

Appendix E: Study Protocol

Protocol for Biosampling Children with Leukemia (Acute Lymphocytic and Acute Myelocytic Leukemias) plus a Comparison Population in Sierra Vista, Arizona

The protocol for Sierra Vista is based on the previously approved Protocol #3195 for the study, "Cross Sectional Exposure Assessment of Case Children with Leukemia (Acute Lymphocytic and Acute Myelocytic Leukemias) and a Reference Population in Churchill County, Nevada."

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RESEARCH STUDY OVERVIEW

The Arizona Department of Health Services (ADHS) and the Cochise County Health Department (CCHD) requested assistance from the Centers for Disease Control and Prevention (CDC) in the investigation of an elevated number of cases of childhood leukemia (acute lymphocytic leukemia [ALL] and acute myelocytic leukemia [AML]) in Sierra Vista, Arizona. It was decided that CDC's National Center for Environmental Health (NCEH) and National Center for Infectious Diseases (NCID) will provide assistance in developing consent forms and a questionnaire, conducting laboratory analysis of biologic samples (blood, urine and buccal cells) for several chemical, radioactive, and infectious agents and genetic markers, and performing statistical analyses of questionnaire data and laboratory results.

The questionnaire will collect information about pertinent risk factors and medical history. A number of chemicals, infectious agents and genetic markers will be measured in biologic samples (Table 2). Statistical analyses will compare questionnaire and laboratory results of children with leukemia to those of their immediate family members (parents and siblings only) and to those of comparison families. Results of laboratory analyses will also be compared to results of a representative national sample, as reported in the Second National Report on Human Exposure to Environmental Chemicals (www.cdc.gov/exposurereport). Biologic samples will be stored by CDC for future studies of environmental and infectious markers, and in addition, DNA extracted from blood and buccal cells will be stored by CDC for future studies of candidate genes involved in metabolizing carcinogens and DNA repair from damage by environmental exposure. Based on consultations with experts, more complex genetic analysis may also be conducted with the stored DNA to identify new genes that may be associated with childhood leukemia.

Investigators/collaborators:

Centers for Disease Control and Prevention (CDC),

National Center for Environmental Health (NCEH)

- Michael McGeehin, PhD, MSPH; Division of Environmental Hazards and Health Effects (EHHE)
- Carol Rubin, DVM, MPH.; EHHE, Health Studies Branch (HSB)
- Beverly S. Kingsley, PhD, MPH; EHHE, HSB
- Martin Belson, MD; EHHE, HSB
- Robert L. Jones, PhD; Division of Laboratory Sciences (DLS), Inorganic Toxicology and Nutrition Branch
- Karen Steinberg, PhD; DLS, MBB

National Center for Infectious Diseases (NCID)

- Siobhan O'Connor, MD, MPH; NCID, Office of the Director
- Scott Schmid, PhD; NCID, OD

State of Arizona

- Cathy Eden, PhD; Director, Arizona Department of Health Services
- Tim Flood, MD; Bureau Medical Director, Bureau of Public Health Statistics (BPHS)
- Bob England, MD, MPH; State Epidemiologist
- Rose Connor, M.Ed; Assistant Director Public Health Services
- Will Humble, M.P.H.; Bureau Chief, Bureau of Epidemiology and Disease Control Services
- Richard Porter, M.S.; Bureau Chief, BPHS
- Frank Williams PhD; Local Health Liaison

Cochise County Health Department

- Diane Carper, D.B.A; Director, Cochise County Department of Health (CCHD)
- Gary Spivey, MD, MPH, Epidemiologist, CCHD

Fort Huachuca

- Larry J. Portouw, Colonel US Army, Commander US Army Garrison

Agency Responsibilities

The Cochise County Health Department (CCHD) will serve as the lead agency for this investigation. Dr. Gary Spivey will serve as the Principal Investigator.

Arizona Department of Health Services will be responsible for:

- Providing contact information for families of children with leukemia to the Cochise County Health Department;
- Calculating and providing comparison rate of childhood leukemia in the state
- Providing technical and epidemiological expertise to Cochise County Health Department in conducting biosampling;
- Co-presenting with CDC and Cochise County the results of the biosampling research study to participating families and the community.

Cochise County Health Department will be responsible for:

- Screening and enrolling the comparison population;
- Providing physical space for interviewing participants and collecting biologic specimens;
- Administering questionnaire to participants;
- Providing personnel to collect biologic samples;
- Interim cold storage (if needed), and oversight for shipping biologic specimens according to CDC protocols;
- Analysis and interpretation of results together with CDC and ADHS
- Presentation of results to the community together with CDC and ADHS

CDC will be responsible for:

- Writing the investigation protocol and submitting it to IRB;
- Developing the method for selecting the comparison population, a brief questionnaire, and consent forms;
- Providing collection materials and training local collaborators on sample collection, processing, and shipping protocols;
- Analyzing biologic samples, entering questionnaire data, analyzing laboratory and questionnaire data, and writing report(s); collaboration with CCHD and ADHS on data analysis and interpretation
- Consulting with ADHS and CCHD in analyzing, interpretation and reporting data
- Storage of biologic specimens (blood, urine, and buccal cells) for future study.

INTRODUCTION

Leukemias are cancers of the blood-forming tissues. They may be subdivided according to the particular cell type involved, the major types being lymphocytic and myelocytic (granulocytic) leukemias.

Leukemias are also classified by their behavior, as either "acute" or "chronic." Childhood leukemias are mostly acute, with the lymphocytic form predominating ([Rudolph 1996](#)). In the U.S., childhood leukemia rates are highest among Filipinos, followed by white Hispanics, non-Hispanic whites and blacks. Reliable rates could not be computed for children in the remaining racial/ethnic groups. The ratio of mortality-to-incidence rates is higher for adult leukemias than for childhood leukemias. Because treatment for childhood leukemias is quite successful, mortality from this cancer is comparatively low among children ([Ries, et. al., 2001](#)).

Several comprehensive reviews of risk factors for childhood cancers have been published in recent years and form the basis of the following discussion ([Sandler and Ross, 1997](#); [Pritchard-Jones, 1996](#); [Zahm and Devesa, 1995](#); [Ross et. al., 1994](#); [Savitz and Chen, 1990](#); [NJDHSS and ATSDR \(Dover\), 1998](#); [Legakos et al., 1986](#); [Massachusetts Department of Health \(Woburn\), 1997](#)). Established causes of leukemia include ionizing radiation (such as occurs from x-irradiation), certain drugs used in the treatment of cancer, and some chemicals (most notably benzene) used largely in industrial settings. Ionizing radiation has been associated with all forms of leukemia except the chronic lymphocytic form. It is suspected that many childhood leukemias may result from parental exposures before the time of conception or during early fetal development ([Savitz and Chen, 1990](#)).

The following table presents the national ALL incidence rates reported by the Surveillance, Epidemiology, and End Results (SEER) registry and incidence rates of ALL within international settings, according to International Classification of Childhood Cancers (ICCC): ([Ries, et al. 2001](#)).

Age category	Incidence Rates of ALL: 1975-1998 ¹	ALL Survival Rates (SEER) (%)	Incidence Rates of ALL:1990-1999 ²
0-4 yrs	6.1	87	5.5
5-9 yrs	3.0	87	Unavailable
10-14 yrs	1.7	76	Unavailable
15-19 yrs	1.2	58	1.1
0-19 yrs	1.9 – 3.3	Unavailable	2.7

For unknown reasons, the rate of lymphocytic leukemia among children aged 0-14 in the US appears to be increasing.³

During the time period January 1995 through December 31, 2003, the average annual rate of childhood leukemia in children aged 0-14 years was 9.9 per 100,000 in Sierra Vista. During the period 1995-2001, a comparable rate for the state of Arizona was 4.53 cases per 100,000 children per year. Based on these rates, there appears to be a statistically significant elevation in the incidence of childhood leukemia in Sierra Vista.

¹ National SEER data Reported as per 100,000 population and age-adjusted by 5-yr age groups, based on the 1970 standard US population of 0 to 19 year-olds.

² ICCC Reported as per 100,000 population (both sexes, all races) and age adjusted to the 1970 standard US population of 0 to 19 year-olds

³ Flood T and Porter R. ADHS Trend line calculations for US rates, 1973-2000 showing slope of 0.025 which is significantly different than slope of 0.0, with T value = 3.54, and p= 0.0016.

Justification for study:

CDC will assist the Arizona Department of Health Services (ADHS), the Cochise County Department of Health (CCHD), and the Sierra Vista community in investigating the elevated incidence of cases of childhood leukemia in Sierra Vista, Arizona. In addition to providing consent forms and a questionnaire, CDC will analyze biologic samples collected from children with leukemia and their families and comparison children and their parents. Although the results of laboratory analysis will not necessarily reflect previous or cumulative exposure to chemical or infectious agents, the results will provide information about current community exposures and has the potential to identify ongoing elevated exposures to harmful chemicals in the community.

We do not expect that data from this investigation alone will provide etiologic information on the cause(s) of childhood leukemia. However, adding biologic samples from Sierra Vista to the bank of stored samples collected during other investigations of childhood leukemia increases the number of samples available for testing when the state of scientific analytic methods is more advanced.

Intended/potential use of study findings:

- To identify through analysis of biologic samples any ongoing environmental contamination that could be remediated to prevent further exposure to the residents of Sierra Vista, Arizona.
- To potentially contribute to our scientific understanding of the health impacts of certain environmental exposures so we can develop better prevention and control strategies in the future.
- To further our understanding of gene-environment interactions and the risk for the development of leukemia in children.

Objectives:

- To assess chemical, radioactive, and infectious exposures among the participating children and family members.
- To assess variation in genes involved in metabolizing toxic substances and repairing DNA damaged by environmental exposure.

Hypotheses or questions:

- Do children who develop leukemia have different current (as determined by biologic sample analysis) or previous (as determined by questionnaire) chemical, radioactive or infectious exposures than their immediate family or than comparison children and/or their parents?

- Do children who develop leukemia have variant genes that code for the key enzymes involved in metabolizing toxic substances and repairing DNA damaged by environmental exposures, as compared to their immediate family and comparison children and their parents?

The results from the Sierra Vista biosampling research study alone are not intended to test these hypotheses; however, in conjunction with samples and findings from other leukemia clusters, evidence to support or refute these hypotheses may be obtained.

METHODS: STUDY POPULATION

Case definition (for biosampling) :

A child aged 0 to 14 years of age at time he/she was medically diagnosed with childhood leukemia (ALL or AML), during the period from January 1, 1995 through June 30, 2004, and who resided in Sierra Vista prior to the time of diagnosis.

Comparison definition:

Two neighborhood comparison children will be matched to children with leukemia 2-to-1 on the basis of year of birth; each eligible child with leukemia will be matched on birth date, plus or minus one year, and on gender ([Table 1](#)). In addition, parents of selected comparison children will serve as comparison participants. Comparison children will be identified using a standardized search procedure (Semenza et al., 1996), the details can be found on in the section on "Sampling, including sample size and statistical power" below.

The biosampling research study population will be composed of children currently living in Sierra Vista who meet the case definition and their families (parents and all siblings living with the case child full time), and children who are enrolled as comparison subjects and their parents. Twelve children meet the case definition; 10 of these children reside currently in Arizona and are potential participants in this biosampling study. As many as 20 comparison children may be enrolled.

Geographic area and time period of study:

The Sierra Vista area reports higher incidence of childhood leukemia. However, the state and local health departments will determine the geographical boundaries of the biosampling research study.

Should additional cases of leukemia associated with the Sierra Vista cluster be identified after data collection has been completed, questionnaire data and biologic specimens from the children with leukemia and their families will be considered for collection; CDC, ADHS and CCHD will collaboratively make that decision.

Sampling, including sample size and statistical power:

The sample size for the biosampling research study is not sufficient by itself to provide definitive data about possible etiologies related to childhood leukemia due to the small number of cases. However, the biosampling research study can provide valuable information about chemical, radioactive, and infectious exposures in the population studied and potentially the community. It is important to note, however, that the sample size is determined based on available resources and the known number of children with leukemia, and not on statistical power. If this biosampling research study identifies pertinent trends in environmental exposure in the population, follow-up studies will be designed to target specific research questions. Power for those studies will be determined by standard statistical calculations.

Selection of Comparison Population:

The ideal comparison group would be chosen randomly within the boundaries of the study area. As a compromise for practicality, the following procedure will be followed.

Each eligible case child will be matched on birth date, plus or minus one year, and on sex to two comparison children. The recruiter will start at the case residence, facing the street, and determine direction by the flip of a coin. Heads will be left and tails will be right. The recruiter will then proceed along the block in the determined direction using the screening questionnaire until the first match is found for any unmatched case other than the one from that neighborhood. An attempt will be made to recruit that child and parents into the study. The recruiter will continue in like manner until two matches (not necessarily the same case) have been recruited. For eligible case children who are no longer living in Sierra Vista, the residence of the child prior to moving will be used as the starting point.

If two matches are not found by the end of the block, the recruiter will return to the case house and proceed in the other direction to the end of the block. Recruitment will continue to the other side of the street and then to the next block in either direction, the direction determined by flip of a coin. The search will continue to adjoining blocks and adjoining streets as necessary, the direction being determined by coin flip. Because all case families currently live in single family homes, apartment buildings will not be searched.

A card will be prepared for the recruiter's use which will have a time-line for each case showing the case's month and year of birth and the start and end month and year of the 24 month interval around the case's birth date. The screening questionnaire asks for the birth month and year for each child born after January 1, 1989. The recruiter will record the birth month and year for each child within the study dates and compare them to the unmatched case intervals. If more than one child in a household is an eligible match, the oldest will be chosen. Only one child per household will be matched to a case. Because people who are not at home during the day may differ systematically from those who are, final decisions on recruitment will not be made until the recruiter has made a reasonable effort to contact those missed during the day time. A child is not eligible to be a comparison child if he/she is sibling of a case child or has ever been diagnosed with any form of cancer.

Informed Consent:

Consent forms will be developed by CDC. During the research study subjects' appointments with CCHD, staff will explain the investigation and the informed consent process and will obtain consent or assent. Sufficient time will be allowed for questions and concerns to be addressed. Local health care officials, who will have been trained by CDC to speak to children as well as adults on informed consent issues, will explain the biosampling research study and the consent forms.

Enrollment: ([Figure 1](#))

CCHD staff will contact the case families to set an appointment for the family's clinic visit. During the clinic visit, case family members will give consent (and assent, as appropriate) provide blood, urine, and buccal cell samples, and be interviewed.

CCHD staff will identify potential comparison families using a standardized search procedure and will screen them for eligibility. Eligible comparison families will be asked for their contact information so CCHD staff can call them to make an appointment for their clinic visit. The comparison clinic visit will follow the same procedure as case family visits.

METHODS: DESIGN

General approach:

In this biosampling research study, we will collect questionnaire data and biologic samples from children diagnosed with leukemia, their immediate family members (parents and siblings only) and comparison children and their families (parents only). Current exposure will be estimated by analyzing

biologic samples for several chemical and infectious agents. Samples will also be tested for specific genetic markers. Past exposure will be estimated using a brief questionnaire.

Research study Description

Biologic samples and questionnaire data will be collected from case and comparison families. Results of analysis of these data and samples will be compared to determine if there are differences in past (as estimated by data collected in the questionnaire) or current (as estimated by the laboratory analysis of biologic samples) exposures between case and comparison children, between children with leukemia and the members of their families, and between case and comparison families and a representative sample of the US population.

Questionnaire Administration

CCHD staff will be trained to administer a brief questionnaire, developed by CDC, ADHS and CCHD investigators. This questionnaire will be administered to case and comparison families during their clinic visit.

Biologic Specimen Collection

CCHD staff will be trained to collect, process, and ship urine, blood, and buccal cell samples according to CDC protocols. At most, 32.5 ml (2 ½ tablespoons) of voided urine will be collected from each case and comparison study participant. Water and fruit juice will be provided to participants as needed to assist in the production of urine. A trained phlebotomist will obtain 21 ml (1½ tablespoons) of whole venous blood from case and comparison participants. We will work with physicians of the children with leukemia to ensure clinical appropriateness of taking a 21 ml blood sample on the day of the child's visit to the clinic offices.

Blood and urine will be aliquotted for analysis or storage as described in Table 2. Stored blood and buccal cells will be used for future studies of candidate genes involved in metabolizing carcinogens and DNA repair from damage by environmental exposure. More complex genetic analysis may also be conducted with the stored DNA-containing samples, after consultations with experts, to identify new gene variants that are associated with childhood leukemia. Biological samples will be stored for an unspecified amount of time, until sufficient testing has been conducted. Personal identifiers will also be kept as long as the samples are, as outlined above, separately in locked files, in the event that the person from who samples came needs to be notified of biological information.

Chain of Custody of Samples

The Division of Laboratory Sciences at NCEH has a detailed chain of custody protocol for tracking biologic samples. The laboratory meets or exceeds the requirements of CLIA 88 (Clinical Laboratory Improvements Amendments of 1998) for specimen/sample chain of custody. This method will be used while collecting samples in the field through analysis at the laboratory in Atlanta, GA.

Audience and stakeholder participation:

Participating stakeholders in this biosampling research study include the participating families, residents of Sierra Vista, Arizona, and Cochise County, the Mayor of Sierra Vista, Sierra Vista Board of Supervisors, military personnel and their families living at Fort Huachuca, the Arizona Department of Health Services, the Cochise County Health Department, and Congressman Jim Kolbe.

Cost benefit/prevention effectiveness:

Few studies have been conducted to assess chemical, radioactive, and infectious exposures for etiologic linkage to developing leukemia. Information learned from this biosampling research study will contribute to the existing body of knowledge about childhood leukemia and has the potential to help local health providers, public health officials, and parents of children with leukemia understand the possible role of environmental and infectious exposures, and genetics in manifesting this illness. Testing of biologic specimens has the potential to identify an ongoing environmental contamination or exposures, which could then be remediated to prevent further exposure of the residents of Sierra Vista, Arizona.

Biosampling research study time line:

<u>Task</u>	<u>Completion date</u>
Institutional Review Board (IRB) approval	2 nd quarter 2004
Training	3 rd quarter 2004
Subject recruitment and biologic sample collection	3 rd quarter 2004
Biologic/environmental sample analyses	3 rd /4 th quarter 2004
Data analysis and final report preparation	1 st quarter 2005
Presentation of biosampling research study results to participants	1 st quarter 2005

Expedited protocol review request:

We request that this protocol be expedited through the IRB review process. This protocol is based on the previously approved protocol for the Study of Childhood Leukemia in Churchill County, Nevada.

METHODS: DATA COLLECTION PROCEDURES

Biologic samples and questionnaire data will be collected by CCHD, the lead organization for this biosampling research study. Participating families will make an appointment at the CCHD clinic. Once there, they will be administered a brief questionnaire during a face-to-face interview and will provide urine, blood, and buccal cell samples.

Face-to-face interview

An eligible member of the participating child's family (e.g., mother, father, maternal or paternal grandparent, step-parent) will be interviewed. The interview consists of a questionnaire about the medical, pregnancy, and exposure histories of the enrolled child and adult members of his/her family.

Biologic Samples

Urine, blood, and buccal cells will be collected by CCHD personnel or by outside contractors hired by CCHD for the purpose of this biosampling research study. Samples will be collected, stored, and shipped according to CDC protocols. All samples will be shipped via overnight express courier to arrive at the NCEH laboratory in Atlanta, GA the day immediately following collection; days of arrival to the NCEH laboratory will be scheduled Tuesday through Friday only. All aliquotting will be completed at the NCEH laboratory. Biological samples will be stored for an unspecified amount of time, until sufficient testing has been conducted. Personal identifiers will be linked to biological specimens (and survey data) for as long as they are stored, in case there becomes biologically significant information that should be relayed to the person from which the sample came.

Supplemental training to be conducted by CDC staff will be scheduled following IRB approval. CDC staff will travel to Sierra Vista and conduct a refresher course on the specific study sample collection and shipping procedures. CCHD staff will be asked to sign a statement that they have completed the training, and they will need to pass a brief quiz about specific collection and shipping procedures. CDC staff will remain through the sample collection of 1-2 participant families, until it is apparent that CCHD staff is able to collect and ship samples according to specific detailed laboratory directions. Study investigators will acknowledge that the study protocol will be followed; in the event of necessary changes written amendments will be submitted to the CDC and Arizona IRB.

Retraining will include (but not be limited to) the following:

- Blood collection

Processing

Shipping

Sample labeling

Training in special situations that might occur when there is a family that includes step-parents and/or step siblings

METHODS: VARIABLES/INTERVENTIONS

Variables:

The questionnaire will collect information on child and parental medical histories, mother's pregnancy history, and parental occupational histories, as well as general sociodemographic variables. Urine, blood, and buccal cell samples will be collected for analysis for chemical, radiologic, and infectious agents and certain genetic markers.

Training for all research study personnel:

CDC research study team members will train local health providers to collect, process, store, and ship biologic samples, and to administer the brief interview questionnaire consistently and without bias. Each interviewer will successfully administer a mock interview under the supervision of a CDC scientist.

METHODS: DATA HANDLING AND ANALYSIS

Data analysis plan:

CDC scientists will be responsible for analysis and storage of biologic samples once the samples reach the NCEH and NCID laboratories. CDC, ADHS and CCHD will be responsible for analyzing questionnaire data; CDC will analyze laboratory results. The general data analysis plan is to describe characteristics of the population (e.g., sociodemographics, medical history, some occupational history, other exposures) and levels of specific chemicals in urine and serum. These data will be used to characterize the extent of current exposures within the cohort. Tables and figures describing the characteristics of the biosampling research study population, exposures, and biologic chemical levels will supplement the descriptive analyses.

Data Analysis:

Biologic Specimen Analysis

The NCEH laboratory will analyze urine and blood samples using standardized analysis protocols for each analyte. Urine samples will be analyzed for selected pesticides and metals; blood samples will be analyzed for volatile organic compounds and selected metals ([Table 2](#)). DNA will be harvested from blood and buccal cells and stored for future studies that will be determined based on the assessment of environmental exposures. The NCID laboratory will analyze for several markers of infection. Analytes to be measured were determined by consultation with experts for the analysis of biologic samples.

DNA will be extracted from buccal cells at CDC's environmental laboratory and from an aliquot of blood in the field. Serum from at least 7 ml of whole blood collected, and any urine specimens in excess of 20 ml, will be sent to the CDC Specimen Packaging, Inventory and Repository (CASPIR) for storage in the event that further tests are indicated. No personal identifiers will be used to label the specimens.

DNA will be stored at CDC until we evaluate the results of other biologic sample analysis. The advice of experts will be obtained to determine the best approach for the genetic analysis of the specimens collected. The approach may include examining genotypes of participants for genes relevant to detoxification pathways of suspected chemical or radioactive exposures, or in DNA repair from damage by such exposure; and identification of genomic regions of interest through family studies and sib-pair analysis that may lead to hypotheses about new gene variants associated with ALL/AML.

Questionnaire Analysis

CDC, ADHS and CCHD will analyze questionnaire data using standard epidemiological and statistical methods.

Quality control/assurance:

We will adhere to standard laboratory quality assurance and quality control procedures for all biologic sampling, handling, shipping, and analysis. . Documents detailing collection, labeling, processing and shipping are attached in Appendix D . Appendix is in Electronic form CD rom with hard copy due to the large file size (40MB).

Cochise County Health Department (CCHD) has requested that they conduct a pilot study for Protocol #4236 to solicit community support, ensure their ability to sample the study participants correctly, ship their biological samples according to CDC laboratory requirements, and become expert in this process. A pilot biosampling and questionnaire administration of two to three families selected at the discretion

of Cochise County investigators will be conducted following training by CDC laboratorian investigators in this study. The use of a pilot participants (sampling 2-3 participant families) is not intended as research, but as training and as public relations for the Cochise County Health Department. The families will undergo the full biosampling and sample shipping procedure detailed in Protocol #4236 *Biosampling Children with Leukemia (Acute Lymphocytic and Acute Myelocytic Leukemias) plus a Comparison Population in Sierra Vista, Arizona*

Methodology Section.

Samples will be packed and shipped to the CDC for analysis using the same protocol that will be used for future samples from study participants.

Pilot Participants:

The families sampled as “pilot run” families will be informed in a letter sent from CCHD and CDC of the opportunity to participate as designated pilot families if they are interested. They will be given contact telephone numbers if they wish to discuss the matter and/or to schedule testing to participate as pilot families. If the initial families identified do not wish to participate two other families will be chosen at the discretion of the Cochise County investigation team.

The participants will be informed when their results will be available for presentation to them. Since, for the majority of analytes tested, the most consistent laboratory results will be obtained if all samples from study participants are analyzed at approximately the same time, biological samples will be analyzed together when sampling is complete for the study. Study results will be presented to pilot and study participants when testing is complete for all participants. However, the results of the pilot families will not be included in the study analysis and the pilot families will be so informed of this.

If selected pilot families agree to participate, they will be sampled according to proper procedure following additional training of CCHD staff for sample collection, processing and shipping. Pilot participants will be informed about the study, and pilot (non research) consent forms developed specifically for pilot families will be administered, since NCEH laboratory requires signed consent forms to perform their laboratory analyses.

Intermediate reviews and analyses:

The biologic sampling results will be reviewed as available to identify any potential hazard to public health in the Sierra Vista community. All biologic results will be reviewed at CDC prior to release to ADHS, CCHD and participants (parent/guardian). Any potential public health threats identified by intermediate reviews will be reviewed in conjunction with the ADHS and CCHD health officials for possible immediate remedial action.

Bias in data collection, measurement and analysis:

- CDC-trained county health providers will administer the questionnaire using uniform techniques, which will reduce the likelihood for interviewer bias.
- Collection of biologic samples and administration of the brief questionnaire cannot be blinded since the identities of the case families may be known to county health providers.
- Testing of biologic samples will be blinded to those conducting the laboratory analyses, which will reduce the likelihood for measurement bias.
- Larger-sized families have a greater chance to be selected as comparison-families (more likely to have a child that is eligible), which is a selection bias among comparisons. We will only select one comparison child per eligible household to avoid clustering exposure information.

Limitations:

- This biosampling research study targets children who have been diagnosed with ALL or AML, who resided in Sierra Vista, Arizona, prior to the time of diagnosis. Case family results from this biosampling research study may or may not be representative of Sierra Vista exposures.
- Biologic sampling in Sierra Vista will measure concentrations of multiple analytes indicative of current chemical exposures; results of this biosampling research study will not necessarily reflect previous or cumulative exposure to these chemicals.
- Given the small number of cases, it is unlikely that we will be able to detect statistically significant differences in the common gene variants that code for the enzymes that metabolize toxic substances or repair DNA damaged by environmental exposures.

METHODS: HANDLING OF UNEXPECTED OR ADVERSE EVENTS

Response to new or unexpected findings and to changes in the study environment:

Medically significant exposure to chemicals, radioactive elements, or infectious agents, or vital genetic information detected as a result of urine, blood, or buccal cell sample analysis, would constitute unexpected findings in this biosampling research study. In the rare event that such unexpected findings

are found, CDC will report these findings immediately to the ADHS and CCHD. CCHD, ADHS and CDC will then immediately notify the affected research study participant and, if appropriate, recommend that his or her family see their physician. Collaborative review of any such findings would allow the state to assess the need for, and urgency of, remedial actions.

Emergency care:

In the highly unlikely event any subjects require emergency care in relation to the biologic measurements indicative of chemical or radioactive exposure in this biosampling research study, a designated official from CCHD will advise the family to obtain emergency medical care immediately.

METHODS: DISSEMINATING, NOTIFICATION, AND REPORTING OF RESULTS

Notifying participants of their individual results/findings: At the time that research study findings are given to participants, they will receive educational materials to help them understand what their results mean. Genetic research results will be provided to participants only if they are individually clinically relevant. CDC will confidentially contact individuals, provide pre-disclosure counseling, and disclosure, if the parent and child wish to receive this information.

Disseminating results to the public:

We intend to publish the information we learn from analyzing these data in a report to parents of participating children and in a peer-reviewed journal to make it easily accessible to all parties interested in possible linkages between environmental or infectious exposures and childhood cancer. The information will also be presented at professional conferences.

METHODS: COMMUNICATIONS INFRASTRUCTURE

In addition to informal telephone conversations and emails on an as needed basis, weekly telephone conferences will be scheduled throughout sample collection period when CDC is not on site in Cochise County. Investigators required on the telephone will be Gary Spivey (Cochise County PI), Veronica Brown (CCHD staff contact), Beverly Kingsley (CDC PI), Robert Jones (Laboratory supervisor), and Tammy Clark (laboratory staff).

Cochise County Investigators and Staff will be reminded that any important occurrences/events in Cochise County should be reported to the other members of the investigation team in a timely manner. These investigators will include Beverly Kingsley, Timothy Flood, and Robert Jones.

Following IRB approval, the site visit and refresher training will be scheduled. Additional site visits will be scheduled as needed.

PROTECTION OF HUMAN RESEARCH PARTICIPANTS

Risks:

This is a minimal risk biosampling research study. The collection of blood, buccal cells, and urine specimens pose no significant risk to the participants. Any future genetic research will be conducted to look for common variants of genes that code for enzymes involved in detoxification or DNA repair from damage by environmental exposure. Genetic research results will be provided to participants if they are individually relevant.

Anticipated benefits:

All participants will benefit from learning the results of their biologic testing. The scientific community and the general public also benefit from a further understanding of the role of environmental exposure and genetics on the likelihood of developing ALL/AML.

Vulnerable populations:

Children aged 0 to 14 years old will comprise approximately one-third of the population for this investigation.

Implementation/documentation of informed consent and parental permission:

The appropriate informed consent document in [Appendix A](#) will be provided to and explained to the recruited research study participants at the time of their appointment with the CCHD. Families of case or comparison children under the age of 18 will receive the consent form for families ([Appendices A4](#) and [A5](#)). At the time of the interview and specimen collection, recruited case and comparison children aged 7-14 will be asked to assent to participation in the biosampling research study ([Appendices A3](#), [A1](#), [A2](#)). The Adolescent Assent forms are essentially the same as the Adult Case/Comparison forms. On each consent form, research study participants and their families will be invited to call the ADHS, the CCHD or CDC if they have any questions at all about the biosampling research study or the consent process.

Protection of privacy and confidentiality:

Confidentiality of participating children, adults, and families will be maintained to the full extent allowable under the law. Unique identifiers (i.e., biosampling research study id numbers) will be used to

link questionnaires, consent forms, and laboratory data. At the time the families visit the CCHD clinic, labels with the unique ID assigned to this family will be affixed to the consent form and the questionnaire. Labels with the participant's study identification number will be affixed to each biologic sample. Including the urine collection cup and aliquots, and blood collection tubes and aliquots. The consent form and the interview questionnaire will have the participant's name, other personal identifiers, and the participant's unique biosampling research study id number. These forms and pages will be stored in locked files at CDC, separate from the completed data collection forms data. ADHS and CDC will retain the master list that matches the code number to the participant. CDC and ADHS will protect the confidentiality of the participants to the full extent allowable by law. Research study databases will not include personal identifiers; data will be entered under research study id number only. Specific procedures will be taken to protect the confidentiality of the research study data including password protection of electronic files at CDC and unique identifiers that will not include any information that could potentially be used to identify participants. We are asking participants to donate their DNA to CDC for leukemia research. The DNA specimen will be linked to pertinent questionnaire data and the findings from the exposure assessment. The genetic studies would not be useful medically and will only be reported back to the research study participant if they are individually clinically relevant (see "Risks", above; see [Appendix A](#) for informed consent documents – 'What will happen' and 'Confidentiality' sections).

Compensation/incentives:

No one will be paid to be in this biosampling research study (see "Anticipated benefits", above).

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Table 1. Year of Birth and Sex for Cases and Controls in Biosampling Study, Sierra Vista, Arizona

Year of birth Category and Sex		
2002-2003 Girls	1	2**
2002-2003 Boys		
2000-2001 Girls	2*	2
2000-2001 Boys		
1998-1999 Girls	1	2
1998-1999 Boys (as of Dec 2001)		
1996-1997 Girls	1	2
1996-1997 Boys	0	
1994-1995 Girls	2	4
1994-1995 Boys	3	6
1992-1993 Girls	0	
1992-1993 Boys	1	2
1990-1991 Girls	1	2
1990-1991 Boys	0	
Total	12	22

* Female child born 2000-2001 deceased. No comparison will be assigned.

** Due to extremely young age of case, it may be necessary to match using wider age strata, e.g. 4 years.

Table 2: Candidate list of substances to be analyzed for in blood and urine samples from biosampling research study participants.

Tube						
7mL EDTA	Pb / Cd	Whole Blood	250 uL	≤ -70°C	<i>This tube MUST be collected FIRST</i>	Robert Jones
<i>This blood fraction must be aliquoted before spinning down.</i>	Mercury (Total and Inorganic)	Whole Blood	650 uL	≤ -70°C	Whole blood will be aliquoted into a 2 mL cryovial before processing to obtain the plasma and the Buffy coat components	Robert Jones
	Selenium	Plasma	250 uL	≤ -70°C		Robert Jones
<i>This blood must be spun down and aliquoted in the field</i>	Organochlorine Pesticides	Plasma	1.0 mL	≤ -70°C	<i>PCBs could also be analyzed from this aliquot</i>	Wayman Turner 770-488-7974 Wturner@cdc.gov
	Lipids	Plasma	0.5 mL	≤ -70°C		Wayman Turner
	DNA	<i>Buffy coat</i>		≤ -70°C	MUST be frozen -70 C or colder. Buffy coat will be separated from plasma and stored frozen for future genetic studies	Peg Gallagher 770-488-3612 mgallaher1@cdc.gov
	Amp RT DNA – PCR on oncoviruses Serology – Leukemia viruses MOP (primers on cellular RNA) HTLV serology – Bharat FeLV serology	Plasma		≤ -70°C		NCID
	Long Term Storage	Plasma		≤ -70°C		NCEH

continued

Tube	Analyte(s)	Matrix	Required Volume	Storage Temp	Comments	Contact
7mL Gray Top	Blood VOC's:	Whole Blood	7 mL	4°C Do NOT Freeze	<i>Blood must be collected into a specially treated and screened gray-top vacutainer. Blood sample collection must closely follow the VOC blood collection protocol. Sample can not be opened prior to VOC analysis.</i>	Ben Blount 770-488-7894 Bblount@cdc.gov
7 mL EDTA	Long Term storage	Plasma		≤ -70°C	MUST be frozen -70°C or colder.	NCEH
<i>This blood must be spun down and aliquoted in the field</i>	DNA	Buffy coat	<i>Sample aliquoted to 3 separate vials in the field</i>	≤ -70°C	MUST be frozen -70°C or colder.	NCID
Full Void Urine	Mercury	Urine	1.25 mL	≤ -70°C	<i>Must be preserved separately in the field</i>	Robert Jones
	Creatinine	Urine	0.25 mL	≤ -70°C		Robert Jones
	Pesticides: Organophosphates Pyrethroids Chlorphenoxy herbicides Carbamates Atrazine	Urine	25 mL minimum for 5 tests	≤ -70°C	Amount will vary depending upon pesticides requested. More volume is needed to allow for reanalysis.	Dana Barr 770-488-7886 dbarr@cdc.gov
	As	Urine	0.5 mL	≤ -70°C		Robert Jones
	Uranium ²³⁵	Urine	0.5 mL	≤ -70°C		Dan Paschal 770-488-7985 DPaschal@cdc.gov
<i>The urine will be a full void that is collected in a pre-screened collection cup. A 1.2 mL sample will then be aliquoted into the</i>	Antimony Barium Beryllium Cadmium Cesium Cobalt Lead Molybdenum	Urine	2 mL	≤ -70°C		Robert Jones 770-488-7991 770-488-4097(FAX) rljones@cdc.gov

<i>Hg tube.</i>	Platinum Thallium Tungsten Uranium ²³⁸					
	Chromium (Total)	Urine	0.5 mL	≤ -70°C		Robert Jones
	Nickel	Urine	0.5 mL	≤ -70°C		Robert Jones
	Manganese	Urine	0.5 mL	≤ -70°C	<i>Requires method development</i>	Robert Jones
	As (Speciated)	<i>Urine</i>	0.5 mL	≤ -70°C	<i>Requires method development</i>	Robert Jones
Buccal smear	DNA	<i>Buccal smear</i>	2 cytobrushes	Room Temp	Can be shipped on Dry Ice	Peg Gallagher

FIGURE 1. STUDY RECRUITMENT, ENROLLMENT, AND PARTICIPATION

