# CONCENTRATION OF LEAD IN SOIL AND DUST AND BLOOD LEAD LEVELS IN ALAVERDI CHILDREN

***Research Grant Proposal***

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Abstract

Lead is an omnipresent toxic metal. In industrialized countries the adverse health effects of lead exposure are considered among the most important environmental health problems. Armenia has several locations contaminated with lead. These are mostly the places with lead releasing industries. However, no study has been conducted on the source of contamination, media, and factors influencing exposure and susceptibility to lead toxicity. One of the lead contaminated places is Alaverdi, the location of a copper mining and metallurgical complex. Moreover, Alaverdi is located in a valley where temperature inversion may trap air pollutants near the surface of the earth, which may increase exposure. Therefore, the Alaverdi population is at risk of high lead exposure. This necessitates preventive measures to safeguard the population, especially children, from lead exposure.

The aim of this study is to investigate the association between lead concentrations in the environment and blood lead levels in Alaverdi children. The study population will consist of 351 children between the ages of 3 – 10 living in Alaverdi. Environmental lead levels will be measured in the houses, yards, and gardens where the children participating in the study live and spend their leisure time, as well as in the kindergartens they attend. Mothers of these children will be interviewed to determine risk factors of exposure. The study will be conducted using a cross-sectional design. The analysis of the association between the children's blood lead level and environmental lead concentration will be done using a multiple regression model controlling for factors potentially effecting this association. Dose-response relationship will be analysed by Chi-square test of trend.

The proposed study will show if a problem of lead exposure exists in Alaverdi children related to environmental lead contamination. The investigation of site-specific factors enhancing susceptibility to lead dosing will be helpful in elaboration and dissemination of lead exposure prevention measures.

The program will be implemented by the Center for Health Services Research and the Environmental Conservation and Research Center of the American University of Armenia. Key personnel of the program are presented by Project Director, Research Assistant, and Accountant. In addition, Nurses, Sample Collectors/Interviewers, Data Entry/Analyst, and Consultants are involved into the study.

The requested amount of money is 29,872 USD. For the analyses of blood, soil, and dust lead levels the equipment of Environmental Conservation and Research Center of American University of Armenia will be used. The total cost of the equipment is 44,000 USD.

## Specific Aims

The aim of this study is to investigate the association between the concentration of lead in the environment and blood lead level in Alaverdi children. Children aged 3 – 10 and their mothers will participate in the study. Children of this age group were born during the period when the Alaverdi smelter was working at low capacity. During this period, the blood lead level in mothers of these children was assumed to be lower than during the period when the plant was functioning at full capacity. Therefore, the contribution of maternal blood lead to children's blood, as a result of transplacental hemotransfusion or through breast milk, was low. Thus, soil and dust lead concentrations contribute predominantly to blood lead levels in children born during these years.

The hypothesis to be tested by this research is: a positive relationship exists between environmental lead levels in soil and dust and children's blood lead levels.

The study will be conducted using a cross-sectional design. The proposed study has the following objectives:

- Determine the concentration of lead in soil and dust as an indicator of environmental lead contamination.
- Measure the children's blood lead levels.
- Interview mothers to ascertain risk factors for lead exposure in children.
- Assess the relationship between the children's blood lead level and the concentration of lead in the environment considering the variables assumed to influence this association.

Considering the fact that in several areas of Armenia the concentration of lead in soil and dust notably exceeds [1,2] the natural terrestrial background levels of 10 – 20 mg/kg [3], the findings of this study could show if there is a problem of lead dosage in children. Moreover, the assessment of behavioral and hygienic patterns and how these factors effect blood lead level may reveal site-specific situations that enhance susceptibility to lead dosing. Awareness of these factors may be helpful in elaboration and dissemination of lead exposure prevention measures.

## Background and Significance

Armenia has several locations that are contaminated by lead, mostly the places where lead releasing industries are located. The study conducted by the Institute of General Hygiene and Occupational Diseases in Yerevan, Byureghavan, and Gagarinavan have focused on lead concentrations in dust, soil, and air, as well as on blood lead levels in resident populations. High levels of lead have been found in dust, soil, and air at these locations, as well as increased blood lead levels in children living in Byureghavan and Gagarinavan [1].

A study conducted by the Environmental Conservation and Research Center of the American University of Armenia and Johns Hopkins University found increased levels of lead in garden and yard soil and in loose and interior dust in Alaverdi, as well as in some parts of Yerevan city. Lead levels in soil and dust in these areas were twenty- to forty-fold higher than the established background level of 10 mg/kg.

In Alaverdi, the mean concentration of lead in the soil of gardens was 308.12 mg/kg, 492.52 mg/kg in the soil of yards, and 770.6 mg/kg in loose dust. Indoor dust levels were 141.54 $\mu g/ft^2$ in building entrances, 53.14 $\mu g/ft^2$ in hallways, 49.81 $\mu g/ft^2$ in living rooms, and 52.61 $\mu g/ft^2$ on kitchen windowsills [2]. The main source of environmental lead contamination in Alaverdi is the Alaverdi copper mining and metallurgical complex. During the period from 1991 to 2000, the Alaverdi smelter was functioning at low capacity. In 2000, it started operating at full capacity again. Currently, the Alaverdi copper plant is among the main environmental polluters in Armenia [4, 5].

In Yerevan, elevated lead levels were detected near a battery plant located in the southwest part of the city and in 20% of apartment yards surveyed. The increased concentrations of lead in the apartment yards were mainly due to the improper disposal of batteries, the burning of garbage, and fluids from car maintenance. In summary, the average concentration of lead in Yerevan soil was 236 mg/kg [2].

While information exists on Alaverdi and Yerevan environmental lead contamination, how this relates to the body lead burden of people residing in these areas is unknown as no population based survey measuring biological markers of exposure has been conducted. Based on research conducted in other countries showing the correlation between environmental lead concentrations and blood lead level [6, 7] it can be assumed that increased blood lead levels may be observed among the residents of the above mentioned sites. However, some specific life-style factors such as behavioral and hygienic practices may influence lead dosing.

## Literature Review

### *Lead. Overview*

Lead is an omnipresent toxic metal. In industrialized countries the adverse health effects of lead exposure are considered among the most important environmental health problems. According to CDC, blood lead levels at or around 10 μg/dL pose a high risk in sensitive populations. In terms of adverse health effects of lead exposure, the sensitive populations are infants, children, and pregnant women (as surrogates for fetuses) [8]. Adverse health effects of lead exposure are death, impairment of immunological, neurological, reproductive, and other systems, as well as developmental disorders, genotoxicicity, and carcinogenicity [9].

Lead is a naturally occurring element. In small amounts it is found in the earth's crust mostly in the form of sulfide galena [9]. In addition, a variety of other chemical forms of lead are present in all strata of the biosphere. Naturally occurring concentrations of lead in soil and dust, which are not the result of anthropogenic releases, represent baseline levels of lead in the environment. Natural and, predominantly, human activities result in the spread of lead throughout the environment. The primary anthropogenic source of lead in the environment used to be due to leaded gasoline in internal combustion engines [9]. However, after the prohibition of leaded gasoline this source of environmental lead contamination became less important. Instead, soil contamination from industrial releases by smelters, battery and chemical plants, and old constructions coated with lead-based paint constitutes the prevailing part of total lead releases [9].

Natural chemical and physical processes such as weathering, runoff, precipitation, dry deposition of dust, and stream/river flow result in the continuous transfer of lead between air, water, and soil [9]. On average, lead may reside in the atmosphere for up to 10 days [9]. During this time, it can be transported long distances, even up to thousands of kilometers. In contrast, in both water and soil lead is highly persistent and is mostly connected to suspended solids and sediments in aquatic systems and is presented in quite immobile forms in soil [9].

The main sources of lead exposure in the general population are ambient air, some types of food, drinking water, soil, and dust [9]. Potential risk factors for lead exposure are residing in urban areas, especially near stationary emission sources such as iron and steel production industries, non-ferrous (brass and bronze) smelters, battery and chemical plants, consumption of food from family gardens, renovation of homes containing lead-based paint or contact with interior lead paint dust, pica (the abnormal eating manifesting of eating soil, paint chips, and other inedible stuff), occupational exposure, secondary occupational exposure (which is more typical for the families of workers involved in industries dealing with lead), usage of lead-containing health care products and folk remedies, hobbies such as sculpturing and staining glass, in which lead may be used, smoking, and wine consumption [9].

*Toxicokinetics of lead*

In humans, lead is absorbed predominantly through the gastrointestinal and respiratory tracts. Inhaled lead may be deposited in both the upper and lower parts of the respiratory tract [8]. Lead deposited in the upper part undergoes ciliary clearance, then it is swallowed and absorbed from the intestine. Smaller lead particles with diameter less than 1 μm pass to pulmonary portion of respiratory tract and are absorbed from there [8]. Little is absorbed through skin, which is a more typical route for occupational exposure [9].

The extent and rate of gastrointestinal absorption are influenced by physiological status of the exposed individual, such as age, fasting, nutritional calcium and iron status, pregnancy, and physicochemical characteristics of exposure medium [9]. Dietary lead absorption is considerably higher in children than in adults [9]. Studies conducted in infants and children indicate 40 – 50% lead absorption rate from intestinal tract [9], while in adults, the intestinal absorption rate is 20 – 70% in fasted subjects and 3 – 15 % in fed ones [10 – 12]. The influence of diet on lead absorption is discussed below. Increased lead absorption due to physiological changes can be observed during the second half of pregnancy. However, other factors also may be responsible for the increase in blood lead level [14, 15]. Immediately after absorption, lead is widely distributed to blood plasma and soft tissues, then it is redistributed and accumulates in bones [9]. In children, approximately 73% of the total body lead is concentrated in bone [9]. In adults, due to age-specific bone turnover rate changes, bone lead concentration constitutes 94% of the total body lead burden [9]. Lead that is not retained in the body is mainly excreted by the kidneys in the form of salts or through intestines as organometallic conjugates [9]. Overall, lead kinetics in the body is mostly determined by mechanisms influencing lead exchange between blood plasma and bone surfaces, processes of bone growth and resorption, and heteroionic exchange processes in kidneys and intestines [9]

*Blood lead concentration as a biologic marker of exposure*

Blood lead is the most common and most useful indicator of lead exposure in acute and subacute poisoning [10]. Blood lead measurement is also the most widely used method of measuring chronic lead exposure. In general, blood lead reflects recent exposure (exposure 20 – 30 days before measurement) in young children who were not extremely and chronically exposed to lead during their earliest years. In contrast, blood lead levels of heavily exposed children and adults integrate recent and older exposures [10].

*General population and occupational exposure*

The general population can be exposed to lead mostly through the ingestion of contaminated food and drinking water and by inhalation of lead particulates in ambient air. Fruits, vegetables, and grains may contain large concentrations of lead as a result of plant uptake of lead from soil and deposition of lead onto plant surfaces [9]. Occupational exposure is the most common source of lead exposure in adults [9]. The following industries are potentially dangerous for occupational lead exposure: lead smelting and refining industries, battery manufacturing plants, steel welding or cutting operations, construction, rubber products and plastic industries, printing industries, firing ranges, radiator repair shops and other industries requiring flame soldering of lead solder, and gas stations [9].

Children are the most susceptible population to lead exposure [8]. Their susceptibility to lead exposure can be explained by the following interacting factors: first, they have more opportunity for contact with lead sources due to their activities. Children crawl on the floor, they put things in their mouths, they may ingest inappropriate things such as dirt or paint chips, and they spend more times outdoors. In addition, being closer to the ground due to their height and the absence of judgment in avoiding hazards put them at higher risk [8]. Other reasons for children's high susceptibility to lead exposure are easier absorption of lead in a child compared to an adult and the higher vulnerability of a child to adverse development following lead exposure [8, 9].

The major sources of exposure of young children to lead are soil and dust [16 – 18]. It was estimated that an increase in dust lead levels from background levels to 200 $\mu g/ft^2$ increases the percentage of children having a blood lead level greater than 10 μg/dL by 23.3% [8]. An increase in soil lead concentration from background of 10 – 20 mg/kg to 400 mg/kg leads to the increase in the percentage of children having the same characteristic by 11.6% [8]. An analysis of 22 cross-sectional studies in areas with polluted soil revealed an association between lead in soil and children's blood lead level. According to this analysis, mean blood lead concentrations in children exposed to lead levels of 1000 mg/kg was 1.10-1.86 times higher whereas blood lead concentrations in children exposed to soil lead levels of 2000 mg/kg was 1.13-2.25 times higher compared to those exposed to soil lead levels of 100 mg/kg [7]. In addition to the ingestion of soil or dust through normal hand-to-mouth activity, children engaging in pica behavior are at greater risk for ingesting large amounts of lead contaminated soil. It has been estimated that an average child may ingest between 20 to 50 mg of soil per day [19, 20]. A pica child may ingest 5,000 mg or more of soil per day, which is more than 100 times the amount ingested by a child not engaged in pica behavior [19, 20].

Lead in food and drink are also sources of children's lead exposure. Food and drinks can be contaminated by lead as a result of their storing or preparation in lead-soldered cans, in ceramic jars or other pottery covered by lead-based glaze, and crystal decanters and glasses [9]. In addition, lead can be absorbed in food crops in the field or garden from soil or through direct deposition onto crop surfaces, during transportation, processing, and preparation in the kitchen [9]. Mining and industrial discharges into the aquatic environment result in the increased concentrations of lead in fish and shellfish. Cattle grazing in the areas with high lead contamination of soil have increased lead levels in cow milk and meat [9]. Parental smoking also may influence blood lead level in children because of the presence of lead in tobacco [21]. Concentration of lead in tobacco is approximately 2.5 – 12.2 μg/cigarette, of which only 2 – 6% may actually be inhaled by smoker [9].

Household members' occupation can also be a source of lead exposure: workers occupationally exposed to lead may carry lead home on clothing, bodies, or tools. In households with occupationally exposed worker blood lead levels of children are twice those of children living in the same neighborhood whose parents do not involve in occupations with high lead exposure [22, 23]. Children may also be exposed to lead because of some hobbies and artistic activities practiced at home by adults. Hobbies and activities involving the use of lead-containing materials are casting, stained glass, painting, glassblowing, and screenprinting [9]. Children from families engaged in tile-glazing production had significantly higher blood lead level with the median of 6 μg /dL compared to those than those living in homes with no such activity (median 2.1 micrograms/l) [24].

Several studies demonstrated the positive association of children's blood lead level with age [25, 26], sex [26], race [26], proximity to the smelters [27], floor on which apartment is located [25], number of siblings [25], carpet in a child's room [25], consumption of locally grown vegetables [25], socioeconomic status [28], education level of parents [29], household hygiene [21, 25, 30], and season [21].

*Adverse health effects of lead exposure*

A variety of adverse health effects caused by lead exposure can be demonstrated in people of different age groups. Central nervous system in children and fetuses, growth in children, cardiovascular system in older people, heme synthesis, and calcium homeostasis are main systems and processes targeted by lead [6]. High levels of lead exposure may cause severe damage of brain and kidneys in both adults and children. High-level exposure may result in miscarriage in pregnant women and can damage organs responsible for sperm production in men [9].

*Adverse effects of lead exposure on children's health*

Lead exposure during infancy or childhood is associated with anemia [31], neurological impairment, renal alterations, and colic [9], and vitamin D metabolism impairment [32, 33]. In addition, lead exposure *in utero*, during infancy or childhood results in delays or impairment of neurological development, neurobehavioral deficits including IQ deficits, growth retardation, low birth weight, and low gestational age [34 – 36]. Neurobehavioral effects resulted from lead exposure during childhood may persist into adulthood [37]. Increased blood lead levels in infancy and early childhood may be manifested in older children and adolescents as increased attention span, reading disabilities, and failure to graduate from high school [38].

The above stated information from various studies evidences the dangerous effects of the toxic metal lead on human body, especially when the exposure is in childhood. This necessitates preventive measures to safeguard children from lead exposure in the areas with high lead levels in the environment. The elaboration of preventive measures should be based on real site-specific data on the routes of exposure. Therefore, it is proposed to conduct a study investigating if the problem of lead exposure exists in Alaverdi children related to environmental lead contamination with the focus on specific life-style factors such as behavioral and hygienic practices which may influence lead dosing.

## Research Design and Methods

### *Study design and population*

The study will be conducted using a cross-sectional design. Children of Alaverdi, aged 3 – 10 and their mothers will participate in the study. These children were born during the period from 1992 to 1999 when Alaverdi smelter was working at low capacity.

*Inclusion and exclusion criteria*

All children born during the period from 1992 to 1999 registered in Alaverdi polyclinic and residing in Alaverdi will be included into the study. Children registered in other health facilities will be excluded. Children registered in the Alaverdi polyclinic but living out of town also will be excluded. In addition, children who will meet the stated inclusion criteria but have been absent from the city during the last 30 days prior to blood lead test will also be excluded.

*Study instruments*

Two types of questionnaires were designed for the study (Attachments 1 and 2). One type of questionnaire is for mothers and the other is for of day care center personnel. The questionnaire for mothers consists of 50 questions. They are grouped into the following sets of questions: introductory questions, questions related to family members' activity, questions related to family hygiene and questions related to children's hygiene and behavior. The questionnaire for of day care center personnel has 16 questions grouped into sets related to hygiene in day care center and to children's hygiene and behavior at the day care center.

*Sample Size*

Sample size was calculated using Stata Statistical Software package. The command for sample size for continuous data (one sample) is:

sampsi $\mu_0$ Y, sd($\sigma$) onesample alpha($\alpha$) power(power)

where $\mu_0$ – population mean

Y – sample mean

σ - population standard deviation

$\alpha = 0.05$

power = 0.8

Population parameters – mean and standard deviation – are derived from literature [16] and equal 7.7 and 5.1 respectively. The expected sample mean is estimated as 10. This number represents the mean blood lead level in children of Byureghavan study [1]. Thus, putting the numbers into command, estimated sample size of 39 is calculated. However, to make the sample size more robust for the planned analysis this figure should be multiplied by 8. The multiplication increases the sample size to 312. Variables to be included into the multiple regression model are soil lead levels, dust lead levels, gender, occupation of parents, and hygienic factors. Soil and dust lead levels are continuous variables. Gender, occupation of parents, and hygienic factors are dichotomous. The rationale of choosing the above stated variables is the following:

- Boys are more involved in outdoor activities and play with soil more than girls.
- Parents working in smelter plant can bring home leaded stuff or enter home in working clothes, which may be an additional source of exposure besides soil and dust lead.
- Practicing some hygienic activities may serve as protecting factor, decreasing lead dosing.

The study sample will be selected by systematic sampling. The total number of children aged 3 – 10 is 1563, which is the sampling frame; 1563 divided by 312 is 5. Therefore each fifth child from the list will be chosen. If in some cases the fifth child is not available or does not suit for the study the next or previous one will be chosen. The list of children will be obtained from Alaverdi Polyclinic.

*Confidentiality/Human subjects*

The proposed study presents minimal risk for the study population. The proposed procedure of drawing blood is consistent with the procedure of taking blood for regular check-ups and diagnostic purposes accepted in Armenian health care system.

Informed consent (Attachment 3) reflecting the purpose of the study and brief the description of blood, dust, and soil sampling procedures will be presented to Lori Marz health officials, to Alaverdi Polyclinic, as well as to the parents of all study participants. Several steps will be taken to guarantee the confidentiality of information. The name of respondents will be replaced with a code. Study participants, including sample collectors, interviewers, and the health personnel of Alaverdi polyclinic involved in the study, will be instructed to not share any obtained personal information.

All supplies for blood testing will be for single use only. This will ensure sterile conditions of testing and minimize the risk of getting infections transferring through blood. Mothers of children will be allowed to be present during blood sampling to be sure that all procedures are performed according to informed consent.

The study was approved by the Departmental Institutional Review Board of American University of Armenia for further development of grant proposal. In the event the grant is funded by an organization, the consent form and protocol would have to be resubmitted to the committee for further review.

*Preparation*

The duration of proposed study is 7 months (Attachment 4). Prior to the initiation of the study, approval will be obtained from both the Ministry of Nature Protection and the Ministry of Health. The study protocol, informed consent, and the questionnaires will be submitted for approval to the Ministry of Nature Protection and the National Institute of Health. Afterwards, the study protocol and informed consent, as well as the letter stating the study purpose and objectives, will be presented to Lori Marz health officials and Alaverdi Polyclinic. Both informed consent and questionnaires will be tested prior to their field application.

Sample Collectors/Interviewers will be trained on how to sample dust and soil and to use trace metal analyser (Attachment 5) and how to conduct the interviews. A Research Assistant will be trained to use the trace metal analyzer and blood lead analyzer (Attachment 5). Later, the Research Assistant will train two nurses from Alaverdi Polyclinic on blood sampling technique using the LeadCare Analyzer Kit. Training of nurses will be done in Alaverdi.

*Data collection*

The total number of children participating in the study is planned to be 312. During the data collection period, the Research Assistant and Sample Collectors/Interviewers will stay in Alaverdi. Blood sampling will be done by two trained nurses in Alaverdi Polyclinic. Immediately after the blood is taken, the Research Assistant will analyze it using LeadCare blood lead analyzer. Ideally, blood lead testing can be completed in 16 days (the duration of each blood sampling is 0.08 hour, during 8-hour working day 100 children may be sampled and 312 children will be tested in approximately 4 days). However, considering the fact that children will arrive at polyclinic at different times and optimal scheduling will not be achieved, the duration of blood lead testing will be longer than calculated for ideal conditions. In order to avoid long periods of waiting and make the testing procedure more effective and organized, a preliminary schedule will be compiled and appointments may be made with each child. Children will be invited according to the districts of the town. As a result of this approach the blood testing period may last from 10 to 15 days.

Ten environmental Sample Collectors/Interviewers will visit houses of blood-tested children to sample indoor dust and residential soil and conduct interviews with mothers. The Research Assistant will monitor the interviews in order to minimize interviewer bias. Prior to the interviews and sample collections informed consent will be presented.

In private houses, soil samples will be collected from gardens (one sample per garden) and dust will be sampled from the yard and the closest street (one sample from each). In case of child residing in an apartment, soil and dust from yard and the closest street will be sampled (one sample of soil and one sample of dust per yard and one sample of dust from the street). Inside, dust samples will be taken from the area close to house/apartment entrance, from hallway, living room, kitchen windowsill, and child's bedroom and playroom. If there is no playroom in the house, a dust sample will be taken from the place where the child spends the most time during the day. Thus, overall 6 samples will be collected inside the house. Overall, approximately 9 samples will be collected during each visit. After analysis of samples, all data will be registered in a special form specifying all mentioned places of dust and soil sampling, as well as the distance of house from smelter. Approximate time spent per visit is 45 minutes. During 8-hour working day each Sample Collector/Interviewer will visit from 8 to 10 households. Combined they will visit from 40 to 50 households per day. Assuming that in each household there is only one child participating in the study, the maximal duration of data collection phase will be from

10 to 15 days. Day care centers of Alaverdi also will be visited to collect dust and soil samples as well as to conduct interviews with the personnel of centers. Measurements in schools will not be done, because the study is planned to conduct in July and August and children will be on summer holydays at least 30 days prior to the initiation of blood lead analysis. In day care center also 9 soil and dust samples will be collected. Overall, the duration of data collection will be from 20 to 30 days.

Children's names and addresses will be registered only in registration log. This information is necessary for home visits. The information in log will be confidential and during the field trips will be kept with Research Assistant who will be responsible for the log. A code will correspond to the name of each child. This code will be on the front page of questionnaires, on containers with blood, dust, and soil samples, and on registration form for recording the results of dust and soil lead analyses (Attachments 6 and 7). These forms will contain also the information about the distance of house/apartment or day care center from the smelter.

The staff of the Center for Health Services Research and Development and the Environmental Conservation and Research Center will be involved in a study as consultants. Data collection will be done by MPH students of the American University of Armenia.

*Limitations*

In some cases, mothers may not remember some information, which will result in recall bias. In other cases, they may knowingly give incorrect information. This may happen particularly with questions related to family hygiene and food preparation and storing activities when mothers being aware of hygiene violations may try to hide their behavior.

Interviewers may encourage respondents to give “positive” responses by their tone, smiles or frowns, which may result in interviewer bias [39]. Proper training of interviewers and monitoring of interviewing process will minimize interviewer bias and correct it as early as possible.

Limitations of PAROS system of vulnerability assessment may contribute to study limitations. However, being registered in PAROS system or not are the only official criteria of socioeconomic status assessment.

Lack of analysis of dietary intake of some nutrients, as well as not measuring of food lead contamination does not allow to observe the influence of diet and food contamination on blood lead level in this study.

*Analysis*

The analysis of findings will start from estimations of the prevalence of high blood lead level and the age-specific prevalence of high blood lead level.

In order to determine the association between independent (dust and soil lead concentrations) and dependent (blood lead level) variables, as well as to study the effect of additional independent variables, univariate regression and multiple regression models are proposed [40]. Additional independent variables that are assumed to influence the association between lead concentrations in dust and soil and children’s blood lead level are the following: age, gender, distance from smelter, type of place of residence, namely house or apartment, floor, socioeconomic status of household, parental education, occupation and hobbies of household members, smokers in household, personal, family, and public hygienic patterns, and behavioral features. Preliminarily, the above-mentioned variables will be analyzed by univariate regression model. Those variable, which will have $p<0.05$ will be studied using multiple regression model. When considering parental, housing, and household related factors, namely distance from smelter, type of place of residence, floor, socioeconomic status of household, parental education, occupation and hobbies of household members, smokers in household, and family hygiene, the mean blood level of children in household will be taken.

In order to estimate the dose – response relationship the following approach will be undertaken. As both exposure and outcome are continuous data, they need to be stratified into categories. The stratification will be done based on findings. After stratification a number of odds ratios will be calculated. Chi-square test of trend indicating whether there is a significant trend in the odds of outcome with increasing levels of exposure [39].

## Management

The program will be implemented by the Center for Health Services Research and the Environmental Conservation and Research Center of the American University of Armenia. Key personnel of the program are presented by Project Director, Research Assistant, and Accountant. They will represent the permanent staff. The Project Director will be responsible for the overall coordination of the program, including negotiations with the Ministry and local officials, development of program documentation, hiring of personnel, assignment of their responsibilities and duties, and supervision of their work. The Research Assistant will be responsible mostly for staff training, fieldwork and data analysis. In addition, he/she will directly supervise the work of Nurses, Sample Collectors/Interviewers and Data Entry/Analyst. The Accountant will perform budgeting and financial reporting. Two Nurses, ten Sample Collectors/Interviewers, one Data Entry/Analyst, and Consultants will be hired on temporary basis.

## Budget

The requested amount of money is 30,322 USD. Budget line items are grouped into the following categories:

| | |
|---|---|
| Salary | 15,298 USD |
| Materials and Supplies | 469 USD |
| Services | 1,050 USD |
| Operational Costs | 4,680 USD |
| Travel | 1,682 USD |
| Rental Costs | 5,700 USD |

For the analyses of blood, soil, and dust lead levels the equipment of Environmental Conservation and Research Center of American University of Armenia will be used. The total cost of the equipment is 44,000 USD.

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# ATTACHMENTS

**Attachment 1**

# *CONCENTRATION OF LEAD IN SOIL AND DUST*

# *AND*

# *BLOOD LEAD LEVELS IN ALAVERDI CHILDREN*

*Survey Questionnaire*

(for mothers)

**ID Number: • • • •**

**Date:_ _ _ _ _ _**
***M M D D Y Y***

**Start Time _ _: _ _**

**End Time _ _: _ _**

Interviewer's Name:______________________________

Data Entry # 1:__________________________________

Data Entry # 2:__________________________________

***Introductory questions***

1. **What is your child's date of birth?** _ _ _ _ _ _
MM DD YY

2. **Gender**
   1. Male
   2. Female

3. **What is the level of education of mother?**
   1. Incomplete secondary (8 years)
   2. Complete secondary (10 years)
   3. Professional technical education
   4. Student/Incomplete university education
   5. University education

4. **What is the level of education of father?**
   1. Incomplete secondary (8 years)
   2. Complete secondary (10 years)
   3. Professional technical education
   4. Student/Incomplete university education
   5. University education

5. **Have you a medical background (graduated from medical university or medical college)?**
   1. Yes
   2. No

6. **Does anyone in your immediate family have a medical background (graduated from medical university or medical college)?**
   1. Yes
   2. No

7. **In what type of house do you live?**
   1. Private residence
   2. Apartment
   3. Other______________________

8. **On what floor do you live? _____________**

9. **Has your child attended day care center during at least the last 1 month?**
   1. Yes
   2. No

10. **What day care center has your child attended (specify the number or address)?**

______________________________________________

11. **Is your family registered in the PAROS system of social vulnerability assessment?**
    1. Yes
    2. No

***Questions related to family members' activity***

**12. Does anybody in your immediate family work in the smelter plant?**
1. Yes
2. No ⟶ SKIP TO THE **QUESTION # 18**

**13. How many of your family members work in smelter? ______________**

**14. Does anyone of them come home in working clothes?**
1. Yes
2. No ⟶ SKIP TO THE **QUESTION # 16**

**15. How frequently does/do he/she/they come home in working clothes?**
1. Always
2. Usually
3. Sometimes
4. Rare

**16. Does anyone of them bring home some objects from his/her/their working place?**

3. Yes
4. No ⟶ SKIP TO THE **QUESTION # 18**

**17. How frequently does/do he/she/they bring home some objects from his/her/their working place?**
1. Always
2. Usually
3. Sometimes
4. Rare

**18. Does anybody in your immediate family practices the following hobbies or activities involving use of the lead-containing materials at least during the last 4 months? (CHECK ALL THAT APPLY)**

| | | |
|---|---|---|
| 1. Stained glass | yes | no |
| 2. Pottery | yes | no |
| 3. Painting | yes | no |
| 4. Glassblowing | yes | no |
| 5. Screenprinting | yes | no |

**19. Are there any smokers in your household?**

5. Yes
6. No ⟶ SKIP TO THE **QUESTION # 22**

**20. How many smokers are there in your household? ______________**

**21. On average, how many cigarettes per day are smoked in your home?**
1. 1 – 4
2. 5 – 14
3. 15 – 24
4. 25 – 49
5. 50 +

99. DK

***<u>Questions related to family hygiene</u>***

**22. How frequently do you clean your house or apartment (mop the floor, dust, etc)?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**23. Do you practice wet mopping when cleaning your house or apartment?**

11. Yes
21. No ⟶ SKIP TO THE **QUESTION # 23**

**24. How frequently do you practice wet mopping?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**25. Do you practice damp sponging of walls when cleaning your house or apartment?**

1. Yes
2. No ⟶ SKIP TO THE **QUESTION # 27**

**26. How frequently do you practice damp sponging of walls?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**27. Do you practice vacuuming when cleaning your house or apartment?**

1. Yes
2. No ⟶ SKIP TO THE **QUESTION # 29**

**28. How frequently do you practice vacuuming?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**29. Do you have carpets at home?**

1. Yes
2. No ⟶ SKIP TO THE **QUESTION # 31**

**30. How frequently do you shake out the carpets?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**31. Do all of your family members (including children) take off shoes when entering home?**

1. Yes ————→ SKIP TO THE **QUESTION # 33**
2. No

**32. If the answer for the question 31 is "no", how frequently do your family members (including children) enter home without taking off shoes?**

1. Always
2. Usually
3. Sometimes
4. Rare

***<u>Questions related to your child's hygiene and behavior</u>***

**33. Does your child play with soil?**

1. Yes
2. No

99. D/K

**34. Does your child have the habit of putting his/her hands into his/her mouth?**

1. Yes
2. No

99. D/K

**35. Does your child have the habit of putting things into his/her mouth?**

1. Yes
2. No

99. D/K

**36. Does your child have the habit of eating inedible or inappropriate things such as dirt or paint chips?**

1. Yes
2. No

99. D/K

**37. Does your child eat food with his/her fingers?**

1. Yes
2. No

99. D/K

**38. How often does your child eat food with his/her fingers?**

1. Always
2. Usually
3. Sometimes
4. Rare
5. Never

**39. Where does your child usually eat his/her meals when at home?**

1. At table
2. Sitting on small chair or couch
3. Sitting on the floor
4. Other_______________________________

**40. Where does your child usually eat his/her snacks?**

1. At table
2. Sitting on small chair or couch
3. Sitting on the floor
4. Other_______________________________

**41. How frequently does your child eat food sitting on the floor?**

1. Always
2. Usually
3. Sometimes
4. Rare
5. Never

**42. Does your child ever eat food after it has dropped on the floor?**

1. Yes
2. No

99. D/K

**43. Do you have pet(s) in the home?**

1. Yes
2. No ——————→ SKIP TO THE **QUESTION # 46**

**44. Does your child play with it/them before meals?**

1. Yes
2. No

99. D/K

**45. Does your child play with it/them during meals?**

1. Yes
2. No

99. D/K

**46. Approximately how many times does your child wash his/her hands each day? _____**

**47. When does your child wash his/her hands?** (CHECK ALL THAT APPLY)

1. before meals
2. after meals
3. before snacks
4. after snacks
5. after going to the toilet
6. before going to bed
7. after coming home from outside
8. other ____________________________________________________________________

**48. How often does your child take bath?**

1. Every day
2. 6 – 5 times per week
3. 4 – 3 times per week
4. 2 – once per week
5. Rare than once per week

**49. How often does your child change his clothes?**

1. Every day
2. Once per every 2 days
3. Once per every 3 days
4. Once per week
5. Rare than once per week
6. When clothes become dirty

**50. How much time does your child usually spend outdoors?**

1. Less than 1 hour per day
2. 1 – 3 hours per day
3. 4 – 6 hours per day
4. More than 6 hours per day

99. D/K

***Thank you for your participation!***

**Attachment 2**

***CONCENTRATION OF LEAD IN SOIL AND DUST***

***AND***

***BLOOD LEAD LEVEL IN ALAVERDI CHILDREN***

*Survey Questionnaire*

(for the personnel of day care center)

**ID Number: • •**

**Date:_ _ _ _ _ _**
***M M D D Y Y***

**Start Time _ _: _ _**

**End Time _ _: _ _**

Interviewer's Name:________________________________

Data Entry # 1:_____________________________________

Data Entry # 2:_____________________________________

Day care center # or address

_______________________________________________________________

***Questions related to hygiene in day care center***

**1. How frequently do you clean the rooms in the day care center?**
   1. Every day
   2. 6 – 5 times per week
   3. 4 – 3 times per week
   4. 2 – once per week
   5. Rare than once per week

**2. Do you practice wet mopping when cleaning rooms in the day care center?**
   1. Yes
   2. No ⟶ SKIP TO THE **QUESTION # 4**

**3. How frequently do you practice wet mopping?**
   1. Every day
   2. 6 – 5 times per week
   3. 4 – 3 times per week
   4. 2 – once per week
   5. Rare than once per week

**4. Do you practice damp sponging of walls when cleaning rooms in the day care center?**
   1. Yes
   2. No ⟶ SKIP TO THE **QUESTION # 6**

**5. How frequently do you practice damp sponging of walls?**
   1. Every day
   2. 6 – 5 times per week
   3. 4 – 3 times per week
   4. 2 – once per week
   5. Rare than once per week

**6. Do you practice vacuuming when cleaning rooms in the day care center?**
   1. Yes
   2. No ⟶ SKIP TO THE **QUESTION # 8**

**7. How frequently do you practice vacuuming?**
   1. Every day
   2. 6 – 5 times per week
   3. 4 – 3 times per week
   4. 2 – once per week
   5. Rare than once per week

**8. Do children or visitors always take off their shoes when entering day care center?**
   1. Yes ⟶ SKIP TO THE **QUESTION # 10**
   2. No

**9. If the answer for the question 8 is “no”, how frequently do children or visitors enter day care center without taking off shoes?**

1. Always
2. Usually
3. Sometimes
4. Rare

***<u>Questions related to children’s hygiene and behavior at day care center</u>***

**10. In the day care center, approximately how many times during the day do children wash their hands? _______**

**11. Do they use soap when washing their hands?**

1. Yes
2. No

99. D/K

**12. When does children wash his/her hands? (CHECK ALL THAT APPLY)**

1. before meals
2. after meals
3. before snacks
4. after snacks
5. after going to the toilet
6. before going to bed
7. after coming home from outside
8. other ____________________________________________________

**13. How much time does children usually spend outdoors during the time when they are in the day care center?**

1. Less than 1 hour per day
2. 1 – 3 hours per day
3. 4 – 6 hours per day
4. More than 6 hours per day

99. D/K

***<u>Questions related to day care center personnel behavior</u>***

**14. Are there any smokers among the day care center personnel?**

1. Yes
2. No ⟶ **END OF QUESTIONNAIRE**
3. D/K

**15. Do any of them smoke in the presence of children?**

1. Yes
2. No ⟶ **END OF QUESTIONNAIRE**
3. D/K

**16. On average, how many cigarettes per day are smoked at day care center in the presence of children?**

1. 1 – 4
2. 5 – 14
3. 15 – 24
4. 25 – 49
5. 50 +

99. DK

***Thank you for your participation!***

**Attachment 3**

**American University of Armenia**

**Department of Public Health**

**Institutional Review Board/Committee On Human Research**

***CONSENT FORM TEMPLATE***

Title of Research Project: **Concentration of Lead in Soil and Dust and Blood Lead Levels in Alaverdi Children**

The Public Health Department of the American University of Armenia is conducting a survey of lead exposure in children living in Alaverdi town. The objectives of this study is to determine the prevalence of elevated blood lead levels in children aged 3 – 7 living in Alaverdi and to investigate the association between children's blood lead level and concentration of lead in the environment.

As an indicator of environmental lead level the concentration of lead in residential soil and dust will be measured. As an indicator of exposure blood lead analysis will be conducted. The measurements of environmental lead level will be done in the houses, yards, and gardens where children participating in the study live and spend their leisure, as well as in kindergartens they attend. In private houses, soil samples will be collected from gardens (one sample per garden) and dust will be sampled from the yard and the closest street (one sample from each). In case of the child residing in an apartment, the soil and dust from yard and the closest street will be sampled (one sample of soil and one sample of dust per yard and one sample of dust from the street). Inside, dust samples will be taken from the area close to house/apartment entrance, from hallway, living room, kitchen windowsill, and child's bedroom and playroom. If there is no playroom in the house, dust sample will be taken from the place where child spent the most time during the day.

Mothers of these children will be interviewed to ascertain risk factors for lead exposure in children.

Blood sampling will be done by health professionals. Blood will be taken from the finger. Only two drops of blood will be taken. The whole procedure will last 3 minutes. All supplies for blood sampling will be for single use only. This will ensure sterile conditions for blood analysis. You will be allowed to be present during blood lead testing and control the accuracy of all procedures.

Soil and dust samples' collection as well as interviews will be done by MPH students Interview questions are related to your family hygiene and your everyday activities.

We appreciate your participation in this study. It will be very valuable to us and to the children of your community.

RISKS/DISCOMFORTS

Finger stick may be inconvenient for both your child and you. However, this procedure is widely practiced in health institutions for screening and diagnostic purposes. Thus, you are familiar with the procedure. Use of single use supplies highly minimizes the risk.

BENEFITS

Considering the fact that in several areas of Armenia the concentration of lead in soil and dust exceeds notably the maximal permissible level, the findings of this study could show if there is a problem of toxicity in children. Moreover, the assessment of behavioural, hygienic and dietary patterns effects on blood lead level may reveal site-specific factors enhancing susceptibility to lead toxicity. Awareness of these factors may be helpful in elaboration and dissemination of lead exposure prevention measures.

CONFIDENTIALITY

The study will be conducted anonymously. Several steps will be undertaken to guarantee the confidentiality of information. Your name will be replaced with code. Any identifying you information will not be used during the research process. Study participants, including sample collectors, interviewers, and the health personnel of Alaverdi polyclinic involved in the study, will be instructed to not share any obtained personal information.

VOLUNTARINESS

It is your decision whether to participate in the study or not. You have the right to stop providing information at any time you wish or skip any question you consider inappropriate.

WHOM TO CONTACT

You may ask the person in charge any questions about the research. You may ask questions in the future if you do not understand anything that is being done. The researchers will tell you any new information they learn which they consider will affect you.

If you want to talk to anyone about this research you should call the person in charge of the study, **[Astghik Grigoryan] [phone number: (374 1) 22 57 64 / e-mail: astghikg@yahoo.com].**

The person in charge of the study will answer your questions. If you want to talk to anyone about the study because you feel you have not been treated fairly or think you have been hurt by joining the study you should contact the American University of Armenia at (374 1) 51 25 12.

Attachment 4

# Concentration of Lead in Soil and Dust and Blood Lead Levels in Alaverdi Children

## Time-table

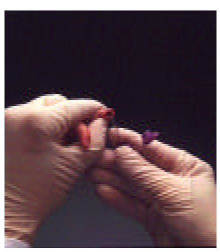
| Activity | Month | | | | | | |
|---|---|---|---|---|---|---|---|
| | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| Preparation | * | | | | | | |
| Data Collection | | * | * | | | | |
| Data Entry/Analysis | | | | * | * | | |
| Final Report Preparation | | | | | | * | * |

Source: http://www.esainc.com/
http://www.esainc.com/theory/ASV/asvtheory.htm

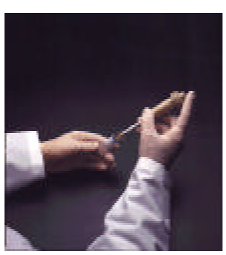
***LeadCare® Childhood Blood Lead Testing***

The LeadCare System is for the determination of lead in whole blood. LeadCare is easy and safe to use. The hand-held analyzer is portable and requires neither manual calibration nor refrigeration. Its unique gold electrode sensor contains no mercury or other toxic materials. The point-of-care LeadCare system was developed by ESA and Andcare with a grant from the CDC. It's the diagnostic tool, which makes sense medically and economically.

*Specifications:*
Test method: Electrochemical with disposable sensors
Blood lead level range: 1.4 - 65 µg/dl
Blood sample volume: 50 µl
Test time: 3 minutes
Steps of Using LeadCare® Childhood Blood Lead Testing



STEP ONE
Draw a capillary or venous blood sample using EDTA or heparin as anticoagulants



STEP TWO

Using the pipette provided with the kit, dispense 50 µl, about two drops of blood, into the reagent and mix.

STEP THREE

Transfer it to the sensor strip. Press the button. Just three minutes later, you have your result

Accuracy of Results

| | |
|---|---|
| **Number of Samples** | **112** |
| **Slope** | **1.07** |
| **Intercept** | **-0.57 µg/dl** |
| **Correlation Coefficient** | **0.97** |

*Source:* *http://www.nitonuk.com/html/multi-source.html*

***NITON XL-723 Multi-Element Analyzer***

The Niton provides quick sample screening for Pb, U, Th, Hg, As, Cr, Co, Cu, Fe, Mn, Ni, Rb, Sr, Zr, Zn in bulk samples (soil, sediments, water, and food products) and in thin film samples (air filters, in situ paint, industrial residues, leaf deposits). Detection limits, accuracy, and precision are sufficient for determining whether samples exceed Maximum Allowable Concentrations in a variety of metals.

**Attachment 6**

# CONCENTRATION OF LEAD IN SOIL AND DUST

# AND

# BLOOD LEAD LEVELS IN ALAVERDI CHILDREN

*Form For Recording The Results of Dust and*

*Soil Lead Analyses in the Households*

**ID Number of household: • • • •**

**Date:_ _ _ _ _ _**
***M M D D Y Y***

**Start Time _ _: _ _**

**End Time _ _: _ _**

Sample Collector's Name:________________________________

Data Entry # 1:______________________________________

Data Entry # 2:______________________________________

Distance from the smelter ________________

Samples taken outside the home:

Lead concentration in soil sample from garden/apt. yard ______________

Lead concentration in dust sample from street/apt. yard _______________

Samples taken inside the home:

Lead concentration in dust sample from…

the area close to house/apartment entrance ___________

hallway ___________

living room ___________

kitchen windowsill ___________

child's bedroom ___________

child's playroom ___________

**Attachment 7**

# CONCENTRATION OF LEAD IN SOIL AND DUST

# AND

# BLOOD LEAD LEVELS IN ALAVERDI CHILDREN

*Form For Recording The Results of Dust and*

*Soil Lead Analyses in the Households*

*Day care center # or address* ____________________________________________________

**Date:_ _ _ _ _ _**
***M M D D Y Y***

**Start Time _ _: _ _**
**End Time _ _: _ _**

Sample Collector's Name:_________________________________

Data Entry # 1:_____________________________________

Data Entry # 2:_____________________________________

Distance from the smelter _________________

Samples taken outside the day care center

Lead concentration in soil sample from playground _______________

Lead concentration in dust sample from playground ________________

Samples taken inside the day care center

Lead concentration in dust sample from…

the area close to entrance ____________

hallway ____________

dining room ____________

kitchen windowsill ____________

bedroom ____________

playroom ____________

**Attachment 8**

# Concentration of Lead in Soil and Dust and Blood Lead Levels in Alaverdi Children

## Budget

| Item | Unit | Time | Cost per unit | Total |
|---|---|---|---|---|
| ***Salary*** | | | | |
| Project Director | 1 | 7 mo | $ 600.00 | $ 4,200.00 |
| Research Assistant | 1 | 7 mo | $ 500.00 | $ 3,500.00 |
| Accountant | 1 | 7 mo | $ 450.00 | $ 3,150.00 |
| Fringe Benefits (25% of salary) | | | | $ 2,712.50 |
| Nurses | 312* | | $ 0.20 | $ 62.40 |
| Sample Collectors/Interviewers | 312* | | $ 4.00 | $ 1,248.00 |
| Data Entry/Analyst | 312* | | $ 0.40 | $ 124.80 |
| Consultants | 3 | 5 days | $ 100.00 | $ 300.00 |
| **Subtotal for Salary** | | | | **$ 15,297.70** |
| ***Materials and Supplies*** | | | | |
| Questionnaire Forms | 4,378 | | $ 0.02 | $ 87.56 |
| Gloves | 312 | | $ 0.10 | $ 31.20 |
| Office supplies | | 7 mo | $ 50.00 | $ 350.00 |
| **Subtotal for Materials and Supplies** | | | | **$ 468.76** |
| ***Services*** | | | | |
| Computer Maintenance | | 7 mo | $ 100.00 | $ 700.00 |
| Communications | | 7 mo | $ 50.00 | $ 350.00 |
| **Subtotal for Services** | | | | **$ 1,050.00** |
| ***Operational Costs*** | | | | |
| NITON XL-723 Multi-element Analyzer | 1 | | | |
| LeadCare Blood Lead Analyzer | 1 | | | |
| Soil Lead Analysis | 312* | | $ 1.50 | $ 468.00 |
| Dust Lead Analysis | 2496* | | $ 1.50 | $ 3,744.00 |
| Blood Lead Analysis | 312* | | $ 1.50 | $ 468.00 |
| **Subtotal for Operational Costs** | | | | **$ 4,680.00** |
| ***Travel*** | | | | |
| Fuel | | | | $ 1,082.00 |
| Per Diems | | 60 days | $ 10.00 | $ 600.00 |
| **Subtotal for Travel** | | | | **$ 1,682.00** |
| ***Rental Costs*** | | | | |
| Office Rental | 1 | 7 | $ 400.00 | $ 2,800.00 |
| House Rental (during field trips) | 1 | 2 | $ 100.00 | $ 200.00 |
| Car Rental | 2 | 9 (7+2) | $ 300.00 | $ 2,700.00 |
| **Subtotal for Rental Costs** | | | | **$ 5,700.00** |
| **Total Costs** | | | | **$ 28,878.46** |
| **Contingency (5% of Total Costs)** | | | | **$ 1,443.92** |
| **Total Project Cost** | | | | **$ 30,322.38** |

* Units are the numbers of blood, soil, and dust samples, conducted interviews, and questionnaires