 through 14 years by contamination source Smelting versus Mining/smelting with Environmental Factors.
Multisite Lead and Cadmium Study, 1991.

Variable	BETA	Standard Error	P-Value
Intercept	0.234	0.279	0.402
Income (<15,000)	0.326	0.080	<0.01
Male	0.173	0.072	0.017
Age	-0.048	0.015	<0.01
Soil Lead	0.14361	0.045	<0.01
Dust Lead	0.06974	0.031	0.026

Model: p = 0.0001, n = 260, adj r^2 = .180.
* Natural Logarithm.

Table 31.—Comparison of natural logs of the mean and standard deviations of lead in adults 15 years to 75 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/dl	s.d.	N	P-Value
Area				
Target	1.12 (3.06)	0.72	317	
Comparison	1.29 (3.63)	0.76	146	0.026
Sex				
Male	1.53 (4.62)	0.65	198	
Female	0.91 (2.48)	0.69	265	<0.01
Household Income				
<15,000	1.17 (3.22)	0.81	100	
>15,000	1.18 (3.25)	0.73	327	0.998
Education Head of Household				
High School or Less	1.23 (3.42)	0.78	204	
College or Technical School	1.06 (2.88)	0.66	182	0.017
Home uses Airconditioning				
Yes	1.08 (2.94)	0.73	363	
No	1.50 (4.48)	0.69	100	<0.01
Age of House				
Before 1950	1.23 (3.42)	0.73	246	
1950 -1991	0.99 (2.69)	0.62	125	<0.01
Cigarette Smoking				
Current Smoker	1.35 (3.85)	0.73	147	
Non Smoker	1.09 (2.97)	0.73	316	<0.01

Table 31.—continued Comparison of natural logs of the mean and standard deviations of blood lead among adults 15 years to 75 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Individual with Occupation or Hobby Using Lead				
Yes	1.18 (3.25)	0.70	195	
No	1.16 (3.19)	0.77	268	0.773
Garden				
Has a Garden	1.20 (3.32)	0.77	198	
Does Not Have a Garden	1.15 (3.16)	0.72	265	.531
Alcohol Consumption				
Yes	1.19 (3.39)	0.73	394	
No	1.05 (2.86)	0.81	69	0.193

Model 7.

Table 32.—Linear regression model for lead* among adults age 15 years and older. Multisite Lead and Cadmium Study, 1991.

Variable	BETA	Standard Error	P-Value
Intercept	0.334	0.118	<0.01
Target	-0.009	0.069	0.900
Illinois	0.269	0.081	<0.01
Pennsylvania	0.428	0.077	<0.01
Air Condition	-0.238	0.075	<0.01
Male	0.563	0.058	<0.01
Smoke Now	0.291	0.062	<0.01

Model F-ratio = 37.59, p=0.0001, n = 423, adj. r^2=.388. Multisite, 1991.

*Natural Logarithm.

Model 8.

Table 33.—Linear regression model for lead* among adults age 15 years and older with Environmental Factors. Multisite Lead and Cadmium Study, 1991.

Variable	BETA	Standard Error	P-Value
Intercept	-0.064	0.300	0.831
Air Condition	-0.224	0.110	0.043
Male	0.583	0.091	<0.01
Age	0.013	0.002	<0.01
Smoke Now	0.231	0.095	0.016
Dust* (Lead)	0.108	0.038	<0.01

Model F-ratio = 17.14, p=0.0001, n = 207, adj. r^2=.299. Multisite, 1991.
*Natural Logarithm

Table 34.—Comparison of natural logs of the mean and standard deviations of blood lead among adults 15 years to 75 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/dl	s.d.	N	P-Value
Area				
Smelting	1.18 (3.25)	0.74	226	
Mining/smelting	0.97 (2.63)	0.65	91	0.017
Sex				
Male	1.46 (4.36)	0.62	125	
Female	0.80 (2.22)	0.66	189	<0.01
Household Income				
< 15,000	1.08 (2.94)	0.82	73	
> 15,000	1.07 (2.91)	0.71	215	0.985
Education Head of Household				
High School or Less	1.04 (2.83)	0.72	128	
College or Technical School	1.03 (2.80)	0.66	128	0.910
Home uses Air Conditioning				
Yes	1.00 (2.72)	0.69	267	
No	1.43 (4.18)	0.82	47	<0.01
Age of House				
Before 1950	1.16 (3.18)	0.69	152	
1950 -1991	0.96 (2.61)	0.65	93	0.017
Cigarette Smoking				
Current Smoker	1.22 (3.38)	0.71	97	
Non Smoker	1.00 (2.71)	0.72	217	0.012

Table 34.—continued Comparison of natural logs of the mean and standard deviations of blood lead among adults 15 years to 75 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/dl	s.d.	N	P-Value
Individual with Occupation or Hobby Using Lead				
Yes	1.10 (3.00)	0.68	128	
No	1.04 (2.83)	0.75	186	0.298
Garden				
Has a Garden	1.00 (2.72)	0.71	74	
Does Not Have a Garden	1.09 (2.97)	0.73	240	0.289
Alcohol Consumption				
Yes	1.09 (2.97)	0.71	264	
No	0.95 (2.56)	0.80	50	0.273

Model 21

Table 35.—Linear regression model for lead* among adults age 15 years and older by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	Standard Error	P-Value
Intercept	0.903334	0.125	<0.01
Smelter	0.211	0.085	0.014
Air Condition	-0.322	0.101	<0.01
Male	0.692	0.075	<0.01
Smoke Now	0.162	0.079	0.042

Model: $p = 0.0001$, $n = 292$, adj $r^2 = .279$. Multisite, 1991.
*Natural Logarithm.

Model 22

Table 36.—Linear regression model for lead* among adults age 15 years and older by contamination source with Environmental Factors. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	Standard Error	P-Value
Intercept	0.495	0.299	0.099
Air Condition	-0.309	0.129	0.018
Male	0.708	0.096	<0.01
Dust* (Lead)	0.101	0.042	0.016

Model F - ratio = 28.33, $p = 0.0001$, $n = 173$, adj $r^2 = .284$. Multisite, 1991.
*Natural Logarithm.

Table 37.—Natural log and Geometric Means for Environmental Media Concentration of Cadmium by Study Areas (i.e., Target, Comparison, Smelting and Mining). Multisite Lead and Cadmium Health Study, 1991.

Category	Target Area			Comparison Area			T-test*
	Mean	s.d.	N	Mean	s.d.	N	P-value
Soil Cadmium	1.93 (6.88)	1.31	655	1.18 (3.25)	0.85	137	<0.01
Dust Cadmium	2.29 (9.87)	1.23	666	1.61 (5.00)	1.75	137	<0.01
Water Cadmium	-0.04 (0.96)	1.17	380	0.46 (1.58)	0.95	140	<0.01
	Smelting Area			Mining Area			
Soil Cadmium	1.87 (6.50)	1.42	502	2.13 (8.41)	0.84	153	<0.15
Dust Cadmium	2.39 (10.9)	1.35	513	1.95 (7.02)	0.62	153	<0.01
Water Cadmium	0.74 (1.84)	0.49	227	-1.20 (0.30)	0.88	153	<0.01

*t-test based on Natural Logarithm

Table 38.—Correlation Coefficients For Adjusted Urine Cadmium And Environmental Media Cadmium Concentration. Multisite Health Study, 1991.

Variables	Adjusted Urine Cadmium	Soil Cadmium	Water Cadmium
Adjusted Urine Cadmium Creatinine >25 µg/g			
Soil Cadmium			
r*	-0.25		
P-Value	<0.01		
Sample Size	908		
Water Cadmium			
r*	0.14	0.26	
P-Value	0.01	0.01	
Sample Size	551	499	
Dust Cadmium			
r*	-0.06	-0.41	0.22
P-Value	0.06	0.01	0.01
Sample Size	913	780	517

* Pearson correlation coefficient.

Table 39.—Frequency distribution of urine cadmium by area of residence. Multisite Lead and Cadmium Study, 1991.

Target Area			Comparison Area	
Urine Cadmium µg/g Creatinine	Number	Cum. %	Number	Cum. %
N/D	593	44.5	227	55.5
0.10-0.20	225	61.4	100	80.0
0.21-0.40	217	77.6	48	91.7
0.41-0.60	150	88.9	15	95.4
0.61-0.81	67	93.3	12	98.3
0.81-1.0	36	96.6	1	98.5
1.01-1.2	15	97.7	3	99.3
1.21-1.4	11	98.6	1	99.5
1.41-1.6	5	98.9	0	
1.61-1.8	3	99.2		
1.81-2.0	3	99.4	1	99.8
2.01-2.2	5	99.8		
2.21-2.4	1	99.8		
2.61-2.8	0		1	100.0
2.81-3.0	1	99.9		
3.21-3.6	0			
3.61-4.4	1	100.0		
Total	1333		409	

Table 40.—Frequency distribution of urine cadmium for target populations by area of residence and contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Study, 1991.

	Smelting Area		Mining/smelting Area	
Urine Cadmium μg/G Creatinine	Number	Cum. %	Number	Cum. %
N/D	293	34.4	300	62.2
0.10-0.20	109	47.2	116	86.3
0.21-0.40	182	68.6	35	93.6
0.41-0.60	132	84.1	18	97.3
0.61-0.81	60	91.2	7	98.8
0.81-1.0	33	95.1	3	99.4
1.01-1.2	15	96.8	0	
1.21-1.4	9	97.9	2	99.8
1.41-1.6	5	98.5		
1.61-1.8	3	98.8		
1.81-2.0	3	99.2		
2.01-2.2	4	99.6	1	100.0
2.21-2.4	1	99.8		
2.61-2.8				
2.81-3.0	1	99.9		
3.01-4.4	1	100.0		
Total	852		482	

Table 41.—Natural log and Geometric Means for Urine Cadmium (Adjusted for creatinine ≥25 mg/dl) for Study Populations by Area of Residence (i.e., Target, Comparison, Smelting, and Mining). Multisite Lead and Cadmium Health Study, 1991.

Category	Target Area			Comparison Area			T-test*
	Mean	s.d.	N	Mean	s.d.	N	P-Value
All Age Groups							
Adjusted Cadmium	-1.71 (0.18)	1.06	1084	-2.25 (0.11)	0.90	389	<0.01
<6 yrs							
Adjusted Cadmium	-1.66 (0.19)	0.99	426	-2.30 (0.10)	0.66	117	<0.01
6-14 yrs							
Adjusted Cadmium	-2.05 (0.13)	1.01	389	-2.63 (0.08)	0.74	148	<0.01
15 yrs +							
Adjusted Cadmium	-1.27 (0.28)	1.07	269	-1.75 (0.17)	1.03	124	0.026

Category	Smelting Area			Mining Area			T-test*
	Mean	s.d.	N	Mean	s.d.	N	P-Value
All Age Groups							
Adjusted Cadmium	-1.33 (0.31)	1.00	679	-2.33 (0.10)	0.84	405	<0.01
<6 Years							
Adjusted Cadmium	-1.16 (0.31)	0.90	249	-2.35 (0.09)	0.65	177	<0.01
6-14 Years							
Adjusted Cadmium	-1.68 (0.19)	0.99	239	-2.64 (0.07)	0.72	150	<0.01
15 Years +							
Adjusted Cadmium	-1.08 (0.33)	1.02	191	-1.73 (0.18)	1.09	78	<0.01

*t-test based on Natural Logarithm

Table 42.—Odds Ratio, 95% C.I. calculation for urine cadmium excretion dichotomized at (<0.1 $\mu g/g$ creatinine vs ≥ 0.1 $\mu g/g$ creatinine) for children less than 6 years of age by area of residence and other variables of interest. Multisite Lead and Cadmium Health Study, 1991.

Category	Detect	Non-Detect	O.R.	95% C.I.
Area				
Target	374	426		
Comparison	33	128	3.4	2.3-5.0
Income				
< 15,000	140	160		
> 15,000	250	360	1.26	0.9-1.7
Education				
High School or Less	241	325		
College or Technical School	162	227	1.03	0.8-1.3
Home Uses Air Conditioning				
Yes	337	450		
No	70	104	1.11	0.8-1.6
Sex				
Male	221	282		
Female	186	272	1.15	0.9-1.5
House Year Built				
Before 1950	199	215		
1950 -1991	208	339	1.51	1.2-1.9
Food Outside				
Yes	195	203		
No	212	351	1.60	1.2-2.1

Table 42.—continued Odds Ratio, 95% C.I. calculation for urine cadmium excretion dichotomized at (<0.1 μg/g creatinine vs ≥ 0.1 μg/g creatinine) for children less than 6 years of age by area of residence and other variables of interest. Multisite Lead and Cadmium Health Study, 1991.

Category	Detect	Non-Detect	O.R.	95% C.I.
House Member Smokes				
Yes	265	297		
No	142	257	1.61	1.2-2.1
Suck Thumbs				
Yes	77	121		
No	330	433	0.84	0.6-1.1
Favorite Toy				
Yes	151	248		
No	256	306	0.73	0.6-0.9
Nonfood in mouth				
Yes	197	274		
No	218	280	0.96	0.7-1.2

Model 9.

Table 43.—**Logistic regression model for urine cadmium dichotomized at (<0.1 μg/g creatinine vs μg/g creatinine) among children age 6 to 71 months. Multisite Lead and Cadmium Study, 1991.**

Variable	Beta	S.E.	O.R.	95% C.I.	P-Value
Intercept	-3.089	0.290	-----	-------	<0.01
Target	0.192	0.251	1.21	0.74-1.98	0.445
Illinois	2.928	0.223	18.70	12.1-29.0	<0.01
Pennsylvania	1.097	0.246	2.98	1.85-4.79	<0.01
Creatinine (>25 μg/g)	1.624	0.190	5.07	3.48-7.33	<0.01

Model: p = 0.0001, n = 961.

Model 10.

Table 44.—Logistic regression model for urine cadmium dichotomized at (<0.1 μg/g creatinine vs μg/g creatinine) among children age 6 to 71 months with environmental factors. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	S.E.	O.R.	95% C.I.	P-Value
Intercept	-1.357	0.231	-----	-------	<0.01
Creatinine (≥25)	0.520	0.272	1.68	1.05 - 3.17	0.056
Water (Cad ≥5)	2.078	1.167	7.99	0.67 - 95.7	0.075

Model: p = 0.0207, n = 342.

Table 45.—Odds Ratio, 95% C.I. calculation for urine cadmium excretion dichotomized at (< 0.1 μg/g creatinine vs $\geq$0.1 μg/g creatinine) for children less than 6 years of age by area of residence and other variables of interest. Multisite Lead and Cadmium Health Study, 1991.

Category	Detect ($\geq$0.1 μg/g)	Non-Detect (<0.1 μg/g)	O.R.	95% C.I.
Area				
Smelting	331	217		
Mining	43	209	7.4	5.3-10.5
Income				
< 15,000	135	138		
> 15,000	222	262	1.16	0.9-1.6
Education				
High School or Less	223	148		
College or Technical School	250	174	1.05	0.8-1.4
Home Uses Air Conditioning				
Yes	76	313		
No	350	61	1.14	0.8-1.6
Sex				
Male	210	206		
Female	216	168	1.19	0.9-1.5
House Year Built				
Before 1950	246	181		
1950 -1991	180	193	1.28	0.96-1.69
Food Outside				
Yes	184	278		
No	190	148	1.82	1.4-2.4

Table 45.—continued Odds Ratio, 95% C.I. calculation for urine cadmium excretion dichotomized at (< 0.1 μg/g creatinine vs ≥ 0.1 μg/g creatinine) for children less than 6 years of age by area of residence and other variables of interest. Multisite Lead and Cadmium Health Study, 1991.

Category	Detect (≥0.1 μg/g)	Non-Detect (<0.1 μg/g)	O.R.	95% C.I.
House Member Smokes				
Yes	247	190		
No	127	236	1.56	1.2 -2.1
Suck Thumbs				
Yes	332	68		
No	94	306	0.78	0.55-1.1
Favorite Toy				
Yes	151	248		
No	256	306	0.73	0.6-0.9
Nonfood in mouth				
Yes	191	208		
No	183	218	1.00	0.8-1.3

Table 46.—Comparison of natural logs of the mean and standard deviations of urine cadmium among children 6 to 14 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/g Creatinine	s.d.	N	P-Value
Area				
Target	-2.07 (0.13)	1.03	377	
Comparison	-2.54 (0.08)	0.76	160	<0.01
Income				
< 15,000	-1.99 (0.14)	1.00	129	
> 15,000	-2.29 (0.10)	0.95	373	<0.01
Education				
High School	-2.20 (0.11)	0.98	252	
College or Technical School	-2.24 (0.11)	0.99	201	0.665
House uses Air Conditioning				
Yes	-2.16 (0.12)	0.98	421	
No	-2.39 (0.09)	0.94	116	0.021
Sex				
Male	-2.27 (0.10)	0.99	299	
Female	-213 (0.12)	0.96	238	0.108
Age of House				
Before 1950	-2.50 (0.08)	0.96	243	
1950 - 1991	-2.18 (0.07)	0.90	177	<0.01
Participant Rides Bike				
Yes	-2.71 (0.07)	0.76	78	
No	-2.13 (0.12)	0.98	459	<0.01

Table 46.—continued Comparison of natural logs of the mean and standard deviations of urine cadmium among children 6 to 14 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/g Creatinine	s.d.	N	P-Value
Sucks Thumb				
Yes	-2.18 (0.11)	0.89	54	
No	-2.21 (0.11)	0.99	438	0.790
Household member Rides Bike				
Yes	-2.61 (0.07)	0.74	86	
No	-2.14 (0.12)	1.00	451	<0.01
Heat Vegetables				
Yes	-2.42 (0.09)			
No	-2.15 (0.17)			<0.01
Food Original Can				
Yes	-2.04 (0.13)	1.07	65	
No	-2.23 (0.11)	0.96	472	0.169

Model 11.

Table 47.—Logistic regression model for urine cadmium dichotomized at (<0.1 $\mu g/g$ creatinine vs ≥ 0.1 $\mu g/g$ creatinine) among children age 6 through 14 years. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	S.E.	O.R.	95% C.I.	P-Value
Intercept	-3.284	0.541	-----	-------	0.037
Target	0.181	0.230	1.20	0.76-1.88	0.429
Illinois	3.672	0.309	39.31	21.4-71.9	<0.01
Pennsylvania	0.737	0.223	2.09	1.35-3.24	<0.01
Age (Continuous)	0.105	0.040	-----	--------	<0.01
Creatinine (>25 μg/g)	0.954	0.309	2.56	1.42-4.77	<0.01

Model: $p = 0.0001$, $n = 652$.

Table 48.—Comparison of natural logs of the mean and standard deviations of urine cadmium among children 6 to 14 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/g Creatinine	s.d.	N	P-Value
Area				
Smelting	-1.68 (0.19)	1.03	377	
Mining/smelting	-2.75 (0.07)	0.76	160	<0.01
Income				
< 15,000	-1.86 (0.16)	1.02	98	
> 15,000	-2.15 (0.12)	1.02	248	0.019
Education				
High School	-2.05 (0.13)	1.01	173	
College or Technical School	-2.12 (0.12)	1.06	148	0.541
House uses Air Conditioning				
Yes	-2.03 (0.13)	1.04	312	
No	-2.26 (0.10)	0.96	65	0.082
Sex				
Male	-2.11 (0.12)	1.03	213	
Female	-2.02 (0.13)	1.02	164	0.413
Age of House				
Before 1950	-2.00 (0.14)	1.02	162	
1950 - 1991	-2.42 (0.09)	0.96	128	<0.01
Participant Rides Bike				
Yes	-2.61 (0.07)	0.84	49	
No	-1.99 (0.14)	1.03	328	<0.01

Table 48.—continued Comparison of natural logs of the mean and standard deviations of urine cadmium among children 6 to 14 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) $\mu g/g$ Creatinine	s.d.	N	P-Value
Sucks Thumb				
Yes	-1.99 (0.14)	0.94	37	
No	-2.09 (0.12)	1.04	340	0.578
Household Member Rides Bike				
Yes	-2.53 (0.08)	0.81	57	
No	-1.99 (0.14)	1.04	320	<0.01
Eat Vegetables Home Grown				
Yes	-2.32 (0.10)	0.96	75	
No	-2.01 (0.13)	1.03	302	0.017
Food Original Can				
Yes	-1.84 (0.16)	1.16	43	
No	-2.10 (0.12)	1.01	334	0.160

Model 24

Table 49.—Logistic regression model for urine cadmium dichotomized at (<0.1 μg/g creatinine vs μg/g creatinine) among children age 6 through 14 years. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	S.E.	O.R.	95% C.I.	P-Value
Intercept	-1.201	0.180	-----	-------	<0.01
Smelter	2.263	0.221	9.61	6.23-14.8	<0.01

Model: p = 0.0001, n = 492. Multisite, 1991.

Table 50.—Comparison of natural logs of the mean and standard deviations of urine cadmium among adults 15 years to 75 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) μg/g Creatinine	s.d.	N	P-Value
Area				
Target	-1.29 (0.28)	1.08	265	
Comparison	-1.70 (0.18)	1.04	128	<0.01
Sex				
Male	-1.44 (0.24)	1.48	178	
Female	-1.41 (0.24)	1.03	215	0.778
Household Income				
<15,000	-1.07 (0.34)	1.00	77	
>15,000	-1.51 (0.22)	1.09	288	<0.01
Education Head of Household				
High School or Less	-1.41 (0.24)	1.06	172	
College or Technical School	-1.51 (0.22)	1.10	159	0.355
Home uses Air Conditioning				
Yes	-1.44 (0.24)	1.11	311	
No	-1.37 (0.25)	0.97	82	0.599
Age of House				
Before 1950	-1.30 (0.27)	1.04	210	
1950 -1991	-1.72 (0.18)	1.11	113	<0.01
Cigarette Smoking				
Current Smoker	-1.06 (0.35)	1.09	117	
Non Smoker	-1.57 (0.21)	1.04	276	<0.01

Table 50.—continued Comparison of natural logs of the mean and standard deviations of urine cadmium among adults 15 years to 75 years of age among variables of interest by area of residence. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/g Creatinine	s.d.	N	P-Value
Garden				
Has a Garden	-1.50 (0.22)	1.03	166	
Does Not Have a Garden	-1.37 (0.25)	1.12	227	0.238
Alcohol Consumption				
Yes	-1.40 (0.25)	1.08	333	
No	-1.57 (0.21)	1.08	60	0.265

Model 12.

Table 51.—Linear regression model for urine cadmium* (adjusted for creatinine ≥25 mg/dl) among adults age 15 years and older. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	Standard Error	P-Value
Intercept	-3.309	0.158	<0.01
Target	0.293	0.116	<0.01
Illinois	0.699	0.131	<0.01
Pennsylvania	-0.049	0.119	0.681
Income (<15,000)	0.227	0.115	0.049
Age	0.033	0.003	<0.01
Smoke Now	0.524	0.103	<0.01

Model F-ratio = 30.583, p = 0.0001, n = 382, adj. r^2 = .329.

* Natural logarithm

Model 13.

Table 52.—Logistic regression model for urine cadmium (<0.5 μg/g creatinine vs ≥0.5 μg/g creatinine) among adults 15 years and older. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	S.E.	O.R.	95% C.I.	P-Value
Intercept	-3.918	0.443	-----	-------	<0.01
Target	0.977	0.280	2.66	1.57-4.69	<0.01
Illinois	1.028	0.261	2.79	1.68-4.66	<0.01
Smoke Now	0.763	0.229	2.14	1.37-3.36	<0.01
Age	0.050	0.007	-----	-------	<0.01

Model: p = 0.0001, n = 467.

* Natural logarithm

Table 53.—Comparison of natural logs of the mean and standard deviations of urine cadmium among adults 15 years to 75 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) $\mu g/g$ Creatinine	s.d.	N	P-Value
Area				
Smelting	-1.08 (0.33)	1.08	265	
Mining/smelting	-1.73 (0.18)	1.04	128	<0.01
Sex				
Male	-1.24 (0.29)	1.12	112	
Female	-1.32 (0.27)	1.05	153	0.523
Household Income				
< 15,000	-1.38 (0.25)	0.89	57	
> 15,000	-0.88 (0.41)	1.10	189	<0.01
Education Head of Household				
High School or Less	-1.34 (0.26)	1.05	111	
College or Technical School	-1.62 (0.27)	1.12	108	0.921
Home uses Air Conditioning				
Yes	-1.30 (0.27)	1.10	226	
No	-1.22 (0.29)	0.94	39	0.624
Age of House				
Before 1950	-1.15 (0.32)	1.08	132	
1950 -1991	-1.62 (0.20)	1.06	82	<0.01
Cigarette Smoking				
Current Smoker	-0.95 (0.39)	1.12	75	
Non Smoker	-1.42 (0.24)	1.04	190	<0.01

Table 53.—continued Comparison of natural logs of the mean and standard deviations of urine cadmium among adults 15 years to 75 years of age among variables of interest by contamination source Smelting versus Mining/smelting. Multisite Lead and Cadmium Health Study, 1991.

Category	Log of the Mean (Geometric Mean) µg/g creatinine	s.d.	N	P-Value
Garden				
Has a Garden	-1.44 (0.24)	1.06	107	
Does Not Have a Garden	-1.19 (0.30)	*1.08	158	0.064
Alcohol Consumption				
Yes	-1.26 (0.28)	1.08	222	
No	-1.44 (0.24)	1.04	43	0.3019

Model 25

Table 54.—Linear regression model for cadmium* (adjusted for creatinine ≥25 mg/dl) in adults age 15 years and older. Multisite Lead and Cadmium Study, 1991.

Variable	Beta	Standard Error	P-Value
Intercept	-1.957	0.136	<0.01
Smelter	0.593	0.149	<0.01
Smoke Now	0.509	0.143	<0.01

Model F-ratio = 13.92, p = 0.0001, n = 266, adj r^2 = .10. Multisite, 1991.

*Natural Logarithm.

Model 26

Table 55.—Linear regression model for cadmium* (adjusted for creatinine >25 mg/dl) among adults age 15 years and older with environmental factors. Multisite Lead and Cadmium Study, 1991.

Variables	Beta	Standard Error	P-Value
Intercept	-0.782	0.304	0.012
Dust* (Cadmium)	-0.206	0.092	0.027

Model F-ratio = 5.04, p = 0.0273, n = 89, adj r^2 = .055. Multisite, 1991.

Table 56.—Comparison of the distribution of Alanine aminopeptidase (UAAP). (adjusted for creatinine ≥50 mg/dl). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	1.715 (5.55)	0.660	-2.29 (0.10)	3.87 (47.9)	858	0.801	0.356
Comparison	1.704 (5.00)	0.603	-1.58 (0.21)	3.66 (38.9)	320		
Children 6-71 months							
Target	2.05 (7.76)	0.551	-0.59 (0.55)	3.87 (47.9)	292	0.600	0.796
Comparison	2.01 (7.46)	0.550	-0.18 (0.84)	3.66 (38.9)	79		
Children 6-14 years							
Target	1.61 (5.00)	0.637	-1.58 (0.21)	3.06 (21.3)	323	0.168	0.677
Comparison	1.69 (5.41)	0.611	-1.58 (0.21)	3.14 (23.1)	130		
Adults 15-75 years							
Target	1.457 (4.29)	0.650	-2.29 (0.10)	2.98 (19.7)	238	0.534	0.970
Comparison	1.500 (4.48)	0.540	0.24 (1.27)	3.11 (22.4)	111		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 57.—Comparison of the distribution of Gamma glutamyl transferase (UGGT) (adjusted for creatinine ≥50 mg/dl). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	3.259 (26.0)	0.534	0.76 (2.14)	4.81 (122.7)	858	0.323	0.119
Comparison	3.536 (34.3)	0.513	1.32 (3.74)	4.92 (137.0)	320		
Children 6-71 months							
Target	3.536 (34.3)	0.478	1.00 (2.72)	4.63 (102.5)	292	0.959	0.730
Comparison	3.539 (34.4)	0.525	1.32 (3.74)	4.92 (137.0)	79		
Children 6-14							
Target	3.189 (24.3)	0.492	1.33 (3.78)	4.81 (122.7)	328	0.165	0.319
Comparison	3.256 (25.9)	0.453	1.52 (4.57)	4.52 (91.8)	130		
Adults 15-75 years							
Target	3.012 (20.3)	0.504	0.76 (2.14)	4.11 (60.9)	238	0.350	0.034
Comparison	2.963 (19.4)	0.432	1.82 (6.17)	4.78 (119.1)	111		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 58.—Comparison of the distribution of N-Acetyl-β-D-Glucosaminidase (UNAG) (adjusted for creatinine ≥50 mg/dl). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	-0.391 (0.68)	0.615	-2.15 (0.12)	2.48 (11.9)	887	0.532	0.984
Comparison	-0.366 (0.69)	0.644	-1.91 (0.15)	2.17 (8.76)	334		
Children 6-71 months							
Target	-0.232 (0.79)	0.543	-1.45 (0.23)	1.75 (5.75)	310	0.993	0.690
Comparison	-0.231 (0.79)	0.502	-1.49 (0.22)	1.27 (5.75)	84		
Children 6-14 years							
Target	-0.526 (0.59)	0.549	-1.84 (0.16)	1.19 (3.29)	338	0.081	0.305
Comparison	-0.478 (0.62)	0.553	-1.60 (0.20)	1.39 (4.01)	135		
Adults 15-75 years							
Target	-0.408 (0.66)	0.735	-2.15 (0.17)	2.48 (11.9)	239	0.849	0.644
Comparison	-0.390 (0.68)	0.808	-1.91 (0.15)	2.17 (8.76)	115		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 59. —Comparison of the distribution of Serum Creatinine. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	-0.412 (0.66)	0.300	-1.20 (0.30)	0.50 (1.80)	1551	<0.01	0.117
Comparison	-0.337 (0.71)	0.340	-1.20 (0.30)	0.74 (2.10)	479		
Children 6-71 months							
Target	-0.619 (0.53)	0.195	-1.20 (0.30)	0.18 (1.20)	748	<0.01	0.015
Comparison	-0.662 (0.52)	0.171	-1.20 (0.30)	-0.22 (0.80)	174		
Children 6-14 years							
Target	-0.341 (0.71)	0.177	-0.92 (0.40)	-0.10 (1.10)	485	0.269	0.506
Comparison	-0.322 (0.72)	0.186	-0.92 (0.40)	0.18 (1.20)	159		
Adults 15-75 years							
Target	-0.034 (0.97)	0.230	-0.92 (0.40)	0.59 (1.80)	318	<0.01	0.234
Comparison	0.034 (1.03)	0.206	-0.36 (0.70)	0.74 (2.10)	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 60.—Comparison of the distribution of Serum Blood Urea Nitrogen (BUN). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	2.553 (12.8)	0.301	1.09 (3.0)	3.61 (37.0)	1550	<0.01	0.055
Comparison	2.610 (13.6)	0.284	1.61 (5.0)	3.74 (42.0)	479		
Children 6-71 months							
Target	2.536 (12.6)	0.311	1.10 (3.0)	3.45 (32.0)	747	<0.01	0.016
Comparison	2.613 (13.6)	0.302	1.79 (6.0)	3.43 (31.0)	174		
Children 6-14 years							
Target	2.582 (13.2)	0.229	1.61 (5.0)	3.13 (23.0)	485	0.959	0.546
Comparison	2.583 (13.2)	0.229	1.95 (7.0)	3.30 (27.0)	159		
Adults 15-75 years							
Target	2.556 (12.9)	0.326	1.39 (4.0)	3.61 (37.0)	318	0.012	0.195
Comparison	2.635 (13.9)	0.313	1.61 (5.0)	3.74 (23.0)	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 61.—Comparison of the distribution of Hematocrit (HCT). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	3.62 (37.3)	0.090	3.24 25.53	3.94 51.41	1592	<0.01	<0.01
Comparison	3.65 (38.5)	0.092	3.35 28.50	3.93 50.90	477		
Children 6-71 months							
Target	3.57 (35.5)	0.066	3.24 25.53	3.85 46.99	793	0.319	0.235
Comparison	3.58 (35.9)	0.052	3.42 30.56	3.73 41.67	171		
Children 6-14 years							
Target	3.63 (37.7)	0.068	3.36 28.79	3.86 47.46	485	<0.01	<0.01
Comparison	3.65 (38.5)	0.058	3.48 32.46	3.89 48.91	163		
Adults 15-75 years							
Target	3.72 (41.3)	0.094	3.43 30.87	3.94 51.41	314	0.157	0.441
Comparison	3.73 (41.7)	0.098	3.35 28.50	3.93 50.90	143		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 62.—Comparison of the distribution of Hemoglobin (HBG). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	2.55 (12.8)	0.091	2.08 8.00	2.94 18.91	1592	<0.01	<0.01
Comparison	2.57 (13.06)	0.091	2.12 8.33	2.83 16.94	477		
Children 6-71 months							
Target	2.50 (12.2)	0.071	2.08 8.00	2.80 16.44	793	0.245	0.165
Comparison	2.51 (12.3)	0.055	2.31 10.07	2.62 13.73	171		
Children 6-14 years							
Target	2.56 (12.9)	0.071	2.27 9.67	2.94 18.91	488	<0.01	<0.01
Comparison	2.57 (13.0)	0.060	2.40 11.02	2.81 16.60	163		
Adults 15-75 years							
Target	2.64 (14.0)	0.100	2.32 10.20	2.90 18.20	314	0.530	0.995
Comparison	2.63 (13.9)	0.111	2.11 8.24	2.83 16.94	143		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 63.—Comparison of the distribution of Mean Corpuscular Volume (MCV). Multisite Lead and Cadmium Study, 1991.

Category	Log Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	4.42 (83.1)	0.152	3.96 52.45	4.66 105.63	159 2	0.343	0.308
Comparison	4.41 (82.3)	0.187	4.12 61.55	4.64 103.54	477		
Children 6-71 months							
Target	4.39 (80.6)	0.071	3.96 52.45	4.52 91.83	793	0.075	0.613
Comparison	4.37 (79.0)	0.055	4.16 64.07	4.50 90.01	171		
Children 6-14 years							
Target	4.42 83.0	0.185	4.29 72.96	4.54 93.69	485	<0.487	0.032
Comparison	4.41 (82.2)	0.047	4.20 66.68	4.53 92.75	163		
Adults 15-75 years							
Target	4.48 (88.2)	0.054	4.14 62.80	4.66 105.63	314	0.677	0.839
Comparison	4.48 (88.2)	0.057	4.12 61.55	4.64 103.54	143		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 64.—Comparison of the distribution of Mean Corpuscular Hemoglobin (MCH). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	3.35 (28.2)	0.075	2.76 15.8	3.57 35.51	1592	0.435	0.242
Comparison	3.34 (28.2)	0.072	2.97 19.49	3.60 36.59	477		
Children 6-71 months							
Target	3.32 (27.7)	0.066	2.76 15.8	3.45 31.50	793	0.51	0.244
Comparison	3.32 (27.7)	0.062	3.04 20.90	3.60 36.59	171		
Children 6-14 years							
Target	3.53 34.12	0.026	3.37 29.07	3.59 36.23	485	0.153	<0.01
Comparison	3.52 33.78	0.029	3.42 30.56	3.58 35.87	163		
Adults 15-75 years							
Target	3.40 (30.0)	0.071	3.17 23.80	3.50 31.11	315	0.901	0.038
Comparison	3.38 29.37	0.079	3.02 20.49	3.48 32.45	143		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 65.—Comparison of the distribution of Mean Corpuscular Hemoglobin Concentration (MCHC). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	3.53 (34.1)	0.075	3.21 24.77	3.66 38.86	1592	<0.01	0.242
Comparison	3.52 33.78	0.072	3.34 28.21	3.64 38.09	477		
Children 6-71 months							
Target	3.53 (34.12)	0.022	3.21 24.77	3.66 38.86	793	0.425	0.244
Comparison	3.53 (34.12)	0.028	3.45 31.50	3.64 38.09	171		
Children 6-14 years							
Target	3.35 (28.50)	0.026	3.38 29.37	3.59 36.23	485	0.153	<0.01
Comparison	3.34 (28.21)	0.024	3.42 30.56	3.58 35.87	163		
Adults 15-75 years							
Target	3.52 (33.78)	0.037	3.41 30.26	3.62 37.33	314	<0.01	0.038
Comparison	3.41 30.26	0.043	3.42 30.56	3.33 27.93	143		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 66.—Comparison of the distribution of Immunoglobulin G (IgG). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	6.76 (862.6)	0.324	187	2165	1567	<0.01	<0.01
Comparison	6.82 (916.0)	0.330	230	2322	484		
Children 6-71 months							
Target	6.58 (720.5)	0.299	187	1394	759	0.745	0.685
Comparison	6.57 (713.4)	0.299	230	1300	176		
Children 6-14 years							
Target	6.90 (992.3)	0.248	192	1978	488	0.275	0.129
Comparison	6.93 (1022.5)	0.269	483	1845	162		
Adults 15-75 years							
Target	6.98 (1074.9)	0.240	493	493	320	0.234	0.340
Comparison	7.01 (1107.6)	0.233	590	590	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 67.—Comparison of the distribution of Immunoglobulin A (IgA). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	4.63 (102.5)	0.695	7	773	1560	<0.01	<0.01
Comparison	4.73 (113.3)	0.746	6	679	482		
Children 6-71 months							
Target	4.22 (68.05)	0.556	7	337	757	0.067	0.029
Comparison	4.13 (62.2)	0.584	6	242	175		
Children 6-14 years							
Target	4.80 (121.5)	0.502	9	455	483	0.815	0.664
Comparison	4.81 (122.7)	0.561	11	416	161		
Adults 15-75 years							
Target	5.35 (210.6)	0.523	13	773	320	0.717	0.968
Comparison	5.37 (214.9)	0.481	51	679	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 68.—Comparison of the distribution of Immunoglobulin M (IgM). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	4.73 (113.3)	0.473	26	572	1567	0.197	0.468
Comparison	4.77 (117.9)	0.511	17	608	484		
Children 6-71 months							
Target	4.63 (102.5)	0.431	33	334	759	0.483	0.511
Comparison	4.61 (100.5)	0.389	35	287	176		
Children 6-14 years							
Target	4.74 (114.4)	0.440	26	407	488	0.184	0.400
Comparison	4.80 (121.5)	0.470	26	407	162		
Adults 15-75 years							
Target	4.98 (145.5)	0.511	33	578	320	0.464	0.675
Comparison	4.94 (139.7)	0.618	17	608	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 69.—Comparison of the distribution of Lymphocyte Count mm³ (LYMPHCT). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value§§
All Ages Combined							
Target	8.08 (3229.2)	0.395	898	9799	1474	<0.01	<0.01
Comparison	7.98 (2921.9)	0.388	317	9605	476		
Children 6-71 months							
Target	8.28 (3944.2)	0.382	1002	9799	732	0.972	0.965
Comparison	8.27 (3904.4)	0.360	1686	9605	170		
Children 6-14 years							
Target	7.95 (2835.6)	0.298	1002	8350	452	0.017	<0.01
Comparison	7.89 (2670.4)	0.275	1224	6248	161		
Adults 15-75 years							
Target	7.82 (2489.9)	0.318	898	8103	290	0.026	0.019
Comparison	7.75 (2321.6)	0.313	863	7480	145		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 70.—Comparison of the distribution of CD4 Count mm^3. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	7.26 (1422.2)	0.407	420	5219	1418	<0.01	<0.01
Comparison	7.19 (1326.1)	0.405	455	5219	469		
Children 6-71 months							
Target	7.42 (1669.9)	0.413	420	5219	694	0.363	0.387
Comparison	7.45 (1719.92)	0.400	679	5219	170		
Children 6-14 years							
Target	7.07 (1117.1)	0.310	464	3984	444	0.170	0.077
Comparison	7.04 (1141.4)	0.286	561	2670	159		
Adults 15-75 years							
Target	7.11 (1224.1)	0.347	464	3790	280	0.014	0.044
Comparison	7.02 (1118.89)	0.350	455	2368	140		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 71.—Comparison of the distribution of CD4/CD8 Ratio. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	0.65 (1.92)	0.363	0.44	6.89	1558	0.085	0.345
Comparison	0.68 (1.97)	0.374	0.38	7.46	481		
Children 6-71 months							
Target	0.70 (2.01)	0.349	0.77	6.75	764	0.207	0.614
Comparison	0.74 (2.09)	0.347	0.97	5.81	178		
Children 6-14 years							
Target	0.53 (1.70)	0.334	0.44	4.90	484	<0.01	0.079
Comparison	0.61 (1.84)	0.292	0.91	4.66	161		
Adults 15-75 years							
Target	0.71 (2.03)	0.393	0.95	6.89	310	0.796	0.883
Comparison	0.70 (2.01)	0.465	0.38	7.46	142		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 72.—Comparison of the distribution of Gamma-Glutamyl transferase (GGT). Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	2.61 (13.6)	0.497	0	194	1538	0.334	0.478
Comparison	2.64 (14.0)	0.615	0	230	148		
Children 6-71 months							
Target	2.43 (11.4)	0.385	0	196	739	<0.01	<0.01
Comparison	2.32 (10.2)	0.403	0	166	174		
Children 6-14 years							
Target	2.58 (13.2)	0.355	5	171	480	0.058	0.857
Comparison	2.52 (12.4)	0.355	5	35	162		
Adults 15-75 years							
Target	3.05 (21.1)	0.633	2	176	317	0.176	0.327
Comparison	3.14 (23.1)	0.727	5	230	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 73.—Comparison of the distribution of AST Serum Glutamic Oxalacetic Transferase (SGOT) Liver Function. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	3.25 (26.8)	0.379	3	437	1541	0.029	<0.01
Comparison	3.21 (24.8)	0.357	5	171	480		
Children 6-71 months							
Target	3.45 (31.5)	0.270	8	437	741	0.689	0.473
Comparison	3.47 (32.1)	0.282	5	171	174		
Children 6-14 years							
Target	3.20 (24.5)	0.310	3	78	482	0.203	0.046
Comparison	3.17 (23.8)	0.220	9	40	160		
Adults 15-75 years							
Target	2.87 (17.6)	0.362	4	106	318	0.013	0.019
Comparison	2.96 (19.3)	0.360	6	134	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 74.—Comparison of the distribution of Serum Albumin Liver Function. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	2.64 (14.0)	0.451	2	473	1540	0.036	0.067
Comparison	2.69 (14.7)	0.437	4	126	480		
Children 6-71 months							
Target	2.62 (13.7)	0.395	5	473	740	0.135	0.044
Comparison	2.67 (14.4)	0.361	4	126	174		
Children 6-14 years							
Target	2.58 (13.2)	0.410	3	119	482	0.694	0.866
Comparison	2.57 (13.1)	0.323	4	48	162		
Adults 15-75 years							
Target	2.78 (16.1)	0.586	2	129	318	0.219	0.419
Comparison	2.78 (17.3)	0.564	5	74	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

Table 75.—Comparison of the distribution of Serum Total Protein Liver function. Multisite Lead and Cadmium Study, 1991.

Category	Mean (Geom. Mean)	s.d.*	Min.†	Max.§	N¶	T-test P-Value**	K-S P-Value††
All Ages Combined							
Target	1.99 (7.3)	0.067	6	10	1551	0.111	0.191
Comparison	2.00 (7.4)	0.065	6	9	480		
Children 6-71 months							
Target	1.97 (7.2)	0.062	6	9	748	0.506	0.782
Comparison	1.96 (7.1)	0.061	6	8	174		
Children 6-14 years							
Target	2.02 (7.5)	0.062	1	6	485	0.964	0.848
Comparison	2.02 (7.5)	0.056	1	6	160		
Adults 15-75 years							
Target	2.02 (7.5)	0.060	6	9	318	0.924	0.995
Comparison	2.02 (7.5)	0.061	6	9	146		

* Standard deviation.
† Minimum.
§ Maximum.
¶ Sample size
** Student t-test p-value.
†† Kolmogorov-Smirnov p-value. A p-value >0.05 indicates that the two distributions are not significantly different.

FIGURES

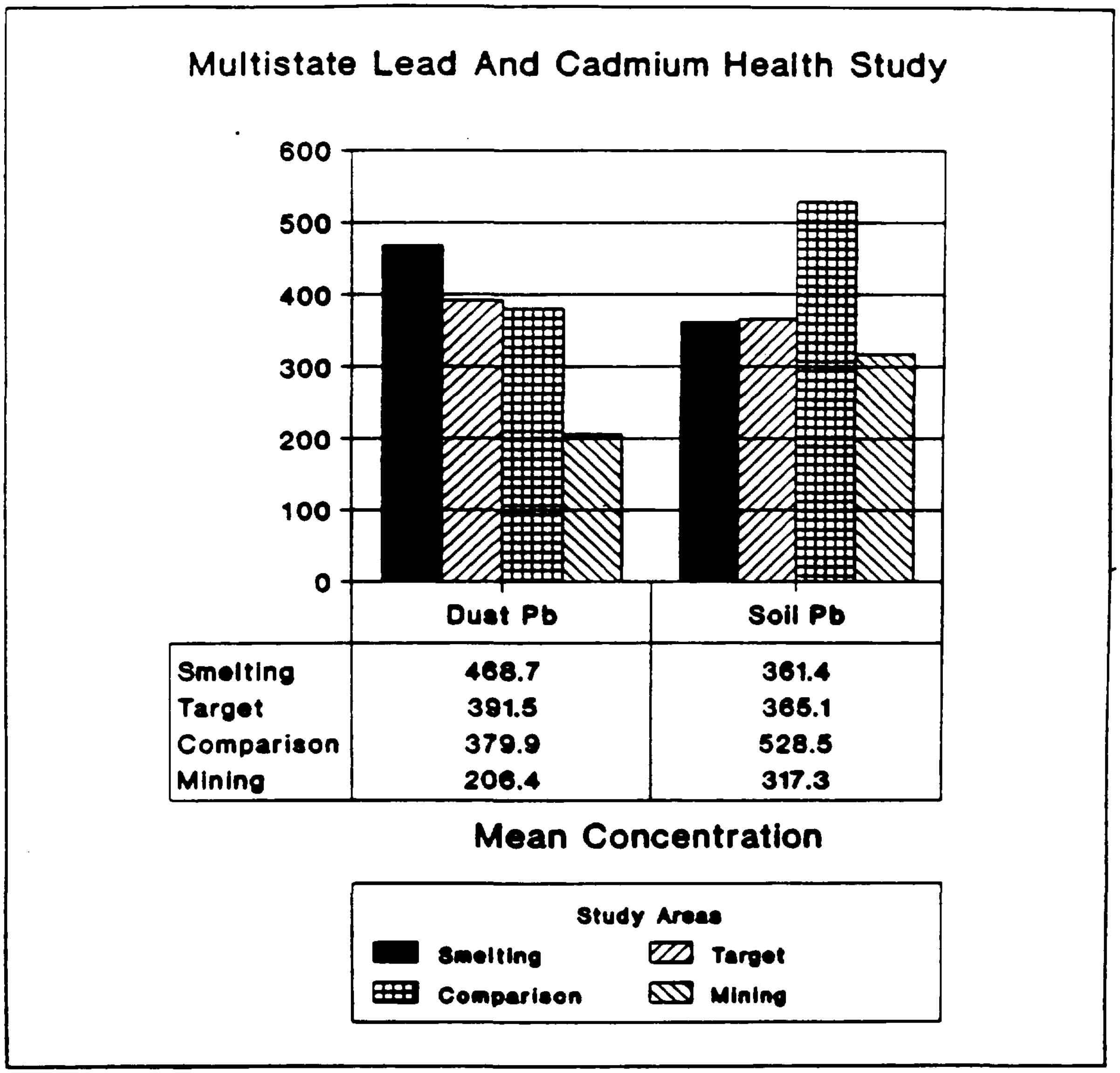


Figure 1. Mean lead concentration in residential soil and house dust measured in (mg/kg) for each study area.

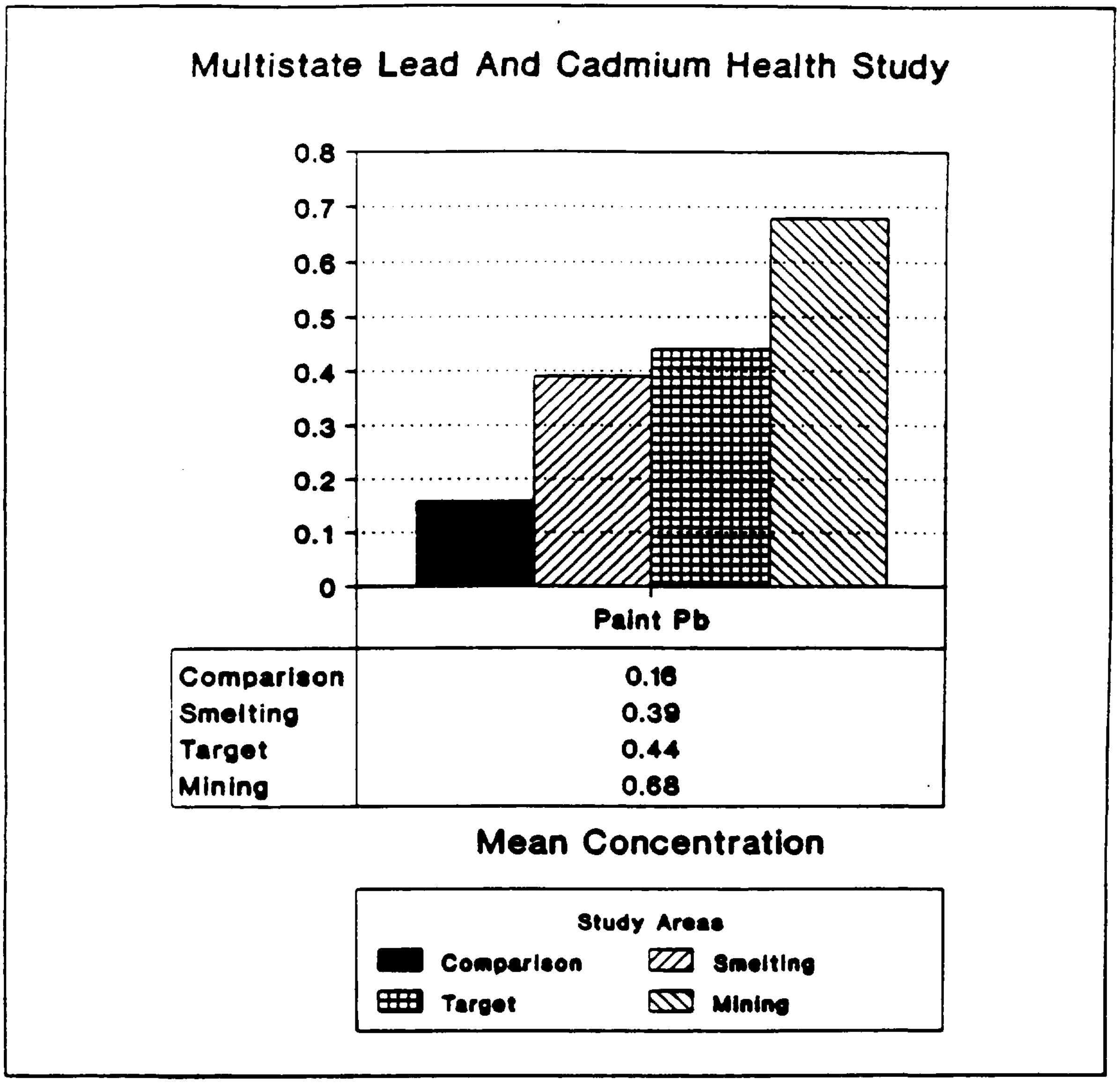
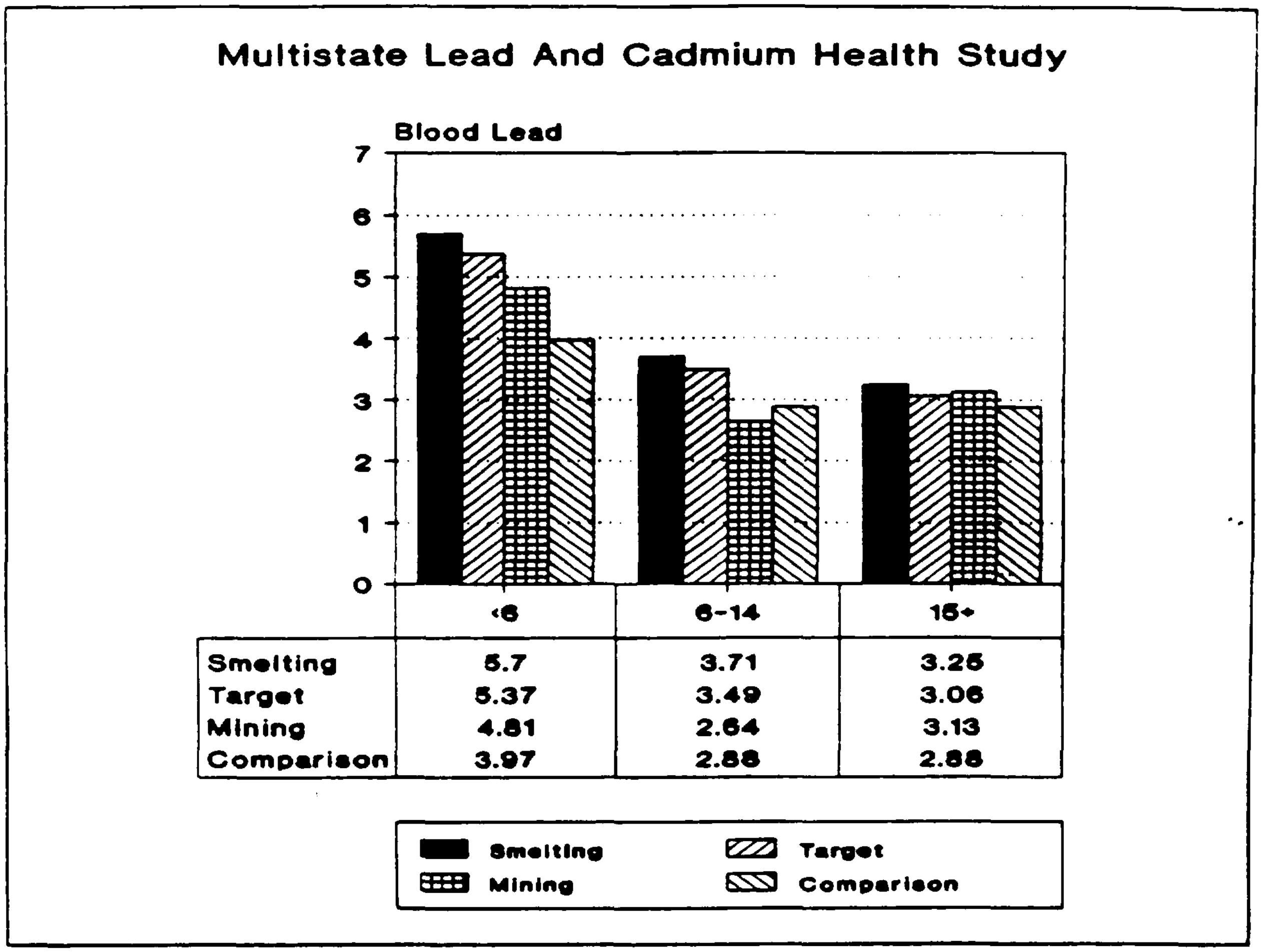
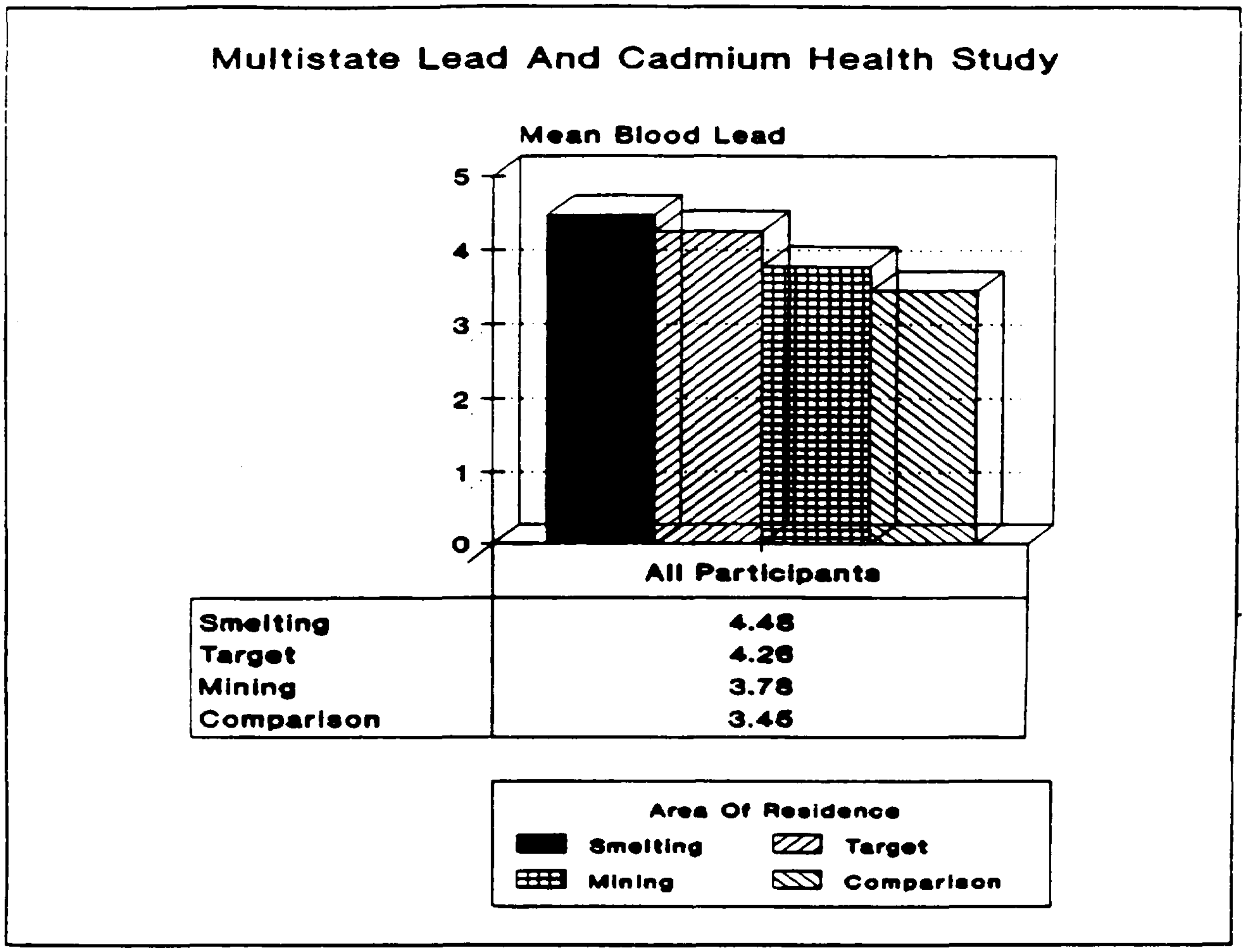


Figure 2. Mean lead concentration in interior paint measured by XRF (mg/cm^2) shown on the vertical axis.



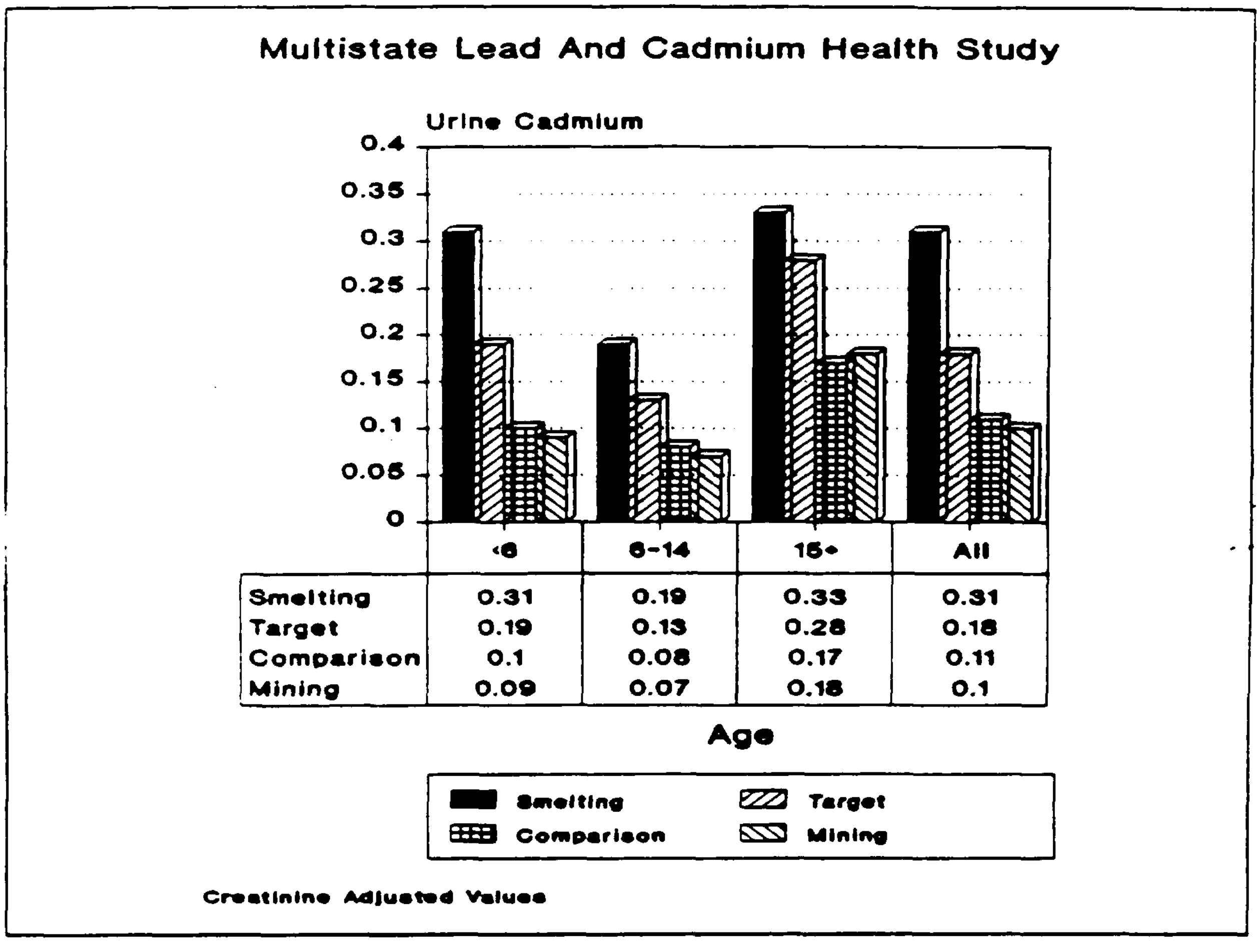
	<6	6-14	15+
Smelting	5.7	3.71	3.25
Target	5.37	3.49	3.06
Mining	4.81	2.64	3.13
Comparison	3.97	2.88	2.88

Figure 3. **Mean** blood lead measured in $\mu g/dl$ for study participants by age **and area** of residence.



	All Participants
Smelting	4.48
Target	4.26
Mining	3.78
Comparison	3.46

Figure 4. Mean blood lead values for participants of all age groups by area of residence.



	<6	6-14	15+	All
Smelting	0.31	0.19	0.33	0.31
Target	0.19	0.13	0.28	0.18
Comparison	0.1	0.08	0.17	0.11
Mining	0.09	0.07	0.18	0.1

Figure 5. Mean urine concentration of cadmium by age of participant group and area of residence. Cadmium excretion is creatinine adjusted (µg/gram creatinine).

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APPENDICES

The contents of these appendices are presented in their entirety as submitted by the author and have not been revised or edited to conform with the Agency for Toxic Substances and Disease registry guideline.

Appendix A-Survey Census Form

MISSOURI DEPARTMENT OF HEALTH
HEAVY METAL EXPOSURE ASSESSMENT
CENSUS FORM

Address ______________________ Interviewer Initials ______________
(Street, RR, Box #)

City ______________________ Telephone Home (______)__________
Zip

Date ______________________ Work (______)__________

Mailing Address ____

____

	NAME	DATE OF BIRTH/AGE	SEX	IF CHOSEN, WILLING TO PARTICIPATE?
A.	Persons 9 months - 5 years old (List by age, oldest first)			
1.				
2.				
3.				
4.				
B.	Persons 6 - 15 years old			
1.				
2.				
3.				
4.				
C.	**Persons 16 - 44 years old**			
1.				
2.				
3.				
4.				

DRAFT

Has everyone listed above lived at this address longer than 60 days. **YES** **NO**

If no, whom? ____

PARTICIPANT ID NUMBER 91- ____________ LAB ID NUMBER __________

MISSOURI DEPARTMENT OF HEALTH

HEAVY METAL EXPOSURE ASSESSMENT QUESTIONNAIRE

FOR PARTICIPANTS ~~9~~ 6 MONTHS - 5 YEARS OF AGE

The following person has been selected to be part of the second phase of the Missouri Department of Health Heavy Metals Exposure Assessment. We need to get some information on this individual.

For this child, I will need to talk to the parent or legal guardian, ______________

______________ (name of parent or legal guardian) preferably the person who can tell us about how (child's name) spends his/her time.

IF THE SELECTED INDIVIDUAL IS NOT AVAILABLE:

When can I return to talk to (parent or legal guardian)?

__________ Day of Week

__________ Month/Day

__________ Time - AM PM

DRAFT

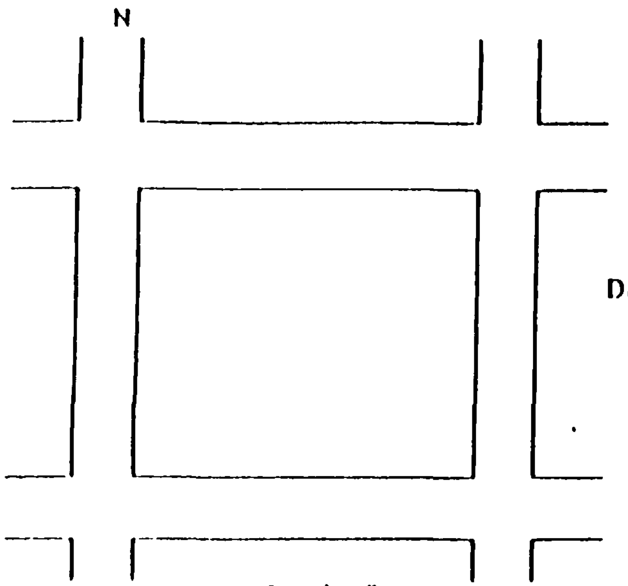
I need to ask a number of questions about (child's name) who was selected for the study.

201. What is the child's full/legal name? ______________________

Lead Exposure Survey

BLOCK SURVEY FORM

Date __/__/9_ Interviewer: ______________________



Block # __

Building#	Premises Address	Residential/ Single Family	Residential/ Multiple Family	Vacation/ Weekend	Business	Vacant	Eligible	Not Eligible	Completed	Refused	Comments

Start with youngest member of household.

Name	Sex 1-M 2-F	Relationship 1-self 2-spouse 3-parent 4-child 5-other family 6-other	Birthdate mo-day-yr	Lived in this house 60 days? 1-yes 2-no	(If NO) Lived in Leeds 60 Days? 1-yes 2-no	Previous address and dates
1. F ________ MI __ L ________	__	__	__ __-__ __-__ __	__	__	
2. F ________ MI __ L ________	__ __	__	__ __-__ __-__ __	__	__	
3. F ________ MI __ L ________	__ __	__	__ __-__ __-__ __	__	__	
4. F ________ MI __ L ________	__	__	__ __-__ __-__ __	__	__	
5. F ________ MI __ L ________	__ __	__	__ __-__ __-__ __	__	__	

Start with youngest member of household.

Name	Sex 1-M 2-F	Rela-tionship 1-self 2-spouse 3-parent 4-child 5-other family 6-other	Birthdate mo-day-yr	Lived in this house 60 days? 1-yes 2-no	(If NO) Lived in Leeds 60 Days? 1-yes 2-no	Previous address and dates
1. F ________ MI __ L ________	__	__	__-__-__	__	__	
2. F ________ MI __ L ________	__ __	__	__-__-__	__	__	
3. F ________ MI __ L ________	__ __	__	__-__-__	__	__	
4. F ________ MI __ L ________	__	__	__-__-__	__	__	
5. F ________ MI __ L ________	__ __	__	__-__-__	__	__	

Appendix B-Environmental Sampling Protocols

QUALITY ASSURANCE PROJECT PLAN

FOR

EPA REGION V SUPPORT

OF THE ATSDR

MULTISTATE LEAD EXPOSURE STUDY

Approved:

David A. Payne for C.T. Elly

Director, Central Regional Laboratory 9/18/91

* Region 5 Quality Assurance Manager 9/18/91

Brad Bradley

Superfund Remedial Project Manager 9/18/91

* Any environmental measurements and samples collected prior to this approval date, were done at the discretion of the Remedial Project Manager and subject to on-site field audit verification, and appear to be collected using the same procedures as are approved herein.

Table of Contents

I. Project Description

Granite City, Illinois is the location of a former secondary lead smelting facility. Metal refining, fabricating, and associated metal processing activities have been conducted at the site since 1903. From 1903 to 1983, secondary smelting occurred on-site. Secondary smelting facilities included a blast furnace, a rotary furnace, several lead melting kettles, a battery breaking operation, a natural gas-fired boiler, several baghouses, cyclones and an afterburner. Most (85 percent) of the air samples taken from Granite City between 1978 and 1981, as part of IEPAs newly instituted air quality testing for lead, showed lead levels higher than levels the federal government considers safe. Metallic pollutants, which have been dispersed throughout the environment in Granite City and the surrounding areas, have heavily contaminated soil in the study area. It is likely that uptake of metallic pollutants by plants and animals, including humans, has occurred. The Agency for Toxic Substances and Disease Registry (ATSDR) has provided funding to the State of Illinois to conduct a comprehensive blood lead/urinary cadmium study on a representative number and distribution of eligible residents nearby the site. The study will include the collection of samples from potential study will include the collection of samples from potential environmental sources of lead and cadmium: soil, house dust, drinking water and indoor paint, from all participant households.

The objectives of the overall study are defined in the ATSDR study protocol (Draft; Summer, 1991; pages 8 and 9). Of the seven objectives listed, the objectives to which EPA participation will contribute are:

> "To determine the level of environmental lead and cadmium contamination found in target areas and compare them with levels of contamination found in comparable non-target areas."

> "To determine the extent to which environmental, behavioral, occupational, and socio-economic factors influence exposure to lead and cadmium in target and non-target populations."

> "To determine the extent to which exposure has occurred in populations living in areas with both mining and industrial emissions compared to populations living in areas with industrial emission only."

In order to contribute to meeting these goals, EPA will collect environmental samples at the residences of selected study participants, as discussed in section IV.A.2, below. Of special interest in the study will be households with children between 6 and 71 months of age.

The specific objectives of EPA participation in the study will be:

1. Collection of representative samples of house dust, drinking water, and play area soil, and in-situ analysis of paint by XRF for Pb (Paint is not considered a major exposure route for Cd), from a randomly selected subset of study participant residences.

2. Provision of data to ATSDR for determination of the probability that a statistically significant relationship, if any, exists between the environmental lead levels in the four sampled media and the human exposure data.

Environmental sampling in this study will be performed by the U.S. EPA Region V contractor, Ecology and Environment (E&E). Environmental samples will be sent by E&E to a CLP lab for analysis. E&E will report analytical results to U.S. EPA Region V. This document describes the procedures and activities which will be applied to such samples.

II. <u>Project Organization and Responsibilities</u>

A. Pat Van Leeuwen, toxicologist, WMD/OSF/TSU, will have the responsibility for maintaining overall communication with ATSDR and the Illinois Department of Public Health and for providing input on questions having toxicological aspects.

Brad Bradley, Remedial Project Manager, WMD/OSF/IL/IN Section, will be the EPA contact to E&E, which will perform project sampling, and will provide input on questions having technical aspects.

B. The Illinois Department of Public Health (IDPH) shall, through designated representatives, interface with Mr. Bradley to provide listing of names, addresses, and telephone numbers of all households where environmental sampling is to occur. Identification and notification of households with children exhibiting elevated blood lead or urinary cadmium levels shall be the responsibility of IDPH.

C. As noted above, environmental sampling in this study will be performed by the USEPA's contractor, Ecology and Environment. The contractor, in accepting the assignment to support this Study, agrees to perform sampling activities as outlined in this Plan and in conformance with applicable Region V CLP SAS - and E & E field SOPs as approved for their Region V ARCS Contract, and other guidance which may be provided by EPA for performance of Study-related activities.

D. Sample receipt, storage, handling, and custody within the laboratory will be the responsibility of the selected CLP laboratory.

E. The selected CLP laboratory will receive and analyze the environmental samples and report analytical results to Region V representatives, following procedures outlined in this Plan.

F. Final data review and validation will be the responsibility of E&E.

G. Transmittal of reviewed and validated data on disk to U.S. EPA Region V will be the responsibility of E&E.

H. Transmittal of final data in a brief report to U.S. EPA Region V will be the responsibility of E&E.

I. Brad Bradley will be responsible for the dissemination of applicable environmental data to the appropriate entities in the State of Illinois, for responding to questions from the State, and for addressing public questions relating to the study from the Federal perspective.

J. ATSDR will assume final Federal responsibility for the Study data because of the greater protection of individual privacy afforded ATSDR data bases; EPA final data is subject to FOIA request actions. ATSDR will perform statistical review of the environmental data vis-a-vis human exposure data. All study data shall be made available to EPA upon request, for purposes such as evaluating the Pb uptake/biokinetic model.

K. Program and field sampling QA/QC oversight will be the responsibility of E&E.

III. Data Quality Objectives

A. The data quality objectives (DQOs) for this project are to generate data that are of sufficient quality to enable the objectives of this project to be met. The sampling and analytical methods selected for this project (SOPs are referenced elsewhere) are consistent with these objectives in terms of accuracy, percision, and representativeness. Of the quantitative DQO components, the quantity of data, or completeness, is typically based on assumptions regarding the statistical variability of the study population to be sampled. For this project there are insufficient data available to make these assumptions with any degree of confidence. Precision and Accuracy objectives for the study data are consistent with those specified in the SAS requests (Appendix B).

B. An additional completeness goal for the laboratory will be the generation of useable analytical data for at least 95% of the samples received in acceptable condition. This means that out of the total amount of data that might potentially be generated for all samples analyzed, no more than 5% of the data will be unusable due to failure to meet analytical accuracy, precision, or detection limit goals stated in the referenced SASs, caused by analytical problems such as matrix interferences, or problem such as laboratory accidents, holding times or preservation violations, etc.

C. To minimize variability in the data reported as part of the Study, it is incumbent upon field samplers and their supervisors to become familiar with all sampling guidelines and procedures included herein or referenced, so as to ensure that the data reported from this Study will represent the overall environment form which the analyzed samples are taken. Any sub-sampling procedures performed in the laboratory will be done in accordance with the SAS requests.

D. To insure the comparability of data produced for this Study to that produced under other plans or studies, EPA accepted sampling and analytical methods, as documented in SOPs referenced herein, will be used whenever possible. All SOPs referenced are available in the E&E ARCS contract QAPP and Appendix A.

E. Method detection limits are dependent upon the specific properties of, and interferences present in, a given sample, and so may not always be achieved. Detection limit goals are to be one tenth the action levels specified in the table below for both metals in various media.

These detection limits will permit evaluation of field sample data against the following limits, so as to determine whether the samples are above background levels with a 95% confidence level.

Sample Medium	Action Level Lead	Cadmium
House Dust	500 ug/g	136 ug/g
Paint	0.7 mg/sq.cm.	N/A
Drinking Water	15 ug/L	5 ug/L
Play Area Soil	500 ug/g	136 ug/g

Note the detection limits of one-tenth the action levels noted may not be achieved if the minimum sample amounts discussed in Section IV, Sampling Protocols, are not collected. Also, available analytical methods may not permit analysis of Cd in water at concentration as low as 0.5 ug/L. A detection limit of 2.0 ug/L will be acceptable for lead in water.

IV. Sampling Protocols

A. **Environmental Sampling Design Considerations**

1. **Selection of Residences to be Sampled:**

   a. In order to meet the Study goals outlined above, EPA Region V will collect environmental samples: soil, house dust, drinking water and paint, from all households in the Study area at which biological sampling is scheduled. In order to identify high biomedical metal levels, an action level of 10 ug/dL of Pb in blood and/or 8 ug/L Cd in urine will be used.

   b. Environmental sampling will be conducted at all households where biomedical testing occurred. The names, address, and telephone numbers of residences to be sampled shall be forwarded to EPA by IDPH as soon as practicable. EPA plans to perform environmental sampling in one sampling event which is scheduled to begin the first week of September, and will last approximately four weeks.

c. Residential environmental sampling will be conducted as summarized in the table below:

Sampling Area	Total Households in study	# of Sampled Households
Granite City and the adjacent areas of Madison and Venice, IL	250	100% of homes
Control area - Pontoon Beach, IL	250	100% of homes

B. Pre-Sampling Verification Interview and Briefing

Prior to sampling, the IDPH will contact the study households to obtain access agreements for environmental sampling. IDPH shall then forward the names, addresses, and telephone numbers of households to be sampled to the EPA, which shall forward appropriate information to E&E.

If possible, E&E shall confirm sampling plans with a given household within one week of the scheduled sampling event. Upon arrival, the E&E sampling teams will briefly speak with the homeowner or other adult resident about the purpose and nature of the visit, and provide them with information written by ATSDR, to include telephone contacts for additional information.

If for some reason a household cannot be sampled (e.g. one is home), an attempt to reschedule sampling will be made.

C. Sample Collection, Documentation, and Handling

1. Sampling Number System: All samples will be assigned a unique identification number according to Region V CLP protocol. EPA will report data to ATSDR using such identification numbers, along with sufficient documentation for ATSDR to correlate the data with biomedical metal levels in study participants, and any other data collected by ATSDR or IDPH. All analyses shall be performed "blind" by the CLP laboratory staff; correlation or analytical data with site location information shall be performed after the analytical results are complete, as part of generating the final report to be forwarded to other project participant organizations.

2. Sample Containers: Sample containers and associated supplies will be obtained by E&E and prepared and utilized per Region 5 SARA Sample Handling Manual or E & E SOPs, with the exception that one liter poly bottles will be used for the collection of water samples.

3. Sample Collection Procedures:

Note: See the attached Appendix A, which shall supercede the language below in the event of any inconsistencies.

One field duplicate sample will be collected for every ten field samples of drinking water, soil and house dust.

a. Drinking Water samples will be collected in accordance with Appendix A: all samples of drinking water will be first-draw samples, as specified in the EPA's Final Rule for Lead and Copper in Drinking Water, Federal Register, June 7, 1991. These samples may be collected by the residents in sample containers without appropriate preservatives, supplied by E&E in advance, and picked up at the time of the dust, soil and paint sampling. Alternatively, E&E may choose to send a sampler first thing in the morning to all residences to be environmentally sampled that day to draw the samples, after pre-arranging with the residents so that the water is not turned on prior to sampling. Either method is acceptable, but the method chosen must be applied consistantly to all residences sampled during the project, and the choice of method must be documented in writing by E&E in the final project report. E&E will acid preserve these samples at the end of each day's activities.

One field blank (deionized water) will be submitted blind for laboratory analysis at a frequency of one in each set of twenty field samples.

b. Indoor House Dust: field sampling personnel will collect residential dust samples form primary play areas (areas most likely to impact on a child's hands or result in ingestion during indoor activity). A minimum of three areas should be sampled: at the main entrances to the household, and two additional areas most likely to be use by children in the household for play areas. Additional areas for sampling may include secondary entrances to the home (back or side doors), dust on window sills, furniture, and carpet in additional play areas or areas of frequent activity by the children. Bedroom, Kitchen, and living room floor samples will be collected first, followed by floor samples from the entry way. Finally, samples from window wells will be collected.

Once the individual sampling areas are determined, they should be noted on the sampling sheets, including the total area sampled for the household. One composite sample of dust will be taken and analyzed per household.

Vacuum equipment to be used will be equipped with a pre-weighed glass fiber filter (the weight of each filter will be noted in indelible ink on its zip-lock by the laboratory prior to shipment to the field) to trap the dust. The filter will be removed between residences and placed in a zip-lock bag for laboratory analysis. Alternatively, a modified portable "dustbuster" type vacuum cleaner may be used (Sirchee-Splittier method), with the dust removed after sampling each residence and placed in a zip-lock bag. Other necessary sampling equipment are zip-lock baggies containing pre-weighed filters with the weight noted on the bag in indelible ink, and a cylinder of compressed air to decontaminate sampling equipment.

c. Indoor Paint: Indoor paint shall be analyzed in-situ by a portable X-Ray Fluorescence (XRF) instrument, operated per manufacturer's instructions. Measurements will typically be made in play areas below three feet in elevation from the floor, indoor walls, door frames, window sills, and banisters, with special attention given to areas indicating peeling or chipped paint, or evidence of chewing on the surface by the resident children. A minimum of five locations will be measured and recorded on the field sheets. The condition of each painted surface sample will be noted on the field sheets by the instrument operator. The mean of the several individual readings will be reported as the paint lead value for the residence. Additional information is provided in Appendix A.

d. Play Area Soil: Field sampling personnel will identify play areas on the property used by children in the household through information available from the previous household survey (area census), pre-sampling questions of the residents, and visible signs of use (e.g. bare soil under a swing set). For each site a site sketch will be made on the sampling form indicating the position of the main building and any other buildings such as sheds or garages, paved areas, and play areas.

A representative number of such location(s), comprising not less than ten aliquots, will be proportionally sampled based on their relative areas and apparent degree of use; these are then composited to produce the one sample forwarded to the lab representing the entire play area. Exact locations to be sampled at a given residence will be chosen per the professional judgement of the sampling team leader, and will be fully documented on the field sheets. A corer shall be used to sample the top one inch of soil. Debris and leafy vegetation will be removed from the top of the core, but not soil or decomposed matter; this part of the soil sample is likely to be the highest in metal contamination. Samples will not be taken from locations within one foot of the house foundation per story of the residence unless there is clear indication such areas are in use as play areas, as chipped or peeling exterior paint may produce a typically high readings in such locations.

4. Field Sample Documentation:

   a. Field Sheets: Field sheets per SARA Sample Handling Manual or E&E SOPs shall be used to document locations and times of sampling, as well as all other appropriate details. In particular, sketches should be made of the locations sampled, especially dust and soil samples taken in the play areas, as noted above. E&E shall retain field sheets until instructed otherwise by EPA.

   b. Sample Chain of Custody: Sample chain-of-custody forms will be prepared per E&E SOPs.

D. Sample Delivery

All samples to be analyzed under this play will be delivered to the CLP Laboratory in accordance with E&E SOPs or Region 5 SARA Sample Handling Manual. Each set of samples will be delivered along with appropriate field documentation, Chain-of-Custody forms, and "Analytical Services Request Form(s)".

V. <u>Sample Receipt and Custody</u>

A. Immediately upon receipt of Study samples the CLP personnel will unpack and inspect the shipment, sign the Chain of Custody form, initiate appropriate internal tracking records, and store the samples in a secure area. If inspection of the shipment causes either the integrity or condition of the samples to be questioned (e.g. samples not cooled, broken containers, etc.), such observations will be noted on the

Chain of Custody Record and brought to the attention of the Region 5 RSCC or SAS Request Contract.

B. The CLP lab personnel or other appropriate personnel will be responsible for the custody, storage, handling, and disposal of all samples received for analysis under this plan.

1. Prior to analysis all non-aqueous samples received for analysis under this plan will be stored at ambient temperature. All aqueous samples will be stored per CLP SAS protocols.

2. Samples will be analyzed and the data will be reported within sixty days of receipt of the samples. Digestates will be disposed upon completion of data review and approval.

3. Approval must be granted before the required analyses may be considered to be complete for each sample. Such approval will be based upon the report of complete and appropriate data, as described in the SAS Request.

VI. Analytical Methodology

A. Preparation and analyses of the samples collected in this Study will be performed according to SAS Requests (Appendix B). Use of GFAA or ICP will be necessary to meet the required levels of accuracy, precision, and sensitivity (detection limits) noted above. Laboratory Quality Control shall be performed per SAS Requests data will be reviewed according to CLP Functional Guidelines for Evaluating Inorganics Data.

VII. Data Reduction and Validation

A. The reporting units and data reduction procedures used will be those specified in the action level table in Section III.E above. The data will be reviewed per CLP Functional Guidelines for Evaluating Inorganics Data, with this document being the basic reference for data usability.

VIII. Data Reporting

After data review, reduction, and validation, as a primary deliverable, a disk or "tape" of the data shall be supplied to EPA within 120 days of the completion of the field sampling operations.

for transmittal to ATSDR. A draft report summarizing the environmental data collected and an evaluation of the quality of such data shall be supplied to EPA within 150 days of the completion field sampling operations, for transmittal to the individual(s) noted in Section II above. The report will include statements that samples do or do not meet applicable criteria as spelled out in this document and applicable SOPs. Following receipt of U.S. EPA and ATSDR comments on the draft report, a final report shall be submitted to Brad Bradley within 30 days.

IX. Quality Control (QC) Checks

A. The laboratory QC procedures are incorporated into specific methodologies referenced in Appendix B, SAS Requests.

B. Field QC will include 10% duplicates (of each matrix), and 5% field blanks (at least one per day).

X. Performance and System Audits

Neither field audits nor laboratory audits beyond the routine QA/QC oversight of the appropriate supervisors is anticipated for this project, unless specifically determined to be necessary.

The CLP lab audits are the responsibility of EMSL - LV and Region 5, CRL. Field audits are the responsibility of the RPM, CRL and CDO.

XI. Preventive Maintenance (PM)

Lab preventive maintenance will be performed in accordance with manufacturer's specifications and applicable laboratory policies and SOP's.

Field - XRF - per manufacturer's specifications.

XII. Analysis of QC Data

All QC data will be reviewed by E&E personnel using the calculations and statistical methods specified in Region V protocols. This review will include an evaluation of accuracy, precision, completeness, sample representativeness, and comparability, using the methods discussed in Section IX., Internal Quality Control Checks, above.

XIII. Corrective Actions

All questionable data will be tracked by the analyst at the CLP lab to identify potential out-of-control situations. When an out-of-control situation is identified, it will be addressed per resolution with the SAS request contract or Region 5 RSCL.

APPENDIX A: FIELD SAMPLING PROTOCOLS

Note: In the event of inconsistencies between the following protocols and the QAPP, the protocols shall govern.

Preparation for the environment sample collection begins at the field office. The environmental team will be given an assignment for the morning or the entire day. Once the assignment is received, the environmental team members will check the accuracy and completeness of the data on each environmental sample form. The Dwelling ID Number and other identifying information should be on all the environmental forms.

The environmental team will then calibrate the Paint XRF instruments (Princeton Gamma-Tech XK-2 or XK-3). Either the Princeton Gamma-Tech XK-2 or the XK-3 instruments, or both, will be used. Both instruments operate on the same principle. The newer model, the XK-3 is capable of reading only to a maximum of 10 mg Pb/sq. cm. Paint in the older housing may have higher concentrations of lead, thus, when monitoring teams visit older housing, i.e., those built before 1940, the XK-2 should be used, if available. If the XK-2 is not available, an attempt should be made to extrapolate values greater than 10 mg Pb/sq.cm. with the XK-3.

After the necessary calibration of equipment, the environmental monitoring team should make certain that all equipment and supplies are ready for use.

All members of the team should wear appropriate identification.

Exterior and interior samples will be collected. Exterior samples to be collected are soil samples. The interior samples and information to be collected is as follows:

1) Collection of tap water samples.
2) Sketching a floor plan of the residence.
3) Collection of interior surface dust samples.
4) Screening for lead in painted surfaces; walls and trim, avoiding metal doors outlets, etc.

I. Soil Sample Collection

The Primary method of determining the lead content of the soil will be by acid digestion and graphite furnace atomic absorption spectrometry.

A. Site Description

For each location, a detailed drawing should be made that shows the boundary of the lot, the position of the main building and any other buildings such as storage sheds or garages, the position of the sidewalks, driveways, and other paved areas, the position of the play areas if obvious, and the position of the areas with exposed soil (grassy or bare), roof rain spouts and general drainage patterns.

In addition to the diagram, briefly describe the location, including the following information:

- Type of building construction (brick, wood, etc- 1 or 2 story)
- Condition of main building
- Condition of property (debris, standing water, vegetation cover)
- Presence and type of fence
- Animals on property
- Apparent use of yard (toys, sandbox, children present)
- Location of 10 soil aliquots

B. Sample Collection

Sample Collection shall be performed as outlined in the QAPP, with the exception that all aliquots will be of equal volume and will be mixed in a stainless steel bowl prior to packaging. Assemble composite soil core segments in 8 ounce glass jars suitable for prevention of contamination and loss of the sample. Record the sample identification number on the bag and the sample record sheet. Store the composite soil sample at ambient temperature until submitted to the laboratory for analysis.

Clean the corer after collecting each sample composite by reinsertion of the corer into the soil of the next sampling area.

C. Sample Handling and Storage

Seal the sample jars to prevent loss or contamination of the sample and store samples in a dry location at ambient temperature.

Record-keeping and Sample Custody: Initiate soil sample records for each location. Record sample numbers on location diagram, soil area description, and sample record sheet. Send the sample to the laboratory and release the sample to the laboratory personnel for analysis.

II. Surface Dust Collection

A. Sample Collection

A portable "dustbuster" type vacuum cleaner will be used; due to the sample size required, the Sirchee-Splittler modified dustbuster will not be used. Use a new bag for each household, to avoid cross-contamination. In order to ensure that the sample size is sufficient, either weigh the sample using a field scale or collect a large enough sample to ensure that three to five grams of dust have been collected.

B. Sample Areas

The interior surface dust sample will consist of a composite of sub-samples taken from the following areas in the residence:

Entry (E): A floor area inside the residence directly adjacent to the main entry to the residence.

Floor (F): At least 3 floor areas which should include but are not limited to a sample from a high-traffic area in the main living area and a sample from the child's bedroom. If carpet is present in the residence it shall be the first choice of sample area. If carpet is not present, a mixture of non-carpet floor areas will be sampled.

Window (W): At least three window areas (window sills and window wells), including but not limited to a window in the main living area and a window in the child's bedroom.

The main entry sample is collected from the floor close to the entry door. The entry mostly used by the family should be used. The identification of sample sites from the most frequently occupied room and the child's bedroom will be determined partly by the floor covering present in these rooms. If the floor is carpeted, a larger sample can readily be collected from almost any pathway in the room. A pathway might consist of an area immediately inside of a doorway into the room or an obvious pathway from one side of the room to the other. In rooms where there is no carpeting, the most likely place to find an adequate supply of surface dust would be an area immediately adjacent to a wall. For each floor surface, an approximately one meter square area should be vacuumed. Additional living areas (e.g. additional floor areas, around furniture, etc.) Should be vacuumed, if necessary, to obtain an adequate sample size. In no event shall dust be obtained from household areas where dust generally collects for long periods of time, such as behind major appliances, under beds, etc.

The sample sequence should be as follows: collect the bedroom, kitchen and living room samples first. Then, collect the floor sample from the entry way. Then, collect the window well samples. Finally, if necessary, collect the samples from additional living areas.

C. Sketch of Residence

In order to more fully describe where samples have been collected, a top view of the residence will be made by the sampling crew. This sketch should show the primary features of the residence, including a north arrow indicator and the relationship of the various rooms to each other. The sampling areas should also be indicated. Rooms should be labeled according to their apparent function.

III. Water Sampling

Residents will be provided with clean, capped bottles and instructed to collect water on the day of scheduled environmental sampling. The sampling team or its manager should give the following instructions to the resident who will collect the sample:

The tap water sample should be taken from the cold water faucet of the kitchen. It should be a first flush sample of water that has been standing in the pipes from 6 to 18 hours. There are two options for the time a sample is taken: (1) it can be taken first thing in the morning, or (2) if all of the residents of the household have been out of the house for the entire day, it can be taken at the end of the day (i.e. dinner time). Labelled plastic bottles will be provided for the sample. The bottle should be completely filled with water. The sampling team will pick up the sample at a convenient time on the day of scheduled environmental sampling.

Before dropping off a water collection bottle, the appropriate member of the sampling team will fill out and affix the label provided. The chain of custody form will be initiated when the collectors pick up the water sample. Region V will record pH and conductivity prior to acidifing the sample.

At the end of each collection day, water samples will be acidified with nitric acid, per required protocol. After the addition of the nitric acid to the water sample, the initials of the person adding the acid to the sample and the time and date will be recorded. In no event will the nitric acid preservative be provided to the residents. Nitric Acit (1:1) will be added to reach pH<2.

manager

WATER SYSTEM EVALUATION

An evaluation will be made of the plumbing under the kitchen sink in order to determine the composition of water lines servicing the kitchen sink. The water supply beneath the kitchen sink generally consists of hot and cold water pipes coming from either the wall behind the sink or, occasionally, up through the floor into the cabinet beneath the sink. These supply lines generally terminate at shut-off valves beneath the sink. The supply lines continuing from the shut-off valves are generally of different material than the supply lines going to the shut-off valves.

Supply lines in residential construction can be copper, galvanized, PVC, or lead pipe. PVC pipe is easily identified because of its plastic composition. Copper pipe can be identified by scraping the surface corrosion from the pipe to reveal the bright copper color. Galvanized pipe can be recognized by the threaded fittings if present and visible or by the hard surface of the pipe. Lead pipe can be recognized by the softness of the material. It is easily bent into shape and can be scratched with a knife blade or other hard tool. When scratched, the exposed surface is silvery in color.

The supply lines running from the shut-off valves to the sink generally are copper, chrome-plated brass or PVC. The PVC is easily recognized because of its plastic composition. Chrome-plated brass is also easily recognized because of the shiny surface. Copper can be identified by scratching the surface to reveal the copper color. Identifying the composition of the plumbing system beneath the sink completes the evaluation of the plumbing system. All information should be recorded.

IV. Paint Sampling Protocol Using an XRF Analyzer

A. Background and Selection of Surfaces

The concentration of lead in paint will be determined by using an X-ray fluorescence analyzer. Two types of instruments may be used, the XK-2 or the XK-3, both manufactured by Princeton Gamma-Tech, Inc. The XK-3 with a range of 0-10 mg of Pb per cm^2 will be the primary instrument used. If available the XK-2 will be a backup and also used in the event a reading on the XK-3 exceeds 10 mg/sq cm^2.

In each residence two surfaces, a painted woodwork and a painted walls in each of three rooms or areas most frequently occupied by the subject child will be evaluated (e.g. child's bedroom, kitchen, living room). One reading will be taken at three different locations on each type of surface. The identity of the rooms and the Pb found in the paint will be recorded. In addition, a copy of a floor plan of the

residence will be available to the technician and on which the sample location will be noted. All unpainted surfaces, such as paneling, wallpaper, and unpainted woodwork will not be tested. In the event a room selected is unpainted an alternate room will be selected and this information recorded.

In order to characterize the paint and surfaces in a given room at least one painted wall and one painted trim in the room (door or window sill) should be screened. When screening the woodwork, three separate readings will be taken at three different locations on the woodwork. A similar procedure will be used for screening painted walls within a room. One reading will be taken on each of three separate wall areas, either on the same wall or on different walls within a room. If all walls are painted the same color, then the three readings can be taken from one wall. If the walls are painted different colors, then a reading from the different colored walls should be included. Whenever changing areas or locations, one reading should be taken to clear the machine prior to taking the actual reading to be recorded. The arithmetic mean of the eighteen readings should be recorded as the reading for the house. Each individual reading will also be recorded to provide data for future follow-up actions, if necessary.

XRF readings will be taken by placing the instrument on the designated surface and opening the shutter. (More accurate readings can be obtained from flat surfaces so curved surfaces will be avoided). Once the shutter is opened the lead content of the paint will appear as a visual numerical display on the instrument. The operator will read the number for the other team member to record. This will be repeated back to the operator.

In addition to the paint lead screening, the environmental monitors will make an evaluation of the condition of painted surfaces. This evaluation will be a rating scale of 1 to 4:

1) Intact
2) Slightly Peeling
3) Moderate Peeling
4) Extremely Deteriorated

B. Operation of the XRF Analyzer to Determine the Concentration of Lead

At the start of each day the performance of the XRF instruments are evaluated using standard procedures. Prior to taking readings at the residence, calibration checks will occur using reference material (1.5 mg/cm^2 Pb and a Zero Check) prepared by the Department of Housing and Urban Development. After the designated areas in the home have been sampled and before the team is ready to leave, the instrument's calibration will once again be checked. All calibration information should be added to the FORM 07 XRF Lead Paint Screening work sheet, if available, or equivalent form. The HUD Guidelines for Lead in Paint, Sept 19, 1990 (Revision 3) are followed.

Following is the Operating Procedure for the XK-3 unit:

1. Remove the battery pack, coiled cable, and XK-3 unit from the carrying case.

2. Connect the battery pack to the XK-3 unit, using the coiled cable.

3. Locate the LOCK SWITCH underneath the handle toward the rear of the unit and push it forward. A red light over the display window will now glow to indicate that the instrument is ready to perform its analysis as soon as the shutter is opened.

4. Depress the RED RESET button on the back plate of the unit, just above the coiled cable connection, and hold for 8-10 seconds.

5. Grasping the wooden handle, position the face-plate of the instrument against the surface to be measured and push down firmly and evenly on the handle to spring the shutter open. The red light over the window will now blink to indicate that the shutter is open and that the measurement is taking place. As soon as the shutter opens, the previous read-out in the window vanishes, leaving the window blank except for a single decimal point.

6. Keep the handle firmly depressed until the new read-out appears.

7. When the new read-out appears, release pressure on the handle. The display window retains read-out until the handle is pushed down again to begin another measurement.

8. Push the lock switch back to the lock position when readings are completed.

9. If the calibration check results exceed +/- 0.02 mg/cm^2, the instrument RESET is pushed before continuing. The XK-3 is calibrated by the manufacturer.

Appendix C-Laboratory Quality Control

ERYTHROCYTE PROTOPORPHYRIN

1. Description

   a. Analyte name: Erythrocyte Protoporphyrin
   b. Method code: 01640A
   c. Specimen matrix: Whole Blood
   d. Supervisor: Elaine W. Gunter ___
      Statistician: ______
      Branch: Nutritional Biochemistry Branch
      Date: February 14, 1988 (Updated 9/5/90)

2. Special Safety Precautions

This method should be performed under an exhaust hood (not a laminar-flow hood) because the ethyl acetate-acetic acid and hydrochloric acid fumes are very irritating. Gloves should be worn at all times. All specimens should be treated as potentially HIV- and Hepatitis B-positive. All leftover acid solutions should be disposed of as hazardous wastes. All leftover blood specimens should be autoclaved before disposal, as well as all plastic, glassware, and paper products which have come in contact with the blood.

3. Specimen collection and storage procedures

Specimens for erythrocyte protoporphyrin analysis should be fresh or frozen EDTA-whole blood. Heparinized blood may be used, but is not preferred because of the tendency of the blood to form microclots upon prolonged storage. Specimens may be stored in glass or in plastic vials, as long as vials are tightly sealed to prevent desiccation of the sample. Hematocrit data should be collected if possible to correct for the effects of anemia and report the final FEP concentration as ug/dL RBC. Protoporphyrin is stable for years at -20°C and below. Quality control pools for the HANES Lab are normally stored at -70°C for maximum stability. Several freeze-thaw cycles appear to have minimal effect on the specimen. However, after prolonged storage at 4-8°C, the blood specimen undergoes necrosis, with the resulting formation of fluorescent compounds which can interfere with FEP analysis.

4. Principle

Free erythrocyte protoporphyrin (FEP) is measured by a modification of the method of Sassa et al [1]. Protoporphyrin is extracted from EDTA-whole blood into a 2:1 (v/v) mixture of ethyl acetate-acetic acid, then back-extracted into diluted hydrochloric acid. The protoporphyrin in the aqueous phase is measured fluorometrically at excitation and emission wavelengths of 404 and 655 nm, respectively. Calculations are based on a processed protoporphyrin IX (free acid) standard curve. The final concentration of protoporphyrin in a specimen is expressed as micrograms per deciliter of packed red blood cells (ug/dL RBC); a correction for the individual hematocrit is made.

5. Instrumentation

   a. Perkin-Elmer model 650-10 spectrofluorometer, with R928 photomultiplier tube, xenon lamp, and custom-made microcell (10-x 75-mm) holder positioned to allow the passage of light through the aqueous phase only (Perkin-Elmer Corp., Norwalk, CT)
   b. Model 56 recorder (Perkin-Elmer Corp.)
   c. Cary model 19 double-beam spectrophotometer (Beckman Instruments, Fullerton, CA)
   d. Vortex mixer (Fisher Scientific Co., Fairlawn, NJ)
   e. Mettler model H18 analytical balance (Mettler Instruments Corp., Hightstown, NJ)

f. Beckman TJ-6 centrifuge (Beckman Instruments Co.)
g. Digiflex automatic dispensers, with 2.0 and 10.0-mL syringes (Micromedic Systems, Div. of Rohm and Haas, Horsham, PA)
h. Micromedic high-speed automatic diluter, with 1.0-mL dispensing pump (Micromedic Systems)

6. Materials

a. Protoporphyrin IX, dimethyl ester, 99.3% purity, grade 1 (Sigma Chemical Co., St. Louis, MO), or 99.9% purity (Porphyrin Products, Logan, UT)
NOTE: Store at -20°C over a desiccant. Purchase of one lot is recommended, with aliquotation into ampoules if possible..
b. Ethyl acetate, high-pressure liquid chromatography (HPLC) grade. (J.T. Baker Co., Phillipsburg, NJ).
c. Acetic acid, glacial, "Baker Analyzed" (J.T. Baker Co.).
d. Hydrochloric acid, (HCl) concentrated, "Baker Analyzed" (J.T. Baker Co.).
e. Kimble 10-x 75-mm disposable glass culture tubes (Kimble Div., Owens-Illinois Co., Toledo, OH).
f. Parafilm M (American Can Co., Greenwich, CT).
g. Actinic glass volumetric flasks (Corning Glassworks, Corning, NY).
NOTE: All nondisposable glassware used in this assay should be washed in 10% (v/v) hydrochloric acid and rinsed six times with deionized water.
h. Formic acid, 88%, reagent grade (J.T. Baker Co.).
i. Deionized water, greater than or equal to 1.0 megaOhm-cm at 25°C (Continental Water Co., Atlanta, GA).

7. Reagent Preparation

a. 7.0 mol/L hydrochloric acid (HCl) (for hydrolysis)
Dilute 551 mL concentrated HCl to volume with deionized water in a 1-L volumetric flask.
b. 1.62 mol/L HCl (for daily absorbance readings)
Dilute 141 mL concentrated HCl to volume with deionized water in a 1-L volumetric flask.
c. 0.43 mol/L HCl (for analysis-extraction)
Dilute 68 mL concentrated HCl to volume with deionized water in a 2-L volumetric flask.
d. 1.5 mol/L HCl (for blanking spectrophotometer)
Dilute 118 mL concentrated HCl to volume with deionized water in a 1-L volumetric flask.
NOTE: These dilutions assume concentrated HCl to be 12.7 mol/L. The molar concentration of different lots of HCl should be calculated by using the following formula:

$$\text{mol/L} = \frac{\text{relative density X \% HCl}}{36.435}$$

e. 2:1 (v/v) ethyl acetate-acetic acid
Working under a hood, combine 200 mL ethyl acetate and 100 mL glacial acetic acid. Mix the solution well; this volume is sufficient for the standards, controls, and 80 specimens in duplicate. (Prepare the reagent daily, immediately before sampling the whole blood.)

8. Standards Preparation

NOTE: Prepare all standard solutions in actinic glass volumetric flasks, in very reduced light. At present there are no NBS SRM's available for FEP standardization. The standard material used for the HANES method uses the highest purity standard material available, and that purity is confirmed by TLC, HPLC, fluorescence, and spectroscopy.

a. Protoporphyrin IX standards

Concentrations are expressed in terms of protoporphyrin IX free acid. The millimolar absorptivity of protoporphyrin IX has conventionally been determined in 1.5 mol/L HCl; thus, the daily absorbance reading of the hydrolysate is determined at this acid concentration [2].

(1) <u>200 mg/L protoporphyrin IX free acid hydrolysate</u> (stock standard) Weigh 42.0 mg protoporphyrin IX dimethyl ester (PPIX DME). Dilute to volume in a 200-mL actinic volumetric flask with 7 mol/L HCl, washing PPIX off weighing paper with a few drops of formic acid. Add a small stirring bar, cover the flask with aluminum foil, and mix contents at 20-25°C for 3 h, using a magnetic stirrer. (Prepare weekly, use once and discard.)

(2) <u>10 mg/L intermediate stock</u>
After 3 h, dilute 25.0 mL of 200 mg/L solution with deionized water to volume in a 500-mL actinic volumetric flask, to yield a 10 mg/L solution in 0.35 mol/L HCl. (Prepare weekly. Store in actinic bottles at 4°C. Allow to reach consistent room temperature before using.)

(3) <u>1 mg/L standard for daily absorbance readings</u>
Dilute 10.0 mL of 10 mg/L intermediate stock (brought to ambient temperature before dilution) to volume in a 100-mL actinic volumetric flask with 1.62 mol/L HCl to yield a 1 mg/L protoporphyrin IX standard in 1.5 mol/L HCl. Use an aliquot of this standard for absorbance readings. (Prepare daily.)

NOTE: The theoretical concentration of this solution with respect to protoporphyrin IX free acid (PPIX FA) is calculated as follows:

(a) $\frac{42 \text{ mg PPIX DME}}{200 \text{ mL}} \times \frac{562.27 \text{ mg PPIX FA}}{590.72 \text{ mg PPIX DME}} = .1999 \text{ mg PPIX FA/mL}$

(b) $\frac{.1999 \text{ mg PPIX FA}}{\text{mL}} \times \frac{25 \text{ mL}}{500 \text{ mL}} \times \frac{10 \text{ mL}}{100 \text{ mL}} = .0009995 \text{ mg/mL PPIX FA}$ (or 99.95 ug/dL)

(c) $\frac{99.95 \text{ ug}}{1 \text{dL}} \times \frac{1 \text{ mmol}}{562.27} \times \frac{10 \text{ dL}}{1 \text{ L}} \times \frac{1 \text{ mg}}{1000 \text{ mg}} = .00178 \text{ mmol/L PPIX FA}$

(4) <u>0-80 ug/dL working standards</u>
Prepare the following working standards daily by diluting the 10 mg/L standard with 0.43 mol/L HCl according to the following dilution scheme using the Micromedic Digiflex dilutor and reagent dispenser.
NOTE: Be sure to work under very subdued lights when diluting and extracting the standard materials, since they are photo-labile.

Working Standard Concentration (ug/dL)	Volume 10 mg/L Standard (uL)	Volume 0.43 mol/L HCl Diluent (uL)	final Volume (uL)
80	400	4600	5000
70	350	4650	5000
60	300	4700	5000
50	250	4750	5000
40	200	4800	5000
30	150	4850	5000

20	100	4900	5000
10	50	4950	5000
0	0	5000 -	5000

9. Procedure

To protect hands against acids and solvents during sampling, wear latex gloves. To avoid evaporatio of degradation of specimens, process samples as rapidly as possible. After centrifugation, samples ar stable for 1-2 h.

a. Thaw specimens and quality control materials of frozen EDTA-whole blood at room temperature.
NOTE: Control pools with elevated levels of FEP are prepared from blood (EDTA-anticoagulated) collected from cows that have been fed lead acetate.

b. Using the spectrophotometer and quartz cuvettes, measure absorbance at wavelength-maximum (approximately 407-408 nm) of the 1 mg/L in 1.5 mol/L HCl standard solution against a blank of 1.5 mol/L HCl, scanning from 380-420 nm. This measurement will be used in determining standard concentrations. Clean cuvettes with 5% Contrad detergent solution after use, and rinse them thoroughly with deionized water followed by ethanol to remove water droplets.

c. Prepare the working standard dilutions from the 10 mg/L standard solution, using 0.43 mol/L HCl as a diluent. These dilutions are unstable; therefore, prepare them as rapidly as possibl

d. Prepare the 2:1 ethyl acetate-acetic acid mixture, and fill a dispenser bottle of the first Digiflex dilutor for delivering 1.0 mL of reagent. Fill the dispenser bottle of the second Digiflex dilutc with 0.43 mol/L HCl for delivery of 1.0 mL. (When using dilutors, place them under a hood to minimize fumes.)

e. Before sampling, vortex thoroughly each standard dilution, quality control pool, or whole blood specimen. Using the Digiflex in sample-transfer mode, transfer 10 uL of the sample to a 10-x 75-mm disposable glass tube, in duplicate.

f. Add 1.0 mL of the 2:1 ethyl acetate-acetic acid mixture to each sample. Mix thoroughly for 10 sec.

g. Add 1.0 mL of the 0.43 mol/L HCl to each sample. Wrap tube with Parafilm, and mix thoroughly for 10 sec.

h. Sample in this order: Standards, quality control pools, and whole-blood specimens in duplicate.

i. Prepare four blank tubes (O standards) with 1.0 mL each of ethyl acetate-acetic acid and 0.43 mol/L HCl, with 10 ul of 0.43 mol/L HCl as sample.

j. When all sampling is completed, centrifuge all tubes for 4 min at 1400 rpm.

k. Perkin-Elmer 650-10 spectrofluorometer settings:

Parameter	Setting
Slit(s) width	10 nm
Photomultiplier tube	R928 Hamamatsu
Cuvettes	10-X 75-mm in microcell adapter
Range	1
PM gain	normal
Response	normal
Mode	normal
Scan	off
Wavelengths	404 nm excitation 658 nm emission

l. Allow 1 h for the 650-10 to warm up and stabilize after the xenon lamp has been ignited.

m. Following the instruction manual, zero the model 56 recorder with "Recorder Zero" and "MEAS."

n. With shutter closed and sensitivity set on "1," zero the 650-10 spectrofluorometer by using "Ze Adjust" with "Zero Suppression" OFF.

o. Open shutter. Turn "Zero Suppression" on. Put tube with blank solution in sample compartment, and zero the digital readout carefully by using the zero suppression knob. NOTE: Because of tube-to-tube variance, be sure to check several blank tubes and take the average amount of the blank to be zeroed out.

p. Adjust sensitivity of fluorometer by reading 50 and 60 ug/dL PPIX standards and adjust "fine" sensitivity to read each value, respectively, as 50 and 60 fluorescent units (Standard curve has a tendency to lose linearity on the 70 to 80 ug/dL standards by approximately 2 fluorescent units

q. Proceed to read the standard curve, quality control pools, and samples.

r. For samples outside the range of standard curve, turn the sensitivity range down to "0.3" or "0.1". Rezero the instrument and perform steps O-Q again without adjusting the "fine" sensitivity setting.

10. Calculations

The millimolar absorptivity of protoporphyrin IX free acid in 1.5 mol/L HCl has been determined in our laboratory to be 297 +/- 1 (600 observations from 1976 to 1988) [4,5]. The purity of our materia has been confirmed by elemental analysis and high-performance liquid chromatography of the extracted protoporphyrin IX free acid. Calculate the actual concentration of the 1 mg/L (.00178 mmol/L) working standard, using the following equation:

$$A=ebc$$

Where:

A= absorbance reading
b= cuvette pathlength, 1 cm
c= concentration, in mmol/L
e= 297, the millimolar absorptivity of protoporphyrin IX free acid in 1.5 mol/L HCl

For example, if the daily absorbance reading of the 1 mg/L standard at wavelength maximum is 0.520, then:

$$C= \frac{0.520}{(297\ \text{L/mmol-cm})\ (1\text{cm})} = .00175\ \text{mmol/L}$$

Then:

(.00175 mmol/L)(562.27 mg/mmol)(1000ug/mg)(1L/10dL) = 0.9840 mg/L PPIX FA

Consider 0.9840 as a percentage of 100 ug/dL (1 mg/L), and correct the standard curve accordingly:
10 ug/dL X 0.9840 = 9.84
20 ug/dL X 0.9840 = 19.68 etc.

Perform a linear regression, with x = corrected standard concentration and y = fluorescent intensity reading. Using the slope of the standard curve and assuming zero intercept, calculate the concentration of protoporphyrin IX per deciliter of whole blood for each specimen. To correct for hematocrit and express results as ug/dL of RBC, use this formula:

<u>ug/dL whole blood</u> X 100 = ug/dL RBC

hematocrit

11. CDC Modifications

The following modification of the original methods are based on CDC optimization experiments: (a) sample size increased from 2 uL to 10 uL; (b) ethyl acetate-acetic acid and 0.43 mol/L HCl volumes increased from 0.3 mL to 1.0 mL; (c) processed protoporphyrin IX standards used; (d) hydrolysis time for the dimethyl ester decreased from 48 h to 3 h, on the basis of the work of Culbreth et al.[3]; and (e) 0.43 mol/L HCl was chosen as a diluent for maximum fluorescent intensity and stability of the extracted protoporphyrin IX.

12. Quality Control System

FEP is a "batch" method, i.e., all specimens, standards, and q.c. pools, are treated to the same processes simultaneously, such as extraction. An average analytical day consists of 40-60 specimens analyzed in duplicate, with quadruplicate analyses of 3 levels of bench q.c. (low-human, medium-bovine, and elevated-bovine). In every rack of 20 specimens, one blind q.c. specimen will be inserted randomly. Blind q.c. pools are prepared in the same manner as the unknown specimens are, using the same types of labels and vials. Two levels are prepared, low-normal and high-normal, to verify values reported in the near abnormal concentration ranges for a given analyte.
Quality control limits are established with the programs "QCLIMIT" and "QC". Preliminary limits established with 20 consecutive runs and updated annually thereafter.

Blind q.c. are examined for similar criteria. The supervisor also evaluates the slope, intercept, R values for trends. The overall coefficient of variation for this method has been 4-5% over the entire analytical range.

The system is declared "out-of-control" if any of the following events occur:

(1) Means Chart:

(a) A single run mean for one or more pools falls outside the upper or lower 95% limit.

(b) The run means for two of the two or more pools fall either both above or both below the lower 95% limit

(c) Two successive run means for a single pool fall either both above or both below the lower 95% limit

(d) Eight successive run means for a single pool fall either all above or all below the center line, establishing a trend.

(2) Range Chart:

(a) A single within-run range falls above the upper 99% limit.

(b) The within-run ranges for two of the to or more pools fall above the upper 95% limit

(c) Two successive within-run ranges for a single-pool fall above the upper 95% limit

(d) Eight successive within-run ranges for a single pool fall above the center line.

If the system should be declared "out of control," the following remedial action(s) should be taken: after troubleshooting procedures have been completed and the system has been verified to be "in control," all specimens for that analytical run should be reanalyzed, with the repeated values reported rather than the original values.

13. Preparation of quality control material

Quality control materials are prepared from whole blood collected with K_3EDTA as an anticoagulant. Donations from normal humans are used, as well as from normal and lead-dosed cows to achieve elevated levels. The pools are well-mixed and prescreened for FEP concentrations. Aliquots of 0.5 mL are usually dispensed in 2.5-mL glass Wheaton vials or high-density polypropylene vials. Pools are stable at -70 °C for more than three years; we normally do not prepare more than a 3-yr supply at one time because of space limitations.

14. Reference Ranges

The following ranges for FEP are used in evaluating data:

Females tend to have higher average values than males; children have higher values than adults.

Children < 6 years old : 36-97 ug/dL RBC
Children 6-14 years old : 37-83 ug/dL RBC
Males 15-74 years old: 33-81 ug/dL RBC
Females 15-75 years old: 37-93 ug/dL RBC

Source:
5th and 95th percentiles from data tables in:

Fulwood R, Johnson CL, Bryner JD, Gunter EW, McGrath CR. Hematological and Nutritional Biochemistry Reference Data for Persons 6 Months-74 Years of Age: United States, 1976-80. Natior Center for Health Statistics. Vital and Health Statistics. Series 11- No. 232. DHHS Pub. No. (PHS) 83-162. Public Health Service. Washington. U.S. Government Printing Office, December 1982.

15. Special Method Notes

Once the analyst begins extracting the standards, specimens, etc., he/she should continue through th analysis to completion to minimize error due to incomplete extraction. Some small variations in fluorescence values may be due to poor quality 10-x 75-mm tubes used as cuvettes. Blood specimen are very stable; standards are somewhat labile and must be processed under reduced light. Accurate weighing of the PPIX DME is critical, as is avoiding prolonged hydrolysis time (i.e., > 3 h).

If hematocrit correction is not used for reporting data, traditional cutoff level is 35 ug/dL. Although we have not found this practice necessary, both New York State and the Wisconsin State Laboratory of Hygiene recommend a 1:5 dilution of fresh whole blood with saline in microtiter plates to ensure complete lysis of cells. A concumitant dilution of standard concentration is also ewmployed.

16. Literature References

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BLOOD LEAD

1. Description
   a. Analyte Name: Lead
   b. Method Code: 1080A
   c. Specimen Matrix: Blood
   d. Supervisor: Mary M. Kimberly, Ph.D. ________________
   e. Statistician: Sam Caudill, Ph.D. ________________
   f. Branch: Nutritional Biochemistry Branch
   g. Date: 9/28/88; updated 4/27/90 and 9/17/90

2. Special Safety Precautions:

   a. Wear gloves, lab coat, and safety glasses while handling all human blood products. Disposable plastic, glass, and paper (pipet tips, autosampler cups, gloves, etc.) that contacts blood is to be placed in a biohazard autoclave bag. These bags should be kept in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished. The use of the foot pedal on the Micromedic Digiflex is recommended because it reduces analyst contact with work surfaces that have been in contact with blood and keeps the hands free to hold the specimen vials, autosampler cups, and to wipe off the tip of Micromedic Digiflex.
   b. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis.
   c. Special care should be taken when handling and dispensing concentrated nitric acid. Always remember to add acid to water. This material is a caustic chemical capable of severe eye and skin damage. Wear metal-free gloves, a lab coat, and safety glasses. If the nitric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.

3. Specimen collection and storage procedures:

   a. A 1-mL sample of blood is required. Blood is collected by standard venipuncture procedures into 3-mL lavender-top Vacutainer brand tubes. If there is more than one analyte of interest in the specimen and it needs to be divided, transfer the appropriate amount of blood into a sterile Nalgene cryovial labelled with the particpant's ID. This procedure should be performed under clean conditions to avoid contamination of the sample. It is important that each lot of collection tubes and shipping and storage containers be screened for lead contamination.

      A fasting sample is preferred.

   b. Specimens collected in the field should be frozen, then shipped on dry ice by overnight mail. Once received, they should be frozen at ≤20 °C until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn should be refrozen at ≤20 °C. Samples thawed and refrozen several times are not compromised.

4. Principle of measurement

Lead is measured in blood by atomic absorption spectrometry by using the method described by Miller, et al. (1). Quantification is based on the measurement of light absorbed at 283.3 nm by ground state atoms of lead from either an electrodeless discharge lamp (EDL) or from a hollow-cathode lamp (HCL) source. Blood samples, human and bovine blood quality control pools, and matrix-matched blood standards are diluted with a matrix modifier (nitric acid, Triton X-100, and ammonium phosphate). The lead content is determined by using a Perkin-Elmer Model 5000 atomic absorption spectrophotometer with deuterium background correction. Lead contamination must be carefully avoided throughout all procedures. All materials used for collecting and processing specimens are screened for possible lead contamination. All processing work is performed under clean conditions, including laminar flow hoods.

5. Instrumentation

a. Perkin-Elmer (Norwalk, CT) model 5000 atomic absorption spectrophotometer, including HGA 500 furnace, lead source lamp, EDL Power Supply (if EDL sources are to be used), AS-40 Autosampler, PRS-10 printer-sequencer, and a UV starter source.

Parameter	Setting
Wavelength	283.3 nm
HCL Current*	10 mA
EDL Power Supply*	10 W
EDL Mode	continuous
Slit	0.7 nm (low)
Signal Mode	Peak Area

* Only one source is used.

Furnace Temperature Program

Step	Temperature °C	Ramp sec	Hold sec	Gas Flow mL/min
Dry*	100	5	10	300
Dry	180	5	20	300
Char	700	5	30	300
Atomize	2000	1	5	20
Burnout	2400	1	4	300
Cool	20	1	4	300

Baseline:	-8 sec
Read:	0 sec
Recorder:	-1 sec

* Dry temperature and hold time may vary with different graphite tube and platform combinations.

b. Micromedic Digiflex Automatic pipet equipped with 2000-uL dispensing syringe, 2000-uL and 200-uL sampling syringes, 0.75 mm tip, and the foot pedal (Micromedic Systems, Inc., Horsham, PA).
c. Mettler PL 200 top-loading balance (Mettler Instrument Corp., Hightstown, NJ).
d. Milli-Q water purification system (Millipore Corporation, Bedford, MA).
e. Vortex-Genie vortex mixer (Fisher Scientific, Atlanta, GA).
f. Eppendorf fixed-volume micropipets: 1000, 500, 250, 200, 50, and 40-uL volumes (Brinkmann Instruments, Inc., Westbury, NY).
g. Magnetic stirrer (Corning Glass Works, Corning, NY) and stirring bars (Fisher Scientific).
h. Oxford automatic Dispensor (Monoject Scientific, St. Louis, MO).

6. Materials

a. Stock solution of lead: NIST SRM 2121-2, 10,000 mg/L, (National Institute of Standards and Technology, Gaithersburg, MD).
b. Redistilled concentrated nitric acid (G. Frederick Smith Chemical Co., Columbus, OH).
c. Triton X-100 (Fisher Scientific, Fairlawn, NJ)
d. Ammonium phosphate, dibasic ("Baker Analyzed", J.T. Baker Chemical Co., -- or any source found to be low in lead contamination).
e. Ultrapure water (from the Milli-Q water purification system).
f. Argon, 99.996% purity (supplied as a compressed gas by Holox or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).
g. NIST SRM 955 (four levels), trace elements in bovine blood (National Institute of Standards and Technology). These are to be run periodically to verify accuracy.
h. Bovine and human blood quality control pools spiked with low and high levels of lead which have reference values established by IDMS.
i. A low-level bovine blood to be used for preparation of matrix-matched standards.
j. Pyrolytic graphite tubes, solid pyrolytic graphite L'vov platforms, insertion and alignment tools, and graphite contact rings (Perkin-Elmer).
k. Small plastic weighing boats (Scientific Products, McGaw Park, IL).
l. Pipet tips: 1-100 uL and 1-1000 uL sizes (Rainin Instrument Co., Inc., Woburn, MA).
m. Acid-cleaned volumetric flasks (1000, 100, and 10-mL volumes). The glassware is soaked in a soapy solution (2% solution of Isoclean detergent, Akron, OH) for at least 24 hours, rinsed, soaked in 25% nitric acid for 48 hours, rinsed with ultrapure water, and dried under clean conditions.
n. Conical-bottom 2-mL polystyrene autosampler cups (Lancer, St. Louis, MO).
o. Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA).
p. Cotton swabs (Hardwood Products Co., Guilford, Maine).
q. Dehydrated alcohol, USP (Midwest Grain Products of Illinois, Pekin, IL).
r. Vinyl examination gloves (Travenol Laboratories, Inc., Deerfield, IL).
s. Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA)
t. Bleach (10% sodium hypochlorite solution) - any vendor.
u. Dental mirror (Perkin-Elmer).
v. Printer-sequencer tape (Perkin-Elmer).

7. Reagent Preparation

<u>Matrix Modifier</u> (0.2% (v/v) nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v) ammonium phosphate)
Using Eppendorf pipets, dilute 2 mL redistilled concentrated nitric acid, and 5000 uL (5.0 mL) Triton X-100 in approximately 750 mL ultrapure water in an acid-cleaned 1000-mL volumetric flask. Weigh out 2.0 g of dibasic ammonium phosphate and add it to the flask by washing down the weighing boat with ultrapure water delivered from a wash bottle. Add a magnetic stirring bar and stir the solution on a stirring plate until the Triton X-100 has dissolved. Remove the stirring bar and bring the solution to volume with ultrapure water. After preparation, this solution should be checked for contamination at the beginning of each analytical run and discarded if absorbance greater than 0.005 Abs-sec is observed. Store at room temperature and prepare as needed in a flask dedicated to this solution.

8. Standards Preparation

a. <u>1000 mg/L stock lead standard</u>
If using NIST SRM 2121-2, dilute 1.00 mL (delivered using either an Eppendorf pipet or the Micromedic Digiflex) to 10 mL with ultrapure water in an acid-cleaned volumetric flask. Store at room temperature and prepare every six months in a flask dedicated to this solution.

b. <u>10 mg/L intermediate lead standard</u>
Using either an Eppendorf pipet or the Micromedic Digiflex, dilute 1.00 mL of the 1000 mg/L stock lead standard to 100 mL with ultrapure water in an acid-cleaned volumetric flask. Store at room temperature and prepare monthly in a flask dedicated to this solution.

c. <u>Working lead standards</u>
Using the Micromedic Digiflex, transfer the following volumes of intermediate standard to 10-mL volumetric flasks and dilute to volume with ultrapure water:

Intermediate Stock (uL)	Working Standard Concentration (ug/L)	Sample Concentration (ug/L)
0 (*)	0	0
50 (*)	50	5
100 (*)	100	10
150 (*)	150	15
200 (*)	200	20
250	250	25
300 (*)	300	30
400 (*)	400	40
500	500	50
600 (*)	600	60
750	750	75
800 (**)	800	80
1000	1000	100

Store at room temperature and prepare weekly in flasks dedicated to these solutions.

(*) Use these standard concentrations for NHANES III studies.
(**) Use this standard when the SRM 955 control materials are to be run.

d. Calibration standards

1. Using the Micromedic Digiflex, dispense 500 uL of matrix modifier into four autosampler cups for use as process blanks. Place these cups in positions AZ, 1, 9, and 17 on the autosampler tray.
2. Using the Micromedic Digiflex, dispense 100 uL of each of the lead working standards into separate autosampler cups by dispensing 100 uL of the standard and 400 uL of the matrix modifier.
3. Using the Micromedic Digiflex, add 100 uL of the base blood and 400 uL of the matrix modifier to each of these autosampler cups. The total dilution of the blood is tenfold (1+9).
4. Place the standard blank in position 2 and the standards, in increasing order, in positions 3-9 on the autosampler tray.
5. When the NIST SRMs are analyzed, an additional calibration standard is used. The standards are placed in positions 3-10 on the autosampler tray.

9. Procedure

a. Preliminaries

1. (For information regarding the range of linearity and how to handle results outside this range, refer to the Calculations section of this document.)
2. Allow frozen blood specimens, quality control specimens, and blood calibration material to reach ambient temperature and mix on a vortex mixer for 10 seconds.
3. While the specimens are thawing, rinse enough autosampler cups for an analytical run with ultrapure water delivered from a wash bottle. Drain the cups upsidedown on Kay-Dry paper towels.
4. Prime the Micromedic Digiflex pumps with the matrix modifier solution.
5. Wipe the tip of the Micromedic Digiflex after inserting it into any blood specimen.

b. Sample preparation

1. Using the Micromedic Digiflex, dilute the specimens and controls tenfold (1:10) with the matrix modifier solution into clean autosampler cups. Use 100 uL of specimen and 900 uL of matrix modifier in the following procedure: pull up 100 uL of blood into the tip, wipe the tip with a Kim-Wipe tissue, dispense the blood and 450 uL of matrix modifier into a cup. Pull up 100 uL of air into the tip and wash out with an additional 450 uL of matrix modifier into the same cup. Observe the tip and release any air bubbles that may become trapped before dispensing into the autosampler cups. To do this, remove the tip from its clamp and hold the tip end up to release air. If air becomes trapped in a blood sample in the tip, dispense it into the waste beaker and take another sample.
3. Place the autosampler cups containing the specimens in positions 10 and following, with the controls first, on the autosampler tray.
4. Place an cup containing the water used in the preparation of the run on the tray as a sample.

c. Instrument setup for the Model 5000 and AS-40.

1. Turn on the argon gas (40 psi).
2. Turn on the cooling water supply.
3. Turn on the HGA 500 furnace. Switch the furnace to the run mode by pressing the STANDBY key.

4. If necessary, program the temperature program into the memory of the HGA 500 furnace. Use the TEMP, RAMP TIME, and HOLD TIME keys to program in each step. Press the STEP key to move to the next step for programming. The default gas flow at all steps is 300 mL/min. At the atomization step (step 4 in the program), type in an alternate gas flow of 20 mL/min and press the INT FLOW key. Also program into the atomization step the BASELINE, REC, and READ functions. Store the program by typing in a number and pressing STORE.
5. Install a source lamp in position on the turret.
6. If using an EDL, turn on the EDL power supply: Turn up the power to three-quarters of maximum and wait for the EDL to spontaneously light. (If the lamp does not light, remove it from the turret and expose it to the UV starter source until it lights and return it to its position in the turret.) After the lamp lights, run the power at a high level momentarily, then turn it down to the recommended wattage.
7. Turn on the Model 5000 spectrophotometer. Press the PRINT key on the keyboard. The red light beside the key will illuminate.
8. Program the Model 5000.
   If using a HCL, type in the lamp current on the keyboard and press the LAMP MA key.
   Type in 0.7 and press the SLIT LOW key.
   Type in the 283.3 and peak up onto the wavelength by pressing the PEAK key. Press the SET UP key. The display will read about 50; but as the lamp warms up the value will increase. Press the GAIN key to bring the value to approximately 50 (pressing the GAIN key will either increase or decrease the energy automatically).
   After the lamp has warmed up for about 20 minutes and while still in the SET UP mode, use the knobs on the lamp holder, twist the lamp in the holder, and move it back and forth (without clamping on the black end piece of the lamp) to optimize the lamp position. This procedure may also require use of the GAIN key. As a check, the ENERGY on the 5000 display should read around 60.
   Press the PEAK AREA key.
   Type in 2 and press the AVG key.
   Type in 6 and press the t key.
   Store the program by typing in a number and pressing the STO key.
9. Turn on the AS-40 Autosampler Controller.
10. Program the AS-40 Autosampler Controller.
    Type in 20 and press the SAMPLE VOLUME key on the controller.
    Type in the HGA program number and press the HGA PROG key.
    Type in the Model 5000 program number and press the INST PROG key.
    Type in the number of the position of the last sample and press the LAST SAMPLE key.
11. Install a new graphite tube and platform after two days of analytical runs. Open the furnace by pressing the FURNACE OPEN key on the HGA 500. Install a L'vov platform in the furnace using the insertion tool. Make sure that the platform is properly seated in the tube by holding the tube on end and gently tapping it on a hard surface. If it falls out, reinsert using more pressure. Install the tube in the furnace with the platform at the bottom of the tube. Use the alignment tool inserted in the sample port while pressing the FURNACE OPEN key again to close the furnace. After the furnace closes, press the furnace together to insure that it has properly closed.

12. Check the quartz windows of the furnace to make sure that they are clean. If there is evidence of sample spattering on the windows, remove the windows and clean using a cotton swab soaked in denatured alcohol. Wipe dry with a soft tissue (Kim-Wipe) and carefully reinstall.
13. Press the STANDBY key; the arm of the autosampler will lift out of the wash cup. Check the alignment of the sampling tip by manually moving the arm to the sample port. If the alignment needs adjustment, unlock the autosampler base by turning the knob on the center front of the base. The knobs on the back left side and front left then can be used to move the base to the necessary position. Lock the base in place with the center front knob. Use the dental mirror to observe the position of the tip in the graphite tube. Check the depth of the autosampler tip in the graphite tube. Use the front adjustment knob to the right of the sampling arm to raise or lower the tip as necessary.
14. Press the STANDBY key again to return the sampling arm to its position in the wash cup.
15. Condition the graphite tube: Sequentially type in the following temperatures on the HGA keyboard and hold the MANUAL TEMP key for about 5 seconds. Wait about 10 seconds before typing in the next temperature.

    1000 °C
    1500 °C
    2000 °C
    2400 °C
    2650 °C

16. Check the dry step of the furnace program: type 1 then press the MANUAL key. Use the mirror to observe the sample tip as it deposits the sample on the platform. Continue to observe as the sample dries. Press the MANUAL key again to keep the autosampler from sampling from this cup indefinitely. The drying should be complete 5-10 seconds before the char step begins. If it is dry sooner, decrease the hold time of the second dry step appropriately. If it is not dry in time, two options are available. The temperature of the dry step may be increased or the hold time may be increased. Use the latter only if increasing the temperature will cause the sample to boil and splatter during the dry step.
17. Continue to check the dry step as in 16 until a successful step is observed.
18. When the appropriate dry temperature is determined (even if it is the one already in the program), make sure that the blank is low (less than 0.003 Abs-sec (mean of two determinations).
19. Check to make sure that there is enough paper in the PRS-10. Press the paper feed button on the front of the PRS-10 to advance the paper.

d. Operation
1. Press the START/STOP key on the AS-40 to begin a run.
2. The Model 5000 will first run the calibration curve and then the quality control materials. Check that the quality control materials are within the specified limits.
3. If the values observed for the control materials for this analytical run are in control, proceed with the analysis of the diluted blood specimens. (Refer to the Quality Control System section of this document for criteria.)

4. Turn off the system in reverse order.
Note: when turning off the EDL power supply, first turn off the power switch. When the power indicator decreases to zero, turn off the variable power knob.

e. Recording of Data:
1. Quality Control Data
Use the "HANES LABORATORY BLOOD LEAD STANDARD AND QUALITY CONTROL REPORTING SHEET" to record this data. This reporting sheet has self-explanatory blanks for the standard absorbance data, the linear regression information, and the quality control pool results. Prepare this form in duplicate, using carbon paper.
2. Analytical Results:
Use the "NHANES III ANALYTICAL WORKSHEET" to record the specimen results. These have been prepared with a list of the sample IDs for each preracked run. Record the results for blood lead in ug/dL. If a result is below the detection limit of the method, write "ND" (for nondetectable) in the blank. If a sample is missing from the rack, write "NOSAX" in the blank. If a sample is not satisfactory, i.e. cannot be analyzed, write "UNSAX" in the blank. Prepare these forms in duplicate, using carbon paper.
3. Give both types of forms to the supervisor along with the hard copy of the data printout from the PE 5000 and the hard copy of the printout obtained from running "PESLEAD". After the supervisor checks the data, the carbon copies and the data printouts will be returned for filing in a notebook. The supervisor will keep the original copies of the reporting sheets.
4. Use the "QC" program in the HANES library of ROSCOE to enter the quality control data. This should be updated regularly.

f. Replacement and periodic maintenance of key components:
1. Source lamp: a spare source lamp should be available. Order another if the spare is used for replacement.
2. Printer-sequencer tape: a supply of printer tape should be on hand. Order more when the last is installed.
3. Graphite contact rings: approximately every six months, the graphite contact rings of the furnace housing will need replacement. Indications that this procedure needs to be performed are an apparent loss of temperature control or sounding of the alarm by the instrument indicating a problem with the tube. It is useful to maintain a log book to help keep track of when these replacements occur. At least one spare set of graphite contact rings should be kept on hand.
4. The sampling tip of the autosampler will need to be repositioned and trimmed every few weeks, depending on how the dispensing is proceeding.

10. Calculations

a. The method described here is linear up to 4 umol/L, or 80 ug/dL (1). Use the linear regression program in ROSCOE ("PESLEAD") to calculate the calibration curve and the specimen concentrations. The program calls for the integrated absorbance values of the standards and samples. It will then subtract the blank and calculate the concentrations of the controls and specimens. The linear regression program generates slopes, intercepts, correlation coefficients, standardized residuals, and plotted and fitted curves. The correlation coefficient, r^2, for each curve should be 0.995 or better. For optimum sensitivity, slopes should be more than 0.035; intercepts should be less than 0.003.

b. Repeat a specimen analysis when duplicate integrated absorbances below 0.03 Abs-sec (mean) differ by more than about 0.005 Abs-sec or when duplicate integrated absorbances above 0.03 Abs-sec (mean) differ by more than 0.01 Abs-sec. This corresponds to concentration differences of 0.01 ug/dL and 0.025 ug/dL, respectively. Reanalyze specimens containing more than 30 ug/dL lead for confirmation. When reanalyzing any specimen with a concentration greater than 60 ug/dL, prepare a new specimen by diluting it twentyfold (1+19), rather than tenfold (1+9). The results output from PESLEAD must then be multipled by 10 to account for this higher dilution.

c. The detection limit, based on three times the standard deviation of ten repeat measurements of a sample with low lead concentration, is 0.07 umol/L (or 1.4 ug/dL) (1,2). Results below the detection limit are reported as nondetectable ("ND"; refer to the section, Recording of Data, in this document).

11. CDC Modifications for the NHANES III study

a. A Micromedic Digiflex automatic pipettor is used for many of the dispensing needs in this method because it is less tedious and provides better precision and accuracy than Eppendorf pipets.

12. Quality Control System

The method described in this protocol has been used for several years in the Nutritional Biochemistry Branch for environmental and occupational health studies. The method has proven to be accurate, precise, and reliable. The primary standard used is a NBS SRM. Estimates of imprecision can be generated from long-term quality control pool results.

Two types of quality control systems are used in this analytical method. These two systems are: (1) "bench" quality control specimens that are inserted by the analyst two times in each analytical run (a set of consecutive assays performed without interruption) so that judgements may be made on the day of analysis and (2) "blind" quality control samples that are placed in vials, labelled, and processed so that they are indistinguishable from the subject samples. The results of the blind specimens are decoded and reviewed by the supervisor. With both systems, all levels of lead concentration are assessed by taking these samples through the complete analytical process. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

Two levels of blind quality control pools are used. These pools are prepared in sufficient quantity to last throughout the survey. The levels chosen are in the "low-normal" (approximately 5 ug/dL) and "high-normal" (approximately 20 ug/dL) range so as not to be obvious to the analyst. The pools are prepared in the same way as the bench pools, but they are dispensed in vials identical to those used in the field for NHANES subject samples, labelled with psudoparticipant numbers corresponding to each geographical location of the survey, and stored at ≤20 °C. At least one blind sample is randomly incorporated with every 20 NHANES samples and analyzed according to the method protocol.

The bench quality control pools comprise three levels of concentration spanning the "low-normal", "normal", and high (at the CDC cutoff for lead poisoning in adults) ranges for lead.

Reference materials (blood products with certified values assigned by independent reference methods) are used periodically as a check of accuracy. NIST SRM 955 (levels A, B, C, and D) should be analyzed once a week for this purpose. If the stock of these materials becomes low, another should be ordered in time to analyze it concurrently with the quality control materials currently in use so that a bridge may be formed between the materials. If the material ordered from NIST is from the same lot, a full characterization is not necessary. However, there should be some overlap between the old and new stocks.

Quality control limits are established for each pool. An analysis of variance (ANOVA) is performed for each pool after twenty characterization runs have been performed in which previously characterized NIST SRM and bench quality control pools are used for evaluation. In addition to providing quality control limits, the characterization runs also serve to establish homogeneity of the pools. Once the homogeneity of the bench and blind materials has been established, it is useful to have them analyzed by another independent reference method, e.g. IDMS.

Precision and Accuracy:

Pool	mean	95% limits	99% limits	UCL-R	RUNS	TOTAL CV
SRM 955A	4.74	3.73- 5.75	3.41- 6.07	0.65	16	11.18
SRM 955B	29.0	26.50-31.51	25.70-32.31	1.35	16	4.42
SRM 955C	46.1	41.08-51.13	39.49-52.72	2.94	16	5.87
SRM 955D	68.7	62.29-75.08	60.26-77.11	1.76	16	4.70
BO155	56.5	53.25-59.71	52.23-60.73	2.5	31	2.99
CO130	31.3	28.97-33.62	28.23-34.36	1.42	30	3.89
LOWP	3.42	2.37- 4.46	2.04- 4.79	0.673	35	16.04
HIPB	20.9	19.47-22.35	19.01-22.80	1.5	35	3.81
0488	1.10	0.69- 1.52	0.55- 1.62	0.51	5	21.49
0588	5.92	5.34- 6.49	5.15- 6.68	0.52	5	5.00
0688	15.41	14.56-16.26	14.30-16.53	1.37	5	3.08
0788	35.3	34.04-36.59	33.63-36.99	1.56	5	1.88

After the standards and bench quality control materials are analyzed (at the beginning of an analytical run), the long-term quality control charts for each control material are consulted to determine if the system is "in control". Two types of charts are used. The first chart plots the means of the duplicate determinations and compares them to the 95% and 99% confidence limits as well as to the center line (the overall mean of the characterization runs). The system is out of control if any of the following events occur for any one of the quality control materials:

1. The mean from a single run falls outside the 99% confidence limits.
2. The means from two successive runs fall either above or below the 95% confidence limits.
3. The means from eight successive runs fall either all above or all below the center line.

The second type of quality control chart plots the range of the duplicate determinations and compares them to the 95% and 99% limits as well as to the center line. The system is out of control if any of the following events occur for any one of the quality control materials:

1. The range from a single run falls above the 99% limit.
2. The ranges from two successive runs fall above the 95% limit.
3. The ranges from eight successive runs fall above the center line.

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any analysis of specimens occurs.

13. Preparation of Quality Control Materials

Levels of lead in three units of bovine blood were evaluated. One animal (Cow #34) had never been dosed and, therefore, had a very low level. The blood from this animal is suitable for use as the base blood from which the calibration standards are prepared. The other two animals had been previously dosed, therefore their blood lead levels were somewhat elevated. One of these (Cow #23) was chosen for use as a mid-range level "normally" seen in the adult U.S. population. The other animal (Cow #26) was dosed again to bring its blood lead level up to a high range (near the cutoff limit). One liter of blood was drawn from each animal into evacuated bottles containing EDTA as coagulant.
One unit of blood each was drawn from nonsmoking male and female human volunteers. Both units were in the "low-normal" range, with the blood from the female being lower than that from the male. The blood from the male was used to prepare the "low-normal" blood lead pool.

A pool containing 75% blood from Cow #23 and 25% blood from the female human was prepared. The blood was mixed in an acid-cleaned flask by stirring on a magnetic stirrer for at least one hour. This pool was used to prepare the medium (or "normal") blood lead pool.

Using a sterile technique under a laminar-flow hood, the blood is dispensed using the Oxford automatic dispensor into 5-mL wide-mouth tubing vials. The base pool was dispensed in 1-mL aliquots. The quality control pools were dispensed in 0.5-mL aliquots. After capping with rubber stoppers, the vials are sealed with tear-away aluminum crimped caps. All vials were labelled after crimping. The pools were frozen at -20 °C. Twenty vials of each level were randomly selected for characterization of the quality control limits and for testing of homogeneity.

Two levels of blind quality control pools were dispensed in the same way except that vials and labels identical to NHANES specimen vials were used.

14. Reference Ranges

a. CDC recommendations:

b. Other references:
The average blood lead value for 801 samples was listed as 138 ± 45 ug/L with a 95% range of 70 - 230 ug/L (3).
A Biological Quality Guide has been proposed for groups of the general population (4). The level of exposure is acceptable when the median group value is ≤ 200 ug/L and the distribution is 98% ≤ 350 ug/L, 90% ≤ 300 ug/L, and 50% ≤ 200 ug/L. For preschool children, the distribution should be 98% ≤ 300 ug/L, 90% ≤ 250 ug/L, and 50% ≤ 100 ug/L.
Half of non-occupationally exposed persons have blood lead values < 200 ug/L with a 95 % value of < 350 ug/L (5).

15. Special Method Notes

16. References

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Determination of Lead in Blood Using Electrothermal Atomisation Atomic Absorption Spectrometry with a L'vov Platform and Matrix Modifier

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Accuracy in the determination of blood lead is of primary importance in such diverse activities as screening for childhood lead poisoning, occupational exposure monitoring and population surveys. To meet the stringent requirements of the third National Health and Nutrition Examination Survey (NHANES III), a large normative population study to be held from 1988–1994, we needed a method for the determination of lead in blood that was simple, accurate, rugged and of defined accuracy for both calibration and control materials. The recent availability of the National Bureau of Standards Standard Reference Materials 2121–2 and 955, a lead standard solution (10 000 mg l^{-1}) and a certified lead in blood reference material has made it possible to evaluate a method against definitive values and NBS reference materials.

In the proposed method, sample preparation consists of a simple dilution (1 – 9) with a matrix modifier which contains 0.5% *V/V* Triton X-100, 0.2% *V/V* 16 M nitric acid and 0.2% *m/V* dibasic ammonium phosphate. This matrix modifier stabilises lead so that the majority of the blood matrix may be removed during the char step. Maximum accuracy in dilution is achieved with the use of autopipettes which have been shown to deliver viscous materials such as blood and serum with high accuracy. The method described in this study has a detection limit of about 0.07 µmol l^{-1} (3 SD) and a precision and accuracy of ±2–5% at the 0.24–2.4 µmol l^{-1} concentration level. Linearity has been demonstrated up to about 4 µmol l^{-1}. Comparability has been established with the previous blood lead analytical method used in other surveys via the analysis of 435 specimens by both the previous (modified Delves cup) and proposed methods. The equation of the resulting line is [ETA-AAS] = 1.0007[Delves] – 0.051, r = 0.924.

Keywords: *Lead determination; blood; electrothermal atomisation atomic absorption spectrometry; L'vov platform*

We have developed a method for determining lead in blood that can be used to monitor the prevalence of exposure to lead and to establish normative blood lead values as part of a large health survey, the third National Health and Nutrition Examination Survey (NHANES III). During NHANES III, approximately 60 000 whole blood specimens will be collected in a statistical sampling of the US population during two 3-year collection cycles, 1988–1990 and 1991–1994. On the basis of previous findings from NHANES II[1] and Hispanic HANES,[2] the mean blood lead concentration anticipated for the NHANES III study subjects is about 0.25 µmol l^{-1}. This concentration is in the range of the levels other workers have measured in subjects from remote, relatively unpolluted areas,[3-5] about 0.15–0.4 µmol l^{-1}. Additionally, accuracy should be demonstrable by the use of calibration material and controls that have been determined by methods of defined accuracy, preferably by definitive methods. As several different analysts may be involved during the 6-year survey period, the method should be simple and rugged and minor changes in experimental conditions should have minimum effects on the performance of the system.

Recently, Subramanian[6] thoroughly reviewed methods for blood lead determination by electrothermal atomisation atomic absorption spectrometry. He stated in the conclusion of this review, "Let us hope that with the recent introduction of certified or working reference materials for Pb in blood. . . we will soon have definitive GFAAS methods. . ." Our proposed method is a step in this direction and the results are the first published values obtained by electrothermal atomisation atomic absorption spectrometry (ETA-AAS) on whole blood pools certified by the US National Bureau of Standards.

A large variety of methods have been published for the determination of lead in whole blood and many of these have been reviewed. ETA-AAS methods have been described that use relatively new methods of background correction, notably Zeeman[7] effect and Smith - Hieftje,[8] together with developments in fast electronics and data handling.[9-13]

Simply stated, the following were the requirements for the proposed method: simplicity and ruggedness, with minimum sample preparation; traceability of both standards and control materials to a legally and scientifically defensible accuracy base; transferrability to blood lead determinations performed in previous HANES studies (NHANES II and Hispanic HANES); sufficient sensitivity and precision to allow the low anticipated blood lead concentrations to be measured and small differences in concentration to be reliably measured; linearity up to about 4 µmol l^{-1}, enabling one specimen treatment and one calibration graph to suffice for nearly all specimens and transferrability to other laboratories.

The last requirement should be further clarified. This laboratory has a defined role in providing methods that are transferrable to other laboratories. Hence, during the method development, parameters that did not require instruments with expensive Zeeman background correction were preferred, and background or non-atomic absorption of less than about 0.2 A was required.

Experimental*

Instrumentation

We used a Perkin-Elmer Model 5000 atomic absorption spectrometer equipped with a Model 500 graphite furnace, an AS-40 autosampler and a PRS-10 printer - sequencer. A

* The use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the US Department of Health and Human Services.

Table 1. Instrument parameters for determination of lead in whole blood

Resonance radiation source	Hollow-cathode lamp
Lamp current	10 mA
Wavelength	283.3 nm, slit 0.7 (low)
Sample volume	20 µl

Temperature programme—

Step	Temperature/ °C	Ramp time/s	Hold time/s	Argon flow-rate/ ml min⁻¹
Dry*	130	10	25	300
Char	750	5	25	300
Atomise†	2400	1	4	20
Cool	20	1	4	300

* Temperatures used for drying vary slightly between lots of pyrolytic furnaces and platforms; each lot should be evaluated for smooth drying of the diluted specimen without spattering.
† Base-line function set at −7 s; read at 0; recorder at −1.

Model 056 recorder was used to monitor the analyte peak shapes. For comparison experiments we used a Perkin-Elmer Z3030 Zeeman-effect atomic absorption spectrometer, equipped with a Model 600 furnace and an AS-60 autosampler. The hollow-cathode lamp was from Perkin-Elmer, as were the pyrolytic platforms and furnaces (Part Nos. B0109-322 and 0290-2311). The graphite furnace programme is shown in Table 1. The internal gas flow-rate was 300 ml min^{-1}, except during atomisation (20 ml min^{-1}).

Reagents

All water used was purified to approximately 18 MΩ cm^{-1} with a Milli-Q system (Millipore). Nitric acid was obtained from G. F. Smith (16 M, re-distilled grade, catalogue no. 63); dibasic ammonium phosphate was obtained from J. T. Baker (catalogue no. 1-0784) and used without further purification. Triton X-100 was obtained from Fisher Scientific (catalogue no. CS 282-4). A lead standard solution of 10 000 mg l^{-1} (48.26 mmol l^{-1}) was purchased from the US National Bureau of Standards as SRM 2121-2. A matrix modifier was prepared by adding 1.0 ml of Triton X-100 and 0.40 g of dibasic ammonium phosphate to approximately 100 ml of Milli-Q water and stirring until the Triton X-100 dissolved. The matrix modifier was acidified with 0.40 ml of ultrapure 16 M nitric acid and diluted to 200 ml in a pre-cleaned Class A calibrated flask. A blank determination should be performed on this reagent at the beginning of each analytical run to ensure the absence of significant lead contamination.

Plasticware

Specimens were diluted in 2-ml conical autosampler cups (Fisher, catalogue no. 02544); 4-ml polyethylene Bio Vials were used for dilutions greater than 2 ml (Beckman, catalogue no. 566353). All plasticware was rinsed with copious amounts of purified water immediately before use.

Procedure

The lead standard solution (10 000 mg l^{-1} of lead in 10% *V/V* 16 M nitric acid) was diluted (1 + 9) with Milli-Q water to make an intermediate stock solution of 1000 mg l^{-1} (4.82 mmol l^{-1}). This intermediate solution was prepared bimonthly; on a wet-dry basis, working standards of 10 mg l^{-1} (in 0.5% nitric acid) and 50, 100, 250, 500, 750 and 1000 µg l^{-1} (0.24, 0.48, 1.21, 2.42, 3.62 and 4.82 µmol l^{-1}, respectively) were prepared in matrix modifier from this 10 mg l^{-1} solution. Working standards are diluted with an automatic pipette (Micromedic Model 25000) for maximum precision and accuracy.

Whole blood was well mixed with a vortex mixer and 100-µl aliquots were diluted with two successive 450-µl portions of matrix modifier. The dilution was accomplished with 1-ml sampling and dispensing pumps, at a setting of 10% (100 µl) and 45% (450 µl), respectively, for the whole blood and matrix modifier volumes.

Calibration was accomplished by pipetting 100 µl of blood as previously described with a 400-µl portion of matrix modifier (pump set at 40%) for dilution. Seven dilutions were prepared in a series of pre-rinsed 2-ml autosampler cups. Then, after the sampling delivery tip had been rinsed 6–8 times to remove all traces of blood, 100 µl of matrix modifier or 50, 100, 250, 500, 750 or 1000 µg l^{-1} of working lead standards (in matrix modifier) were added. This was followed by an additional rinse with 400-µl of matrix modifier. This calibration procedure resulted in a standard additions curve with a matrix modifier + blood mix (9 + 1). As noted by Shuttler and Delves,[9] standard additions curves are necessary for maximum accuracy. The whole blood chosen should be a low lead blood with the same anticoagulant as the specimens. We have found that dipotassium or disodium EDTA is the anticoagulant of choice owing to its more permanent action.

Duplicate aliquots of 20 µl of diluted blood, standards and control blood pools were dispensed on to the platform and the integrated absorbances were measured. Using the method of linear regression analysis, we plotted the best straight line through the calibration standard absorbances and used the slope of this graph to calculate unknown blood lead concentrations.

Method Optimisation

Matrix modifier

The matrix modifier chosen, 0.5% Triton X-100 in 0.1% nitric acid and 0.1% $(NH_4)_2HPO_4$, is similar to that described by Fernandez and Hilligoss.[11] The thermal pre-treatment (char) and atomisation temperature optimisation curves are shown in Fig. 1, in which a 1 + 9 dilution of pool DE3B03 (isotope dilution mass spectrometry target value 1.44 µmol l^{-1}) is evaluated. This pool was part of a series of whole blood pools characterised by the US National Bureau of Standards for the Centers for Disease Control (CDC) using the definitive isotope dilution mass spectrometric method of analysis. The amount of lead injected was approximately 600 pg. Pre-treatment temperatures up to 1100 °C could be used; the 750 °C temperature chosen was conservative. At this temperature the background absorbance is approximately 0.05 (0.10 A), which is well within the correction capability of deuterium arc systems.

Although very little carbonaceous residue is left from blood charred with this matrix modifier, the platform should be cleaned after each day of use. We found that about 300–350 firings could be obtained per platform and furnace before the precision was noticeably degraded.

Another feature apparent from Fig. 1 is that the atomisation temperature chosen, 2400 °C, does not give the maximum absorbance. The rationale for this choice is simple: at temperatures of about 1800 °C, the indicated maximum, the build-up of carbon residue was prohibitively high. The use of 1800 °C requires a burn-out step at 2600 °C, a feature we regarded as undesirable.

Temperature programme

Many workers have recommended using maximum power heating at atomisation,[11,12] which involves setting the ramp from char to atomise at zero. We chose not to use this setting, because of several considerations: with maximum power heating, the precision was poorer and an average increase in the coefficient variation of about 2–3% was observed for a given blood specimen; the slope of the calibration graph was greater with a 1-s ramp, allowing a lower limit of detection; and the background absorbance was lower with a 1-s ramp.

Fig. 2 illustrates the analyte and background signals obtained with the two different ramp settings. With a zero ramp the background absorbance is higher, is produced sooner and has a faster rise time. Other workers have noted this effect.[14–16] With a 1-s ramp, the lead peak maximum occurs before the background peak is at a maximum and the rate of change of the background peak is clearly less. Another frequent recommendation made is to use a zero argon flow-rate at atomisation. For volatile elements such as lead this usually increases the analytical sensitivity. The flow-rate chosen, 20 ml min^{-1}, is a compromise between the requirements of adequate sensitivity and linearity. With a 0 ml min^{-1} flow-rate, increased sensitivity is observed, with a calculated characteristic mass of 15.4 pg. The linearity of the proposed calibration is lost at about 3 µmol l^{-1}, however, and the background absorbance increases to about 0.2 A, which is at the limit of accurate correction by deuterium arc systems. If the linearity requirement is slightly relaxed, then a 0 ml min^{-1} flow-rate at atomisation can be successfully used with Zeeman-effect systems.

Results and Discussion

Our laboratory has served as the central laboratory for the NHANES programme since 1971 and has provided blood lead measurements during NHANES II and Hispanic HANES. In addition, over the last few years, our laboratory has served as a reference laboratory for three proficiency programmes. The performance of the proposed method has been evaluated by the determination of a series of whole blood pools, analysed in a blind fashion.

The results of the determinations of 42 pools during approximately 1 year are shown in Fig. 3. Very good agreement was obtained with the consensus mean used as target value for these pools. A further illustration of accuracy and precision is given in the determination of lead in a series of whole blood pools whose target values are established on the basis of a definitive method, isotope dilution mass spectrometry.[17] Within- and among-day or -run precision (SD within runs, SD among runs and SD total) were calculated by two-way analysis of variance. The results of these calculations are shown in Table 2. The pools in the DE series were analysed by the US National Bureau of Standards using isotope dilution mass spectrometry and were used in proficiency testing by another Center for Disease Control activity. The series of pools A–D are from the National Bureau of Standards, SRM 955, lead in blood. These pools were determined by isotope dilution mass spectrometry at the National Bureau of Standards. The target values for these pools in SRM 955 are given as follows: A, 0.27 ± 0.02; B, 1.47 ± 0.02; C, 2.38 ± 0.04; and D 3.53 ± 0.03 µmol l^{-1}. The uncertainties listed are estimates of two standard deviations of the mean and include between-vial variability.

With the use of area integration, the linearity of the calibration graph has been demonstrated up to about 4.0 µmol l^{-1}. Use of peak height reduces the range of linearity to about 3 µmol l^{-1} and is not recommended for theoretical reasons.[14] The characteristic mass is calculated at about 25 pg. Considering that the published value of 11 pg was calculated under conditions of zero argon flow-rate at atomisation, this is a reasonable agreement with our value at a 20 ml min^{-1} flow-rate. The limit of detection is calculated as 0.07 µmol l^{-1} (14 µg l^{-1}) according to the recommendations of Winefordner and Long.[18]

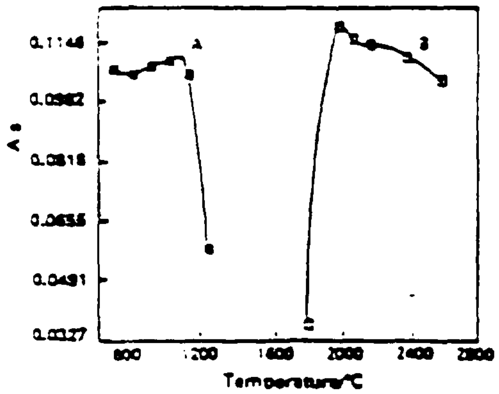

Fig. 1. Thermal pre-treatment and atomisation optimisation. (A) char and (B) atomisation curves for blood lead (pool DE5B03). Target value = 1.44 µmol l^{-1}

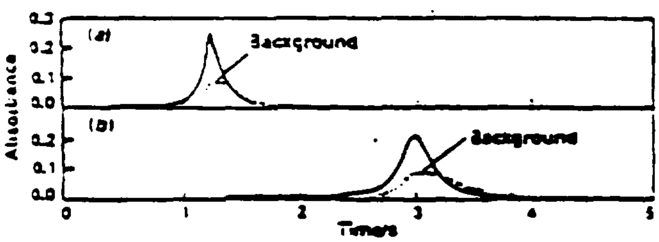

Fig. 2. Atomisation profiles for blood lead with (a) 0 s (maximum power) and (b) 1 s ramp times

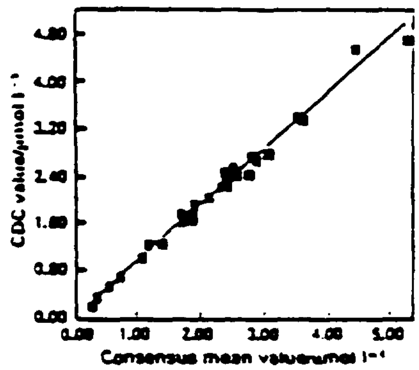

Fig. 3. Accuracy of method for New York and Pennsylvania State Proficiency Programs. 42 pools over 15 months. Centers for Disease Control (CDC), mean values vs. consensus mean values. Equation for the least-squares line: [ETA-AAS/CDC] = 0.955[consensus mean] − 0.063; r = 0.993

Table 2. Precision and accuracy of the ETA-AAS method

Pool	Target value/ µmol l^{-1}	CDC value/ µmol l^{-1}	Within-day SD	Among-day SD	Total SD	No. of runs	No. of determinations
NBS A	0.27	0.270	0.019	0.034	0.039	20	40
DE7C59	0.767	0.753	0.019	0.053	0.058	20	40
DE5B03	1.44	1.44	0.024	0.058	0.063	20	40
NBS B	1.47	1.45	0.029	0.077	0.082	20	40
DE1CO3	2.46	2.50	0.034	0.063	0.072	14	28
DE5B02	2.64	2.64	0.034	0.135	0.140	15	30
NBS C	2.38	2.38	0.072	0.159	0.179	14	28
NBS D	3.53	3.48	0.058	0.174	0.153	10	20

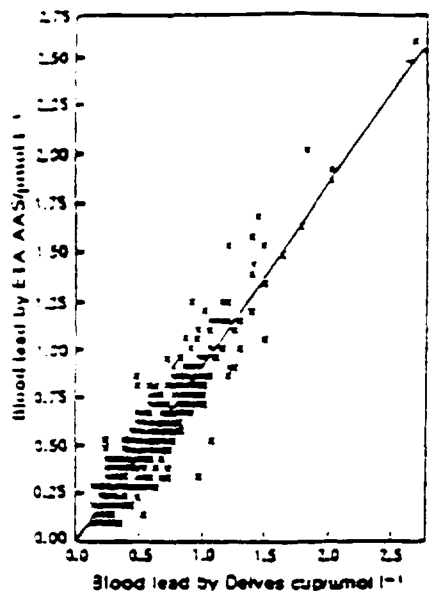

Fig. 4. Comparison of ETA-AAS and Delves cup measurements using 435 specimens from the Hispanic Health and Nutrition Examination Survey (HHANES). Slope = 1.0069, y-axis intercept = −0.051 µmol l⁻¹, r = 0.924, 95% UCL intercept = −0.0243 µmol l⁻¹, 95% LCL intercept = −0.0771 µmol l⁻¹, mean difference (Delves cup − ETA-AAS) = −0.047 µmol l⁻¹

Table 3. Summary of comparison measurements for the modified Delves Cup and the ETA-AAS method

	Delves cup	Graphite furnace
N	435	435
Mean/µmol l⁻¹	0.609	0.562
SD/µmol l⁻¹	0.340	0.342

An additional consideration in developing the proposed method for use in NHANES III was its comparability with our modification of the Delves micromethod[19] previously used for the two other large population surveys, NHANES II and Hispanic HANES. The comparability of these two methods was established to permit meaningful comparisons of population means, prevalence or longitudinal trends among surveys. To accomplish such a comparison, we determined lead in 435 whole blood specimens by both methods. The results of this experiment are shown in Fig. 4. For both methods statistical parameters were calculated by using a Deming regression which assumes error in both variables, the results of which are shown in Table 3. These results indicate a negative bias of about 0.05 µmol l⁻¹ for the ETA-AAS method compared with the Delves method in the range 0.25–1.2 µmol l⁻¹. The accuracy of the proposed ETA-AAS method in the range 0.25–1.5 µmol l⁻¹ has been rigorously evaluated with the determination of lead in selected whole blood pools from (1) the National Bureau of Standards (SRM 955 pool A and pool DE7C59), as indicated in Table 2 and (2) pools from the New York and Pennsylvania State Health Department Lead Proficiency Programs. Over a period of 2 years, 22 pools from these two proficiency programmes with consensus mean target values of less than 1.5 µmol l⁻¹ were evaluated by the proposed method. The results of the statistical analysis of these comparison data by regression analysis indicated an average error of −0.008 µmol l⁻¹ and a correlation coefficient of 0.967. These results are comparable or superior to those obtained by other reference laboratories in these two programmes.

Conclusions

The proposed method has been used for more than 1 year. The relatively high sensitivity and good precision allow the discrimination of blood lead concentrations that are closely spaced at low normal levels. For example, the base blood used for preparing the standard additions calibration has a value of about 0.18 µmol l⁻¹. Repetitive measurements (N = 20) of this and the National Bureau of Standards A pool (target value 0.275 µmol l⁻¹) showed that the two pools could be distinguished in a statistically significant way. This feature establishes the method as highly reliable for evaluating small concentration differences among specimens. We conclude that the accuracy and precision of the proposed method are sufficient to meet the requirements of a large population survey with low anticipated mean blood levels. Additionally, the method is sufficiently rugged to ensure reasonable constancy over the 6-year sampling period.

This work was supported in part by the National Center for Health Statistics through an intra-agency agreement with the Centers for Disease Control.

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GRAPHITE FURNACE PROCEDURE FOR BLOOD CADMIUM

(Revised August 26, 1983)

WORKING COPY MAY BE LOGGED OUT

Determination of blood cadmium is accomplished by flameless (electrothermal) atomic absorption. The specimen is deproteinized with the addition of nitric acid after dilution with water, and cadmium is determined in the acid supernatant. A L'vov platform is used to decrease matrix effects (7) and to increase precision and sensitivity (8). The procedure is based on published work by Stoeppler et al. (10).

EQUIPMENT

<u>Atomic Absorption Spectrophotometer</u>: Perkin Elmer Model 372, with Model 500 graphite furnace, Model AS-1 autosampler, and Model 56 recorder. Pyrolytic graphite furnaces and L'vov platforms are used.

Instrument Parameters:

Parameter	Setting
Wavelength	228.8 nm
Lamp Current	6 ma
EDL Power	6 W
Slit	0.7 (alt)
Signal Model	ABS
Read Time	4.0 s
Inert Gas	Argon
Furnace Type	Pyrolytic/L'vov
Background Corrector	ON
Temperature Program	

DRY	150 C 25 s (5 s ramp)
CHAR	450 C 25 s (5 s ramp)*
ATOMIZE	2000 C 5 s (1 s ramp)
COOL	20 C 10 s (1 s ramp)
Inert Gas Flow	300 mL/min; 20 mL/min @ ATOMIZE

*During the latter part of the CHAR, the baseline is reset to "0" using the BOC function of the 372; the settings at step 2 (char) are: READ 20s; BASELINE 25 s: REC 29s. READ and REC are set "0" during ATOMIZE.

Recorder: Model 56 set at 5 mV; 20 mm/min speed.

Automatic Pipet: Micromedic Model 25000, with 1 mL sampling and dispensing pumps; sampling pump set at "20%" (200 uL), dispensing at "50%" (500 uL).

REAGENTS

Water: Ultrapure water, polished by a Milli-Q system to 18 megaohm/cm purity is used throughout.

Nitric Acid: Redistilled grade nitric (GF Smith) or Ultrex (JT Baker) is used.

Stock and Working Cadmium Standards: A 1000 mg/L stock solution of cadmium acetate dihydrate is prepared from 237 mg of the cadmium salt, dissolved and diluted to 100 mL with ultrapure water. To a 100 mL volumetric flask, add 237 mg of the salt and 500 uL ultrapure nitric acid, diluting to the mark with ultrapure water. This stock solution should be prepared every six months. Intermediate stock of 10 mg/L is prepared by 1:100 volumetric dilution of the 1000 mg/L solution weekly; working and spiking stocks of 1.0 mg/L, 10, 25, 50, and 75 ng/mL are prepared daily from the 10 mg/L intermediate stock.

PLASTICWARE AND GLASSWARE

All plasticware and glassware used is cleaned by soaking 24 h in detergent, followed by soaking for 3 days in 25% v/v nitric acid. The cleaned items are then rinsed thoroughly with ultrapure water, and stored in a dust-free environment.

Venous blood specimens are collected in either 5mL vacutainers (Beckton and Dickinson) or 5 mL "Monoject" tubes (Sherwood Manuf.). Both these containers employ aqueous dipotassium EDTA as anticoagulant.

Eppendorf micropipets (Brinkmann Instruments) are used to prepare spiked aliquots for calibration, disposable polyethylene tips are used as received.

Beckmann Instruments' "Bio-Vials" are used for dilution of blood specimens; these 4 mL containers are cleaned as above.

SPECIMEN COLLECTION

Collection of an uncontaminated whole blood specimen is a critical part of many toxicological investigations. The following guidelines will provide directions which, if carefully followed, will minimize the contamination of whole blood by the many sources from which it may come. It cannot be overemphasized that skin, clothing, dust and many other sources of contamination contain many times the levels of lead, arsenic, cadmium, and other metals which will be determined in the collected specimen.

1. Clean the antecubital area thoroughly with: a) soap and water (Phisohex has been shown to be free from significant metal contamination); followed by b) alcohol (isopropanol or ethanol).

2. Puncture the skin/vein with a sterile, disposable needle capable of multiple sampling. A suggested product is the B + D catalog #5749, 20-gauge needle. In some applications, the first blood specimen collected will be discarded; its use is to "rinse" the collection needle with blood.
3. Collect one or more tubes of whole blood, using an appropriate anticoagulant for the metal of interest. Anticoagulant/metal combinations that have been shown to be compatible are:

Mercury-Heparin or Citrate

Lead-EDTA, Heparin, or Oxalate

Cadmium-Heparin or Oxalate

It is recommended that a few "spares" of the lot of tubes used for collection be sent to the laboratory along with collected specimens. This will allow the laboratory to determine the metal content of the anticoagulant used in that tube lot, and make appropriate blank corrections.
4. It is critical that the collected specimen be thoroughly mixed after collection, to insure the anticoagulant/blood mixture is uniform and that clotting therefore will be prevented. Clotted specimens are nearly useless!
5. Refrigerate the collected specimens, and ship refrigerated by the most expeditious means available. Heparin is by far the least "permanent" of the anticoagulants listed, but will prevent clotting for two weeks if well-mixed on collection and refrigerated after collection and during shipment.
6. Ship the collected specimens in well-padded, insulated containers (freeze safe or the equivalent).

ANALYTICAL PROCEDURE

1. Aspirate 200 uL of blood into the delivery tip of the automatic pipet; dispense blood and 500 uL water into a precleaned 4 mL plastic vial.
2. Aspirate air into the delivery tip, and dispense an additional 500 uL water into the same vial. This "double rinse" should give a quantitative transfer of blood to the plastic vial.
3. Add 50 uL of ultrapure nitric acid to the diluted specimen, cap and mix thoroughly on a vortex type mixer until protein precipitation is complete, as evidenced by the solution color change to a dark brownish red and the dispersion of precipitated protein/RBC's throughout the diluted specimen.
4. Centrifuge @ 2000 RPM for 5 minutes.
5. Decant the acid supernatant with a 250 or 500 uL Eppendorf pipet.
6. Measure the absorbance of the resulting supernatant in duplicate or triplicate, using the AS-1 autosampler to dispense 20 uL of solution into the graphite furnace.

STANDARDIZATION AND CALCULATIONS

Standardization is accomplished by the use of a modification of the method of standard additions. A bovine "base blood", typically containing less than 1 ng/mL cadmium, is diluted per the procedure, and aliquots are spiked with microliter additions of cadmium acetate standards.

Standard Addition Procedure:

1. Base (low cadmium) blood is prepared, using twice the prescribed volumes.
2. Into four precleaned autosampler cups, pipet 10 uL of 10, 25, 50, and 75 ng/mL cadmium standard, using a 10 uL Eppendorf pipet with disposable plastic tips.

3. Add 250 uL of acid supernatant to each of the four cups.

4. Transfer 250 uL of the remaining supernatant to a fifth autosampler cup.

5. Measure the absorbance of the resulting solutions in duplicate or triplicate.

Calculations:

Two methods of calculation have been used with the described procedure, each of which give essentially identical results. Each individual specimen may be analyzed by the procedure outlined above for standard additions. Although potentially highly accurate, this method suffers from extremely low throughput. Any differences in observed slope from spiked aliquots of different specimens will be automatically compensated for by this method. Since no measurable difference in slope was observed (within experimental error) between spiked bovine and human blood, either regression analysis, Method 2 or an "average slope" method, Method 1, are normally used.

Method 1 Average Slope

1. Calculate the average (mean) absorbance values for the solutions measured.

2. Correct the mean absorbances of the cadmium-spiked solutions by a dilution factor, df, calculated as follows:

$$df = \frac{\text{original volume + spiking volume}}{\text{original volume}}$$

In this procedure, the df will be 1.04, which is

$$\frac{250\ \text{uL} + 10\ \text{uL}}{250\ \text{uL}} + \frac{260\ \text{uL}}{250\ \text{uL}} = 1.04$$

3. Construct the following table:

Specimen	Corrected Absorbance	ng/mL added	Corrected Absorbance-A_{base}
Base	A_{base}	0	0
$Spike_1$	$A_{spike\ 1}$ (1.04)	2.5	$A_{spike\ 1}$ (1.04) - A_{base}

etc.

4. Calculate the factor, (ng/mL added)/(corrected absorbance - A_{base}) for each of the four spiked calibrators. The ng/mL additions are calculated as additions to the original blood specimen, and have the values 2.5, 6.25, 12.5, and 18.75 ng/mL.

5. Average the four factors from step 4, and multiply specimen absorbances (controls or unknowns) by this average factor to calculate the cadmium content in ng/mL. Make sure that all specimen absorbances are corrected for blanks (dilute nitric acid) before calculation.

NOTE: The value for the average of (ng/mL) (corrected absorbance -A_{base}) for this procedure usually falls in the range of 0.05-0.07 for the 2.5 ng/mL addition. The calculated factors should be examined for consistency; this is one indication of linearity of the calibration curve.

Method 2-Linear Regression

1. Construct the following table:

X (ng/mL added)	Y ($A_{corrected}$ $-A_{base}$)
0	0
2.5	$A_{spike\ 1}$ (1.04) $-A_{base}$
6.25	etc with spike 2
12.50	etc with spike 3
18.75	etc with spike 4

2. Using a calculator with linear regression curve fitting ability, and with Y-X calculation capability, enter the X, Y pairs of data as in the above table.

3. Calculate the r^2, slope and intercept for the data.

4. To calculate specimen values (controls or unknowns), enter the blank-corrected A (Y) values into the calculater, and calculate X (ng/mL).

NOTE: These two approaches will give essentially equivalent results if:

1) the r^2 value for the regression equation is high (0.98 or higher), and

2) the calculated intercept for the equation is near zero (0-0.05).

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two way analysis of variance, ANOVA, with the construction of quality control charts based on 95 and 99% confidence limits of the mean of duplicate measurements, as well as range charts (1).

Precision and accuracy of the analytical system will be monitored as follows:

1) Ten analytical runs will be performed to characterize all control materials used, with duplicate measurements performed per run.

2) Analysis of variance calculations will be performed on these twenty data points, and quality control charts will be generated by computer for $\bar{X}$ and range R.

3) A minimum of two control materials will be incorporated into each analytical run of twenty unknown specimens, and data obtained for these controls will be evaluated with the $\bar{X}$ and R charts from 2).

Blind Quality Control

Two types of blind quality control specimens will be incorporated into the system:

1) blind duplicate specimens will be prepared, and inserted at an interval determined by the supervisor, usually one blind duplicate per twenty unknown specimens.
2) blinded control or reference material samples will be inserted into each analytical run of twenty specimens, at the minimum rate of one blind control per twenty specimens.

In both cases, the blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Run Format

In all cases, the following format will be used for specimen determination:

SAMPLE #	Sample ID
1	blank
2-5 (or greater)	calibration curve
6	Control I
7-27	Specimens
28	Control II
29-33	Calibration Curve
34	blank

Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (1):

$\bar{X}$ chart

1) A single $\bar{X}$ value falls above the upper 99 percent limit or below the lower 99 percent limit.
2) Two successive $\bar{X}$ values fall either both above the upper 95 percent limit or both below the lower 95 percent limit.
3) Eight $\bar{X}$ values in succession fall either all above the center ~~line or all~~ below the center line.

R chart

1) A single R value falls above the upper 99 percent limit.
2) Two successive R values fall above the 95 percent upper limit.
3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

1) Check for errors in recording levels of control samples, and if none are found,
2) Check and calibrate instruments before performing further analyses on analytical samples,
3) Reanalyze patient samples performed during the out-of-control run.

METHOD PERFORMANCE

Limits of Detection

Limit of detection is in a practical sense determined by two factors: 1) the slope sensitivity of the method, i.e., the response (in the case of atomic absorption the absorbance or absorbance-second measurement) for a given concentration or amount of analyte, and 2) the random noise of the instrumental system used in measurement, especially that noise at the measured response for the blank for the determination (2).

According to the recommendations of the above reference, the limit of detection will be defined as that concentration of analyte corresponding to an absorbance or absorbance-second measurement equivalent to three times the standard deviation of this signal measured at an analyte concentration at a "low" level. In symbolic terms, this becomes:

$$c_L = \frac{3s_B}{m}$$

where c_L is the concentration calculated to be the limit of detection, s_B is the standard deviation of the measurement of a blank or low concentration sample; and m is the slope of the calibration curve (change in absorbance or absorbance-seconds/change in concentration or amount of analyte). For a set of measurements at the 0.6 ng/mL level, the standard deviation was 2.08×10^{-3}A, which yields a detection limit of 0.28 ng/mL (N=6).

Accuracy and Precision

At the present moment, there is only one commercial source of blood with a certified target value (4). Since this material is not available to the laboratory, evaluation of precision and accuracy was performed by the determination of cadmium in a bovine blood pool spiked with cadmium. Both the unspiked or "base" material and the spiked material were analyzed for cadmium with the described procedure.

Results were as follows:

POOL	Blood Cadmium ng/mL	Standard Deviation	% CV	N
unspiked	0.50	0.10	20.0	10
spiked	5.9	0.24	4.1	12

The spiking of the pool was performed to provide an approximate increase of 5 ng/mL in the blood; it can be seen from the above data that this goal was accomplished. Performance of the proposed method for EPA quality control water samples, prepared as dilute aqueous nitric acid control materials, has consistently given ± 10% accuracy; similar results have been obtained for National Bureau of Standards' SRM 1643a, Trace Elements in Water.

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Determination of Cadmium in Urine by Graphite Furnace Atomic Absorption Spectrometry with Zeeman Background Correction

1. *Description*
   a. *Analyte Name:* Cadmium
   b. *Method Code:* 0360A
   c. *Specimen Matrix:* Urine
   d. *Supervisor:* Mary M. Kimberly, Ph.D.__________
   e. *Statistician:* Sam Caudill, Ph.D.__________
   f. *Branch:* Nutritional Biochemistry Branch
   g. *Date:* 8/8/88 UPDATE: 4/27/90

2. *Special Safety Precautions:*
   a. Wear gloves, lab coat, and safety glasses while handling all human urine products. Disposable plastic, glass, and paper (pipet tips, autosampler cups, gloves, etc.) that contacts urine should be placed in a biohazard autoclave bag. These bags should be kept in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished.
   b. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analytical run.
   c. Special care should be taken when handling and dispensing concentrated nitric acid. Always remember to add acid to water. This material is a caustic chemical capable of severe eye and skin damage. Wear metal-free gloves, a lab coat, and safety glasses. If the nitric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.

3. *Specimen collection and storage procedures:*
   a. A 10-mL sample of urine is collected. It is convenient to collect a larger quantity in a plastic, sterile collection cup and transfer the required sample to to a 15-mL conical-bottom plastic tube. It is important that each lot of collection cups and shipping and storage containers be screened for cadmium contamination. If possible, the urine should be preserved to a concentration of 1% with nitric acid when collected. If it is not convenient to do this in the field, it should be done before specimens are stored (archived) for long periods of time.

A first-void urine specimen is preferred, but random ("spot") urine samples are acceptable.
Since cadmium is a very ubiquitous element, the risk of contamination is very high. The following protocol should be strictly followed in order to obtain samples that are free from external contamination.

Urine collection:

1. Ideally, metal-free disposable gloves should be worn by those handling the urine collection materials and specimens. Remove the cup and cap from its plastic wrapping, being careful not to dislodge the cap or touch the inside of the cup or cap.
2. Ensure that the cap is sealed on the container and affix the participant's preprinted label to the outside of the cup.
3. The collection instructions should be explained to the participant prior to the urine collection. It is important to stress that the inside of the cap not be touched or come in contact with any parts of the body or clothing or external surfaces; exposure to air should be minimized.
4. The participant's hands should be washed with soap and water before specimen collection.
5. The participant should not remove the cap from the collection cup until immediately prior to voiding. Caps should not be left off the cup longer than is necessary to collect the sample. The inside of the cap should not be touched. The cap should be turned up while the participant is voiding; the filled cup should be recapped immediately.

b. Processing procedure:

1. Specimens should be processed promptly to prevent microbiological deterioration.
2. Do not leave the caps off storage containers longer than is necessary to process the sample. Avoid touching the inside of the caps.
3. Gently swirl the specimen in the capped collection container to resuspend any solids.
4. Immediately after mixing, pour 10 mL of urine into the plastic tube that has been labelled with the participant's ID.
5. Cap and tightly seal the tube.

b. Specimens collected in the field should be frozen, then shipped on dry ice by overnight mail. Once received, they should be acidified to 1% with HNO^3 (if not previously done) and frozen at $\leq$20 °C until analyzed. Portions of the specimen that remain after analytical aliquots are withdrawn should be refrozen at $\leq$20 °C. Samples thawed and refrozen several times are not compromised. However, care should be taken if other analytes are to be analyzed in the same sample. The cadmium analysis should be performed first because of the high risk of contamination from repeated opening of, dispensing from, and closing of a sample tube or vial.

4. Principle of measurement

Cadmium is measured in urine by atomic absorption spectrometry by using a modification of the method described by Pruszkowska, et al (1). Quantification is based on the measurement of light absorbed at 228.8 nm by ground state atoms of cadmium from either a cadmium electrodeless discharge lamp (EDL) or from a hollow-cathode lamp (HCL) source. Urine samples, human urine quality control pools, and aqueous standards are diluted with a matrix modifier (nitric acid, Triton X-100, and ammonium phosphate). The cadmium content is determined by using a Perkin-Elmer Model 3030 atomic absorption spectrophotometer with Zeeman background correction. Cadmium contamination must be carefully avoided throughout all procedures. All materials used for collecting and processing specimens are screened for possible cadmium contamination. All processing work is performed under clean conditions, including laminar flow hoods.

5. Instrumentation

a. Perkin-Elmer (Norwalk, CT) model 3030 atomic absorption spectrophotometer with Zeeman background correction, including HGA 600 furnace, cadmium source lamp, EDL Power Supply (if EDL sources are to be used), AS-60 Autosampler, Model 100 printer and UV starter source.

Parameter	Setting
Wavelength	228.8 nm
HCL Current*	8 mA
EDL Power Supply*	6 W
EDL Mode	continuous
Slit	0.7 nm (low)
Signal Mode	Peak Area

* Only one source is used.

Furnace Temperature Program

Step	Temperature (degrees C)	Ramp (sec)	Hold (sec)	Gas Flow (mL/min)
Dry*	150	5	35	300
Char*	650	5	20	300

Atomize	2100	1	5	0
Burnout	2600	1	2	300
Cool	20	1	4	300

The program for the atomize step includes instructions for the instrument to turn on the Zeeman magnet at the beginning of the step (Read) and to activate the record function (Recorder) during the step.

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*Dry and Char temperatures and hold time may vary with different graphite tube and platform combinations.

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b. Micromedic Digiflex Automatic pipet equipped with 2000-uL dispensing syringe and 2000-uL and 200-uL sampling syringes (Micromedic Systems, Inc., Horsham, PA).
c. Mettler PL 200 top-loading balance (Mettler Instrument Corp., Hightstown, NJ).
d. Milli-Q water purification system (Millipore Corporation, Bedford, MA).
e. Vortex-Genie vortex mixer (Fisher Scientific).
f. Eppendorf fixed-volume micropipets: 1000, 500, 250, 200, 50, and 40-uL volumes (Brinkmann Instruments, Inc., Westbury, NY).
g. Magnetic stirrer (Corning Glass Works, Corning, NY) and stirring bars (Fisher Scientific).
h. Oxford automatic Dispensor (Monoject Scientific, St. Louis, MO).

6. Materials
a. Stock solution of cadmium: either NBS SRM 3108 or SRM 2121-1, both 10,000 mg/L, (National Bureau of Standards, Washington, DC).
b. Redistilled concentrated nitric acid (G. Frederick Smith Chemical Co., Columbus, OH).
c. Triton X-100 (Fisher Scientific, Fairlawn, NJ)
d. Ammonium phosphate, dibasic ("Baker Analyzed", J.T. Baker Chemical Co., -- or any source found to be low in cadmium contamination).
e. Ultrapure water (from the Milli-Q water purification system).
f. Argon, 99.996% purity (supplied as a compressed gas by Holox or other contract agency) equipped with approved gas regulator (Matheson Gas Products, Secaucus, NJ).
g. NBS SRM 2670, trace elements in urine (elevated) (National Bureau of Standards, Washington, DC). This is to be run periodically to verify accuracy.
h. Bench and blind human urine quality control pools including a base pool and pools spiked with "high-normal" and high levels of cadmium.

i. Pyrolytic graphite tubes, solid pyrolytic graphite L'vov platforms, insertion and alignment tools, and graphite contact rings (Perkin-Elmer).
j. Small plastic weighing boats (Scientific Products, McGaw Park, IL).
k. Metal-free pipet tips: 1-100 uL and 1-1000 uL sizes (Bio-Rad Laboratories, Richmond, CA).
l. Acid-cleaned volumetric flasks (1000, 100, and 10-mL volumes). The glassware is soaked in a soapy solution for at least 24 hours, rinsed, soaked in 25% nitric acid for 48 hours, rinsed with ultrapure water, and dried under clean conditions.
m. Conical-bottom 2-mL polystyrene autosampler cups (Lancer, St. Louis, MO).
n. Polystyrene 15-mL conical centrifuge tubes with polyethylene seal (Falcon 2099 Tubes, Becton-Dickinson, Oxnard, CA).
o. Floppy disks for storage of analytical software and methods (Maxell Corporation of America, Moonachie, NJ).
p. Kay-Dry paper towels and Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA).
q. Cotton swabs (Hardwood Products Co., Guilford, Maine).
r. Dehydrated alcohol, USP (Midwest Grain Products of Illinois, Pekin, IL).
s. Metal-free disposable gloves (Oak Technical Inc., Ravenna, OH).
t. Biohazard autoclave bags (Curtin Matheson Scientific, Inc.)
u. Bleach (10% sodium hypochlorite solution).
v. Polypropylene screw-cap 6-mL vials (Packard Co., Chicago, IL).
w. Model 100 printer ribbons (Perkin-Elmer).

7. Reagent Preparation

a. Before using any disposable pipet tip, first rinse it with 1% nitric acid by drawing up the volume of acid into the tip and then dispensing it into the sink or a waste beaker.
b. <u>0.10% (v/v) Triton X-100</u>
Using an Eppendorf pipet, dilute 1 mL of Triton X-100 in approximately 900 mL of ultrapure water in an acid-cleaned volumetric flask and mix well using a stirring bar and stirring plate. Remove the stirring bar before bringing the flask to volume. Store at room temperature and prepare as needed.
c. <u>1.0% (v/v) nitric acid</u>
Using an Eppendorf pipet, add 2 mL of redistilled concentrated nitric acid to 200 mL of ultrapure water in a clean plastic wash bottle and mix well. Using an Eppendorf pipet, add 1 mL of redistilled concentrated nitric acid to 100 mL of ultrapure water in another clean plastic bottle and mix well. Store at room temperature and prepare as needed.
d. <u>Matrix Modifier</u> (2% (v/v) nitric acid, 0.001% (v/v) Triton X-100, and

0.25% (w/v) ammonium phosphate)
Using Eppendorf pipets, dilute 2 mL redistilled concentrated nitric acid, and 1 mL 0.10% Triton X-100 in approximately 75 mL ultrapure water in an acid-cleaned 100-mL volumetric flask. Add 0.25 g of dibasic ammonium phosphate to the flask by washing down the weighing boat with ultrapure water delivered from a wash bottle. Bring the solution to volume with ultrapure water. After preparation, this solution should be checked for contamination and discarded if an absorbance value greater than 0.01 Abs-sec is observed for the water-matrix modifier blank. Store at room temperature and prepare daily in a flask dedicated to this solution.

8. Standards Preparations

a. <u>1000 mg/L stock cadmium standard</u>
Dilute 1.00 mL NBS SRM 2121-1 (delivered using either an Eppendorf pipet or the Micromedic Digiflex) to 10 mL with ultrapure water in an acid-cleaned volumetric flask. Store at room temperature and prepare every six months in a flask dedicated to this solution.

b. <u>10 mg/L stock cadmium standard</u>
Using either an Eppendorf pipet or the Micromedic Digiflex, dilute 1.00 mL of the 1000 mg/L stock cadmium standard to 100 mL with ultrapure water in an acid-cleaned volumetric flask. Store at room temperature and prepare monthly in a flask dedicated to this solution.

c. <u>1 mg/L intermediate cadmium standard</u>
Using either an Eppendorf pipet or the Micromedic Digiflex, dilute 1 mL of the 10 mg/L stock cadmium standard to 10 mL with ultrapure water in an acid-cleaned volumetric flask. Store at room temperature and prepare daily in a flask dedicated to this solution.

d. <u>Working cadmium standards</u>
Using the Micromedic Digiflex, transfer the following volumes of intermediate standard to 10-mL volumetric flasks and dilute to volume with ultrapure water:

Intermediate Stock Concentration (ug/L)	(ug/L)	Working Standard Concentration (uL)	Sample
	125	12.5	1
	250	25.0	2
	500	50.0	4
	750	75.0	6

Store at room temperature and prepare daily in flasks dedicated to these solutions.

e. Calibration standards

1.Prepare 8 mL twofold diluted (1+1) matrix modifier by mixing 4 mL of ultrapure water (delivered using an Eppendorf pipet) and 4 mL of matrix modifier (delivered using an Eppendorf pipet) in a 15-mL polystyrene centrifuge tube and mix well.

2.Using an Eppendorf pipet, dispense 20 uL of each of the cadmium working standards into separate autosampler cups.

3.Using an Eppendorf pipet, add 500 uL of the diluted matrix modifier to each of these autosampler cups as well as to an empty cup (to be used as a blank).

4.Place the blank in position 1 and the standards, in increasing order, in positions 2-5 on the autosampler tray.

9. Procedure

a. Preliminaries

1.(For information regarding the range of linearity and how to handle results outside this range, refer to the Calculations section of this document.)

2.Allow frozen urine specimens and quality control urine specimens to reach ambient temperature and mix on a vortex mixer for 10 seconds.

3.While the specimens are thawing, rinse enough autosampler cups for an analytical run with 1% nitric acid delivered from a wash bottle. Drain the cups upsidedown on Kay-Dry paper towels.

4.Before using any disposable pipet tip, first rinse it with 1% nitric acid by drawing up the volume of acid into the tip and then dispensing it into the sink or a waste beaker. Before using any plastic container, rinse it with 1% nitric acid delivered from a wash bottle.

b. Sample preparation

1.When the SRM 2670 is to be used for a quality control specimen, it must first be diluted tenfold (1+9). Using Eppendorf pipets, pipet 100 uL of the SRM into an autosampler cup. Add 900 uL of ultrapure water. Use this diluted urine as a quality control specimen.

2.Using either the Micromedic Digiflex or an Eppendorf pipet, dilute the specimens and controls twofold (1+1) with the matrix modifier solution in clean autosampler cups. Use 250 uL of specimen and 250 uL of matrix modifier.

3.Place the autosampler cups containing the specimens in positions 7 and following, with the controls first, on the autosampler tray. Fill an empty autosampler cup with ultrapure water and place it in position 6.

4.NOTE: It is important to have the same concentration of matrix modifier in both the standards and in the specimens (controls and unknowns).

c. Instrument setup for the Model 3030 and AS-60.

1.Turn on the argon gas (40 psi).
2.Turn on the cooling water supply.
3.Turn on the HGA 600 furnace.
4.Install a source lamp in position on the turret.
5.Turn on the spectrophotometer (Zeeman/3030). After the software has automatically loaded (the element selection page will come up on the screen), press the PRINT key on the keyboard. The red light beside the key will illuminate.
6.Turn on the PR 100 printer.
7.Turn on the source lamp.
If using an EDL, turn on the EDL power supply: Turn up the power to three-quarters of maximum and wait for the EDL to spontaneously light. (If the lamp does not light, remove it from the turret and expose it to the UV starter source until it lights and returns to its position in the turret.) After the lamp lights, run the power at a high level momentarily, then turn it down to the recommended wattage.If using a HCL, the 3030 software will light the lamp as part of setup.
8.In the SETUP mode, dial in the wavelength until the bar graph on the screen indicates a maximum. It may be necessary to press the GAIN key to bring the bar graph to approximately 50% (pressing the GAIN key will either increase or decrease the energy automatically).
After the lamp has warmed up for about 20 minutes and while still in the SET UP mode, use the knobs on the lamp holder, twist the lamp in the holder, and move it back and forth (without clamping on the black end piece of the lamp) to optimize the lamp position. This procedure may also require use of the GAIN key.
9.Press the USER INDEX key and look up the number of the method for cadmium. Type in the method number and press the RECALL key.
10.Enter the Programing Mode by pressing the PROG key. Check the Instrument parameters indicated on this page of the screen. Update, if necessary. An example of the Instrument page is attached.
11.Press the PROG key again to get to the HGA 600 page. Check the HGA 600 parameters and update if necessary. An example is attached.
12.Press the PROG key again to get to the Autosampler page. Check the parameters and update if necessary. An example is attached.
13.Install a new graphite tube and platform after 200 firings (about two full days' analyses). Open furnace by pressing the FURNACE key on the HGA 600. Install a L'vov platform in the furnace using the insertion tool. Make sure that the platform is properly seated in the tube by holding the tube on end and gently tapping it on a hard surface. If it falls out, reinsert using more pressure. Install the tube in the furnace with the platform at the bottom of the tube. Use the alignment tool inserted in the sample port while pressing the FURNACE key again to

close the furnace. After the furnace closes, press the furnace together to insure that it has properly closed.

14.Check the quartz windows of the furnace to make sure that they are clean. If there is evidence of sample spattering on the windows, remove the windows and clean using a cotton swab soaked in denatured alcohol. Wipe dry with a soft tissue (Kim-Wipe) and carefully reinstall.

15.Press the CONT key to enter the Continuous Mode. Press the AS STANDBY soft key; the arm of the autosampler will lift out of the wash cup. Check the alignment of the sampling tip by manually moving the arm to the sample port. If the alignment needs adjustment, unlock the autosampler base by turning the knob on the center front of the base. The knobs on the back left side and front left then can be used to move the base to the necessary position. Lock the base in place with the center front knob. Move the mirror down so that the tip can be observed. Check the depth of the autosampler tip in the graphite tube. Use the front adjustment knob to the right of the sampling arm to raise or lower the tip as necessary. Move the mirror back to the upright position.

16.Press the AS HOME soft key. The sampling arm will return to its position in the wash cup and flush for several seconds.

17.Condition the graphite tube: Press the RUN key. Type in the following temperatures, pressing the MANUAL TEMP soft key after each entry and waiting for about 5 seconds. Press the MANUAL TEMP again to turn off the furnace. Wait about 10 seconds before typing in the next temperature.

1000 °C
1500 °C
2000 °C
2400 °C
2650 °C

18.Press the RUN key.

19.Press the CHECK soft key.

20.Check the dry step of the furnace program; type 1 then press the MANUAL POSITION soft key. Lower the mirror and observe the sample tip as it deposits the sample on the platform. Continue to observe as the sample dries. (Return the mirror to upright, out of the light path, after the sample dries.) Press the MANUAL POSITION soft key again to keep the autosampler from sampling from this cup indefinitely. (The system will run through the cycle twice.) The drying should be complete 5-10 seconds before the char step begins. If it is dry sooner, decrease the hold time of the dry step appropriately. If it is not dry in time, two options are available. The temperature of the dry step may be increased or the hold time may be increased. Use the latter only if increasing the temperature will cause the sample to boil and splatter during the dry step.

21.If the parameters in the dry step need to be changed, press the PROG key twice to get to the HGA 600 page. Change the parameters as necessary.
22.Continue to check the dry step as in 20 until a successful step is observed.
23.When the appropriate dry temperature is determined (even if it is the one already in the program), make sure that the blank is low (less than 0.01 Abs-sec.

d. Operation

1.In the RUN mode, press the SAMPLER ON/OFF soft key to begin a run.
2.The Model 3030 will first run the calibration curve and then the quality control materials. Check that the quality control materials are within the specified limits.
3.If the values observed for this analytical run are in control, proceed with the analysis of the diluted urine specimens. (Refer to the Quality Control System section of this document for criteria.)
4.Turn off the system in reverse order.

Note: when turning off the EDL power supply, first turn off the power switch. When the power indicator decreases to zero, turn off the variable power knob.

e. Recording of Data:

1.Quality Control Data
Use the "HANES LABORATORY URINE CADMIUM STANDARD AND QUALITY CONTROL REPORTING SHEET" to record this data. This reporting sheet has self-explanatory blanks for the standard absorbance data, the linear regression information, and the quality control pool results. Prepare this form in duplicate, using carbon paper.
2.Analytical Results
Use the "NHANES III ANALYTICAL WORKSHEET" to record the specimen results. These have been prepared with a list of the sample IDs for each preracked run. Record the results for urine cadmium in ng/mL. If a result is below the detection limit of the method, write "ND" (for nondetectable) in the blank. If a sample is missing from the rack, write "NOSAX" in the blank. If a sample is not satisfactory, i.e. cannot be analyzed, write "UNSAX" in the blank. Prepare these forms in duplicate, using carbon paper.
For samples that are repeated, use an additional report sheet (the "NHANES III REPEAT WORKSHEET"). Prepare in duplicate using carbon paper.
3.Give all forms to the supervisor along with the hard copy of the data printout from the Zeeman 3030 and the hard copy of the printout obtained from running "URINCAD". After the supervisor checks the

data, the carbon copies and data printouts will be returned for filing in a notebook. The supervisor will keep the original copies of the reporting sheets.
4.Use the "QC" program in the HANES library of ROSCOE to enter the quality control data. This should be updated regularly.

f. Replacement and periodic maintenance of key components
1.Source lamp: a spare source lamp should be available. Order another if the spare is used for replacement.
2.Printer ribbon: a supply of printer ribbons should be on hand. Order more when the last is installed.
3.Graphite tubes and pyrolytic platforms: at least three months' supply should be kept on hand. Order more when the inventory falls below this quantity.
4.Graphite contact rings: approximately every six months, the graphite contact rings of the furnace housing will need replacement. Indications that this procedure needs to be performed are an apparent loss of temperature control (frequent adjustments to the dry temperature that are not relieved by reseating the platform in the graphite tube) or sounding of the alarm by the instrument indicating a problem with the tube. It is useful to maintain a log book to help keep track of when these relacements occur.
5.The sampling tip of the autosampler will need to be repositioned and trimmed every few weeks, depending on how the dispensing is proceeding.

10. Calculations

a. NOTE: the program used next will correct for the dilution discrepancy between the standard blank and the spiked standards using the dilution factor 1.04.

b. The method described here is linear up to 6 ng/mL. (The NBS SRM 2670 will routinely be above this level, but further dilution of the urine matrix to bring the value into range is not recommended.) Above this level, the calibration curve begins to take on some curvature and linear regression should not be used for calibration. Most of the specimens encountered in NHANES III will be well below this 6 ng/mL. If they are not, they should be handled as described below in 10.e. Use the linear regression program in ROSCOE ("URINCAD") to calculate the calibration curve and the specimen concentrations. Enter the water blank (from position 6 on the autosampler tray) as a specimen. The linear regression program generates slopes, intercepts, correlation coefficients, standardized residuals, and plotted and fitted curves. The correlation coefficient, r^2, for each curve should be 0.995 or better. For optimum sensitivity, slopes should be more than 0.05 and intercepts should be less than 0.02.

d. Repeat a specimen analysis when duplicate integrated absorbance or concentration values differ by more than about 0.015 Abs-sec or 0.3 ng/mL, respectively. Reanalyze specimens containing more than 2.5 ng/mL cadmium for confirmation (2,3).

e. If a survey specimen has more than 6 ng/mL cadmium, it should be reanalyzed at a higher dilution because this value is outside the linear range of the method. Also, some urine specimens may have a particularly difficult matrix due to dissolved solids such as phosphates. This will be manifested by very poor precision in the integrated absorbance of the analyte and/or by background absorbance greater than 1.0 abs-sec. Samples to be reanalyzed for these reasons should be diluted twofold with ultrapure water. Dilute the urine 1+1 with ultrapure water (250 uL of urine and 250 uL of ultrapure water). Use this diluted specimen as the sample for dilution with matrix modifier (proceed as in section 6.c.2). Since the program URINCAD uses a dilution factor of 2, the results for samples which have been diluted in this way must be multiplied by 2 (for an effective dilution factor of 4). On occasion, the diluted specimen will need further dilution for the same reasons described above. If the 1+1 dilution is not high enough, use a 1+4 dilution (100 uL of urine and 400 uL of ultrapure water) and proceed as above. In this case, an additional dilution factor of 5 must be applied after the URINCAD program has been run (for an effective dilution factor of 10).

f. The detection limit, based on three times the standard deviation of ten repeat determinations (4) of a urine containing a low concentration of cadmium, is 0.07 ug/L. Results below the detection limit are reported as nondetectable (use 0.01 ug/L for reporting). If one of the two replicates is lower than the detection limit, then the specimen should be reported as nondetectable, even if the average of the two replicates is $\geq$ 0.07 ug/L. Analysis of specimens with nondetectable cadmium should be repeated, preferably in a separate run. If the repeat analysis determines that there is detectable cadmium (it may be just above 0.07 ug/L), and if the specimen required dilution the additional dilution factor may result in a value much higher than the first analysis of this specimen. So that this sample is not mistaken as contaminated indicate on the repeat run sheet that the sample was diluted because of a matrix interference by placing "(M)" beside the repeat result.

11. **CDC Modifications**

a. **Matrix modifier is added as part of the standard and specimen preparation procedure rather than by separate autosampler addition to save time during the analytical run.**

b. **The plasticware is not dried in an oven. We obtained a low analytical blank by rinsing the pipet tips, autosampler cups, and other plasticware with 1% nitric acid immediately prior to use. There is no dilution of the reagents or specimens because the rinsing solution successfully drains from the surfaces.**

c. Aqueous calibration standards are used with no attempt to matrix-match the standards to the specimens because this technique was found to be accurate. The slopes of urine and aqueous calibration curves are not significantly different. This is also why we can analyze "difficult" matrices (see 10.e.) at a higher dilution using the same calibration curve used for less diluted samples. The same amount of matrix modifier is present in all samples, no matter what the dilution used.
d. A twofold (1+1) dilution of urine specimens and controls is used rather than a fivefold (1+4) dilution for improved sensitivity. A higher dilution is used for samples with problem matrices; the infrequency of these types of samples does not justify using a higher dilution for all of the specimens.
e. Triton X-100 is added to the matrix modifier to improve the precision of the autosampler dispensing as well as the "wetting" of the platform by the sample (5).
f. Maximum power mode is not used for atomization. A one-second ramp works well for elements such as cadmium because it allows for the analyte to be atomized before the majority of the background is burned off the platform.

12. Quality Control System

The method described in this protocol has been used for several years in the Nutritional Biochemistry Branch for environmental and occupational health studies. The method has proven to be accurate, precise, and reliable. The instrumentation used is "state-of-the-art".. The primary standard used is a NBS SRM. Estimates of imprecision can be generated from long-term quality control pool results.

Two types of quality control systems are used in this analytical method. These two systems are: (1) "bench" quality control specimens that are inserted by the analyst two times in each analytical run (a set of consecutive assays performed without interruption) so that judgements may be made on the day of analysis and (2) "blind" quality control samples that are placed in vials, labelled, and processed so that they are indistinguishable from the subject samples. The results of the blind specimens are decoded and reviewed by the supervisor. With both systems, all levels of cadmium concentration are assessed by taking these samples through the complete analytical process. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

Two levels of blind quality control pools are used. These pools are prepared in sufficient quantity to last throughout the survey. The levels chosen are in the "low-normal" (approximately 1 ng/mL) and "high-normal" (approximately 5 ng/mL) range so as not to be obvious to the analyst. The pools are prepared in the same way as the bench pools, but they are dispensed in vials identical to those used in the field for NHANES subject samples, labelled with psudoparticipant numbers corresponding to each geographical location of the survey, and stored at $\leq 20\,^{\circ}C$. At least one blind sample is randomly incorporated with every 20 NHANES samples and analyzed according to the method protocol.

The bench quality control pools comprise three levels of concentration spanning the "low-normal", "normal", and high (at the World Health Organization health-based biological limit) ranges for cadmium.

Reference materials (urine products with certified values assigned by independent reference methods) are used periodically as a check of accuracy. NBS SRM 2670 should be analyzed once a week for this purpose. If the stock of this material becomes low, another should be ordered in time to analyze it concurrently with the quality control materials currently in use so that a bridge may be formed between the materials. If the material ordered from NBS is of the same lot, a full characterization is not necessary. However, there should be some overlap between the old and new stocks.

Quality control limits are established for each pool. An analysis of variance (ANOVA) is performed for each pool after twenty characterization runs have been performed in which previously characterized NBS SRM and bench quality control pools are used for evaluation. In addition to providing quality control limits, the characterization runs also serve to establish homogeneity of the pools. Once the homogeneity of the bench and blind materials has been established, it is useful to have them analyzed by another independent reference method, e.g. IDMS.
Precision and Accuracy: (SEE ATTACHED TABLE)

After the standards and bench quality control materials are analyzed (at the beginning of an analytical run), the long-term quality control charts for each control material are consulted to determine if the system is "in control". Two types of charts are used. The first chart plots the means of the duplicate determinations and compares them to the 95% and 99% confidence limits as well as to the center line (the overall mean of the characterization runs). The system is out of control if any of the following events occur for any one of the quality control materials:

1. The mean from a single run falls outside the 99% confidence limits.
2. The means from two successive runs fall either above or below the 95% confidence limits.
3. The means from eight successive runs fall either all above or all below the center line.

The second type of quality control chart plots the range of the duplicate determinations and compares them to the 95% and 99% limits as well as to the center line. The system is out of control if any of the following events occurs for any one of the quality control materials:

1. The range from a single-run falls above the 99% limit.
2. The ranges from two successive runs fall above the 95% limit.
3. The ranges from eight successive runs fall above the center line.

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any analysis of specimens occurs.

13. Preparation of Quality Control Materials

Urine is collected from "normal" laboratory workers in sterile collection containers. It is pooled in acid-cleaned glassware and kept at 4 C. The level of cadmium in this base pool is evaluated using the analytical method described above. The urine is then clean-filtered in a stack system using the following filter sizes: 0.22, 0.30, 0.45, 0.65, 0.80, 1.20, 3.00 u, and a prefilter. After filtering, the urine is preserved with nitric acid at a concentration of 1%. The acidified urine is mixed well by stirring on a magnetic stirrer for several hours. The acidification process causes the urine to become cloudy from precipitation of proteins. Previous experience in this laboratory has shown further filtering to be ineffective. The base pool so prepared is divided into three portions. The first is kept as is. The other two are spiked to higher levels. The concentration of cadmium added depends upon the concentration of the base pool determined above. The target values for these two spiked pools are a mid-range "normal" value (assuming that the concentration of the base pool is in the "low-normal" range), and 5.0 ng/mL (the WHO health-based biological limit value (6)). Spiking is accomplished by the addition of NBS SRM 2126-1 and mixing on a magnetic stirrer for several hours. Before dispensing the pools, the levels are again evaluated using the analytical method described above.

Bench quality control pools are dispensed into 6-mL polypropylene screw-capped vials that have been previously screened for cadmium contamination. Dispensing is accomplished using an Oxford dispensor. (NOTE: The glassware and tubing of the dispensor are cleaned before use using 1% nitric acid followed by ultrapure water.) Into each prelabelled vial, 2.5 mL of the urine are dispensed and the vials are capped. Twenty vials are selected at random from each pool for characterization of the quality control limits before the pools are frozen at $\leq$20 °C.

Two levels of blind quality control pools are prepared and similarly dispensed except that vials and labels identical to NHANES specimen vials are used.

14. Reference Ranges

a. WHO recommendations

The World Health Organization has recommended 10 ug Cd/g creatinine as the critical level for cadmium in urine. A value of 5 ug Cd/g creatinine has been considered the health-based biological limit (6).

b. Other references

Ewers, et al (7) proposed 2 ug Cd/g creatinine as the upper normal limit of cadmium in urine.

c. CDC experience

The results of the first pilot study from NHANES III ranged from 0.05-3.0 ng mL with a 95% range of 0.1-1.6 ng/mL. A total of 399 specimens were analyzed.

15. Special Method Notes

16. References

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TABLE 3. BIOMEDICAL TESTS (SERUM)

Test	Reference range		
AST (SGOT)	0-6 mo	0-120 IU/L	
	7-12 mo	0-110 IU/L	
	1-5 yr	0-75 IU/L	
	6-10 yr	0-60 IU/L	
	> 10 yr	0-50 IU/L	
ALT (SGPT)		0-50 IU/L	
GGT	Male	0-65 IU/L	
	Female	0-45 IU/L	
AP	0-19	40-300 U/L	
	20-60	20-125 U/L	
	> 60	20-150 U/L	
Albumin		3.5-5.5 g/dL	
Cholesterol*	Adult	Desirable	< 200 mg/dL
		Borderline—high	200-239 mg/dL
		High	≥ 240 mg/dL
	Pediatric	Not established	
Glucose*	< 50 yr	60-115 mg/dL	
	≥ 50 yr	60-125 mg/dL	
Total protein	Newborn	4.6-7.2 g/dL	
	< 2 yr	5.7-8.2 g/dL	
	≥ 2 yr	6.0-8.5 g/dL	
Creatinine	Male	0.2-0.7 mg/dL	
	Female	0.3-0.9 mg/dL	
BUN		7-26 µg/dL	
Electrolytes			
Sodium		135-148 mEq/L	
Potassium		3.5-5.5 mEq/L	
Chloride		94-109 mEq/L	

* These tests may be eliminated due to fasting requirements.

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TABLE 4. ROUTINE BLOOD AND URINE TESTS

Specimen	Test
Blood	CBC to include: Hemoglobin and hematocrit White blood cell count and 5-part differentials* Red blood cell count, indices, and morphology Platelet estimate and reticulocyte count
Urine	Chemical urinalysis (routine dipstick) Microscopic urinalysis Osmolarity/specific gravity

* Two blood slides may be prepared for manual determination of differential.

3.5 QA/QC REQUIREMENTS

The on-site personnel will generate and analyze quality control specimens for the biomedical tests and routine blood and urine tests. "Blind" controls will be obtained from Baxter Scientific Products as follows:

Biomedical Tests—a lyophilized, assayed chemistry control serum (in the normal and abnormal ranges) will be used.

Urinalysis—human urine controls (in the normal, high abnormal, and low abnormal ranges) for physical, chemical (test strips), and microscopic examination will be used.

CBC—hematology controls (in the normal, high abnormal, and low abnormal ranges) will be used.

Controls will be included at the rate of 15% of field specimens in each batch submitted to Roche and the local hospital laboratories for analysis.

Standard laboratory QA/QC procedures and guidelines will be applied to ensure that specimen integrity will be maintained throughout collection, preparation, storage, and transport. These include:

- Training of personnel by MRI in the procedures incorporated into the specimen collection and shipping protocol to be supplied by ATSDR. A copy of the protocol will be available at each collection site for reference.