Appendix A

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Appendix B: Biological Laboratory Methods

BIOLOGIC SAMPLE COLLECTION AND ANALYSIS PLANS:

Collection:

URINE

BLOOD

BUCCAL CELLS

Analysis:

METALS

NON-PERSISTENT AND PERSISTENT PESTICIDES

POLYCHLORINATED BIPHENYLS (PCBs)

VOLATILE ORGANIC COMPOUNDS (VOCs)

Protocol for Collecting Urine Specimens

COLLECTION PROCEDURE

Urine collection cups will be provided for each participant. Instruct each person to do the following for urine collection:

- Hands should be washed with soap and water.
- Do not remove the cap from cup until ready to void.
- Collect at least 30 mL urine in the cup.
- Do not touch the inside of the cup or cap at anytime.
- Recap the specimen and deliver to investigator.
- Place a label for **URINE CONTAINER** on cup.

One aliquot is needed. Any specific analyte can be prepared at CDC from this container.

- Pour 25 mL urine into a 1 oz glass bottle with the green screw cap.
- Place the participant's ID label on each container.

SHIPPING LIST

A collection log is provided to record samples that are collected. Please mark the appropriate spaces indicating which aliquots were collected, date collected and any problems that were encountered in collection, storage, or shipping.

SHIPPING PROCEDURE

1. Pack the shipping box with the boxes of urine samples. Place each box in the zip-lock bags before packing. Fill the shipper with dry ice, cover with the Styrofoam lid and tape down the cardboard outer flaps. Place a dry ice label on the outside of the container and write in the amount of dry ice in the shipper.

2. Ship to the following address:

Charles Dodson
Centers for Disease Control and Prevention
4770 Buford Highway NE
Building 17 Loading Dock
Atlanta, GA 30341

3. Please call (770) 488-4305 on the day the shipment is made. Also, if any questions arise, please call the above number.

Protocol for Collecting Blood Specimens

1) Have the following items on hand and available:

- Blue Absorbent Pad
- Powder free gloves
- Tourniquet
- Alcohol disinfectant swabs (individually wrapped)
- Gauze bandages (sterile, individually wrapped)
- 21g or 23g vacutainer butterfly
- Vacutainer needle holder
- 7 mL PURPLE top tube with Hemagard cap
- 7 mL GREY top tube with Hemagard cap
- 7 mL PURPLE top tube with Hemagard cap
- Bandage
- Sharps disposal container for used needles and butterflies

2) Select the appropriate size butterfly and attach to the Vacutainer needle holder.

3) Wipe the three tube caps with an alcohol wipe **immediately** before collection.

4) Tie the tourniquet onto the upper arm so that it can be quickly released with one hand.

5) Swab the venipuncture area with an alcohol pad.

6) Wipe off excess alcohol with the gauze bandages. Allow to air dry for 5 - 10 seconds.

7) Puncture the vein with the butterfly needle.

8) Insert the **first 7mL purple top tube** into the barrel of the vacutainer needle holder and push until blood enters the tube. The tube will draw only 6.5 mL of blood. When full, remove tube and invert 4-6 times to mix.

9) Insert the **GREY top 7mL tube** into the barrel of the vacutainer needle holder and push until blood enters the tube. When full, remove tube and invert 4-6 times to mix.

10) Insert the **second 7mL purple top tube** into the barrel of the vacutainer needle holder and push until blood enters the tube.

11) Release the tourniquet when the last tube has filled half way. Allow tube to finish filling, remove from holder, invert 4-6 times to mix, and then apply pressure with sterile gauze to venipuncture site as you remove the needle.

12) Carefully remove vacutainer needle or butterfly from holder and dispose of it in the sharps container.

13) Mix all of the blood tubes well by inverting 4-6 times upon removal from the holder to ensure good distribution of the anticoagulant throughout the blood.

14) Immediately upon completion of venipuncture, while pressure is being applied to site, pick up collected tubes and invert 6-10 more times to mix.

15) Place pressure on the venipuncture site for a few minutes with a gauze pad. Cover the venipuncture site with a bandage.

16) Place a bar-coded ID label on the vacutainer tubes. Make sure that the label edge starts on the edge of the tube label and that there is a “window” so that one can see the tube contents. Place the label on the tube so that the barcode looks like a “ladder” when the tube is held upright.

17) Record the sample number on the collection log indicating results of the collection and appropriate collection comments if difficulties were encountered.

18) Place samples in the sample tube rack or box provided. Give the blood and urine specimens to the CDC laboratory personnel.

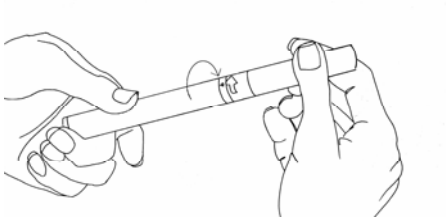
Notes:

If unable to collect blood after two tries, contact the CDC laboratory personnel.

If the CDC laboratory personnel are unable to collect a blood specimen after two additional tries then consult the CDC MD.

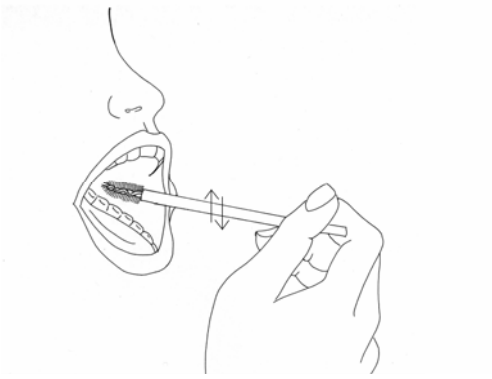
Protocol for Collecting Buccal Cell Specimens

1) Read and sign the enclosed consent forms.



2) Twist it at the blue arrow to remove the cap. Take the brush out of the tube. Be sure **NOT** to touch the brush end.

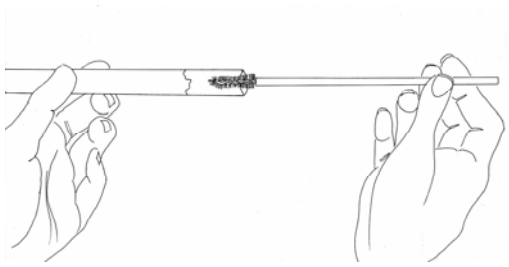
3) Place the brush inside the subject's mouth, against the inside of the cheek. Rub the brush firmly up and down along the inside of the cheek 30 times.



4) Put the brush back into the tube with the brush end first. Cover the tube with the cap. Do not re-use this brush on another person.

5) Place the Barcode label on the tube and write the **date** the sample was collected on the tube label.

6) Repeat steps 2-5 with the second tube. This time, use the brush on the other cheek.



7) Store the tubes on dry ice.

Laboratory Methods for the Elemental Analysis of Blood and Urine Samples

Collection of Blood Samples

The blood sampling was conducted by drawing from all study participants by venipuncture whole blood samples into EDTA-anticoagulated tubes. Blood specimens were refrigerated then aliquoted within two hours into cryovials immediately frozen and shipped on dry ice. All blood samples were stored frozen at or lower than -20ºC until analyzed. All blood collection supplies including butterflies, syringes, needles, evacuated tubes, transfer pipettes and cryovials were prescreened for metal contamination before use.

Analysis of Blood Samples

Lead and Cadmium

Lead was measured by graphite furnace atomic absorption spectrometry (GFAAS). The GFAAS included simultaneous determination of lead and cadmium utilizing Zeeman background correction and resulted in a blood lead limit of detection for lead of 0.3 µg/dL and for Cadmium of 0.3 µg/L. The reported lead and cadmium results were the average of two measurements. The blood lead/cadmium instrument was calibrated by using calibrators prepared by using the National Institute of Standards (NIST) Standard Reference Material (SRM) aqueous lead. Analytical quality control was monitored by utilizing six quality control pools to assure adherence to quality control standards.

Mercury

Whole blood specimens were analyzed for total and inorganic mercury. Specimens were analyzed using an automated cold vapor atomic absorption spectrophotometry instrument (PerkinElmer Flow Injection Mercury System [FIMS] 400 with an AS-91 autosampler). The detection limit was 0.14 parts per billion (ppb) for total mercury and 0.4 ppb for inorganic mercury. Matrix matched calibration methods were used for both blood measurements. The total blood mercury analysis utilized a Maxidigest MX 350 in-line microwave digester connected to the FIMS-400 system. The inorganic mercury analysis utilized stannous chloride as the reducing

agent and the total mercury analysis utilized sodium borohydride as the reducing agent. The blood mercury analysis requires 0.2 mL of blood for the total and an additional 0.2 mL of blood for the inorganic analysis. The reported mercury result was the average of two measurements. NIST SRM 966 was used as a bench quality control material as well as 3 levels of in-house blood pools traceable to NIST SRM 966 for daily QC.

Collection of Plasma Samples

The blood sampling was conducted by drawing from all participants by venipuncture whole blood samples into EDTA-anticoagulated tubes. Samples were spun down and plasma was aliquoted into cryovials within two hours then immediately frozen and shipped on dry ice. All plasma samples were stored frozen at or lower than -20ºC until analyzed. All blood collection supplies utilized for plasma collections including butterflies, syringes, needles, evacuated tubes, transfer pipettes and cryovials were prescreened for selenium contamination before use.

Analysis of Plasma Samples

Plasma Selenium

Plasma selenium concentrations were determined by using Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (ICP-DRC-MS). This multi-element analytical technique is based on quadruple ICP-MS technology and includes DRC™ technology, which minimizes or eliminates many argon-based polyatomic interferences.

In this method, selenium was measured at mass 80 utilizing methane as the reaction gas. Plasma samples were diluted 1+23 with 1% (v/v) double-distilled nitric acid, 0.01% Triton-X-100, 10% ethanol containing gallium for internal standardization. The detection limit for plasma selenium was 3 µg/L. The ICP-MS was calibrated using NIST traceable matrix matched calibrators. The reported selenium concentration was the average of three measurements. Quality control was monitored by utilizing three quality control pools to assure adherence to quality control standards.

Collection of Urine Samples

Urine samples were collected from study participants by collecting a midstream void into a sterile urine collection cup. Urine specimens were then aliquoted into cryovials, frozen and shipped on dry ice. All urine samples were stored frozen at or lower than -20º C until analyzed. All urine collection supplies including urine cups, transfer pipettes, glass bottles, and cryovials were prescreened for all metal contamination before use. The mercury urine specimen was aliquoted into a cryovial that included a preservative added for the stability of the mercury specimen.

Analysis of Urine Samples

Mercury

Samples were analyzed for mercury using an automated cold vapor atomic absorption spectrophotometry instrument (PerkinElmer FIMS-400 with an AS-91 autosampler). The detection limit for mercury was 0.14 µg/L. A matrix matched calibration method was used. The urine mercury analysis required 0.2 mL of urine. The reported mercury result was the average of two measurements. Analytical quality control was monitored by utilizing three quality control pools to assure adherence to quality control standards.

Chromium

Chromium in urine was measured by GFAAS. The GFAAS determination of chromium utilizes Zeeman background correction and results in a limit of detection for urine chromium of 0.3 µg/L. The reported chromium results were the average of two measurements. The urine chromium instrument was calibrated by using calibrators prepared by using the NIST SRM aqueous SRM. Analytical quality control was monitored by utilizing four quality control pools to assure adherence to quality control standards.

Nickel

Nickel in urine was measured by GFAAS. The GFAAS determination of nickel utilizes Zeeman background correction and results in a limit of detection for urine nickel of 1.5 µg/L. The reported nickel results were the average of two measurements. The urine nickel instrument was calibrated by using calibrators prepared by using the NIST SRM aqueous SRM. Analytical

quality control was monitored by utilizing four quality control pools to assure adherence to quality control standards.

Arsenic (total)

Urine arsenic (total) concentrations are determined by using ICP-DRC-MS. This multielement analytical technique is based on quadruple ICP-MS technology and includes DRC™ technology, which minimizes or eliminates many argon-based polyatomic interferences. In this method, arsenic (isotope mass 75) and gallium (isotope mass 69) or tellurium (isotope mass 126) are measured in urine by ICP-DRC-MS using argon/hydrogen (90%/10%, respectively) as a reaction gas. Urine samples are diluted 1+9 with 2% (v/v) double-distilled nitric acid containing gallium or tellurium for internal standardization. The detection limit for urine arsenic is 0.3 µg/L. The ICP-MS was calibrated using NIST traceable matrix matched calibrators. The reported urine arsenic concentration was the average of three measurements. Quality control was monitored by utilizing four quality control pools to assure adherence to quality control standards.

Manganese

Urine Manganese concentrations were determined by using Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (ICP-DRC-MS). This multielement analytical technique is based on quadruple ICP-MS technology and includes DRC™ technology, which minimizes or eliminates many argon-based polyatomic interferences. In this method, manganese was measured at mass 55 utilizing methane as the reaction gas. Plasma samples were diluted 1+23 with 1% (v/v) double-distilled nitric acid, 0.01% Triton-X-100, 10% ethanol containing rhodium for internal standardization. The detection limit for plasma selenium was 0.24 µg/L. The ICP-MS was calibrated using NIST traceable matrix matched calibrators. The reported manganese concentration was the average of three measurements. Quality control was monitored by utilizing three quality control pools to assure adherence to quality control standards.

Multi-Element

Multiple elements in urine were measured by ICP-MS. This ICP-MS method measured the following 12 elements in urine: Beryllium (Be), Cobalt (Co), Molybdenum (Mo), Cadmium (Cd), Antimony (Sb), Cesium (Cs), Barium (Ba), Tungsten (W), Platinum (Pt), Thallium (Tl),

Lead (Pb), and Uranium (U). Urine samples were diluted 1+9 with 2% (v/v), double-distilled, concentrated nitric acid containing both iridium (Ir) and rhodium (Rh) for multi-internal standardization. The detection limits for the urine metals were: Be 0.14 µg/L, Co 0.10 µg/L, Mo 1.7 µg/L, Cd 0.09 µg/L, Sb 0.08 µg/L, Cs 0.22 µg/L, Ba 0.30 µg/L, W 0.04 µg/L, Pt 0.06 µg/L, Tl 0.03 µg/L, Pb 0.22 µg/L, and U 0.006 µg/L. The ICP-MS was calibrated using NIST traceable matrix matched calibrators. The reported urine metals concentration was the average of four measurements. Quality control was monitored by utilizing four quality control pools to assure adherence to quality control standards.

Laboratory Methods for the Analysis of Pesticides and Polychlorinated Biphenyls and/or their Metabolites in Blood and Urine Samples

Collection of Urine Samples

Urine samples were collected from study participants by collecting a midstream void into a sterile urine collection cup. Urine specimens were then aliquoted into cryovials, frozen, and shipped on dry ice. All urine samples were stored frozen at -70^{O}C until analyzed.

Analysis of Urine Samples

Class-Specific Metabolites of Organophosphate Pesticides

Samples (2 mL) were lyophilized then the metabolites were derivatized to their respective chloropropyl phosphate esters. The esters were analyzed using gas chromatography-tandem mass spectrometry. The detection limits ranged from 0.1 to 0.6 ng/mL. A matrix-matched isotope dilution calibration method was used. Analytical quality control was monitored by utilizing two quality control pools to assure adherence to quality control standards.

Chlorinated Phenols, Chemical-Specific Organophosphate Pesticide Metabolites, Carbamate Metabolites, Fungicides

Samples (2 mL) were extracted with n-butyl chloride/diethyl ether then the metabolites were derivatized to their respective chloropropyl ethers. The ethers were analyzed using gas chromatography-tandem mass spectrometry. The detection limits ranged from 0.1 to 1.1 ng/mL. A matrix-matched isotope dilution calibration method was used. Analytical quality control was monitored by utilizing two quality control pools to assure adherence to quality control standards.

Herbicides, Pyrethroids and Repellents

Samples (2 mL) were extracted using solid phase extraction cartridges. The extracts were analyzed using high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. The detection limits ranged from 0.01 to 0.5 ng/mL. A

matrix-matched isotope dilution calibration method was used. Analytical quality control was monitored by utilizing two quality control pools to assure adherence to quality control standards.

Collection of Plasma Samples

The blood sampling was conducted by drawing from all participants by venipuncture whole blood samples into EDTA-anticoagulated tubes. Samples were spun down and plasma was aliquoted into cryovials within 2 hours then immediately frozen and shipped on dry ice. All plasma samples were stored at -70^{o}C until analyzed.

Analysis of Plasma Samples

PCB/Organochlorine Pesticide Analysis

Samples (1 mL) were lyophilized on hydromatrix in an accelerated solvent extraction cell. The cell was subsequently filled with florisil and inverted. The sample was extracted using accelerated solvent extraction with dichloromethane:hexane and received an in-line florisil cleanup during the extraction process. The extracts were analyzed using gas chromatography-high resolution mass spectrometry. The detection limit for the PCBs and organochlorine pesticides were 5 and 20 pg/mL, respectively. An isotope dilution calibration method was used. Analytical quality control was monitored by utilizing two quality control pools to assure adherence to quality control standards.

VOLATILE ORGANIC COMPOUNDS (VOCs)

Laboratory Methods for the Analysis of Volatile Organic Compounds in Blood Samples

Volatile organic compounds (VOCs) are measured in whole blood by solid phase microextraction/gas chromatography/isotope dilution mass spectrometry based on the method described by Blount, et al. (1). The analytes are in equilibrium between the whole blood matrix and the headspace above the sample. A solid-phase microextraction fiber is inserted into the headspace and the VOCs partition into the phase on the outside of the fiber shaft. This fiber is then inserted into the heated GC inlet where the VOCs rapidly desorb due to the increased temperature. Extracted VOCs are focused at the head of the GC column using a cryo-trap. Analytes are separated on a DB-VRX column and quantified using selected ion monitoring mass spectrometry (unit mass resolution). Comparison of relative response factors with known standard concentrations yields individual analyte concentrations. The method is applicable to the determination of VOCs in 3 mL blood with detection limits in the parts per trillion range. Since non-occupationally exposed individuals have blood VOC concentrations in this range, the method is applicable for determining these quantities and investigating cases of low-level exposure to VOCs.

Blount B.C., Robert J. Kobelski, David O. McElprang, David L. Ashley, John C. Morrow, David M. Chambers, and Frederick L. Cardinali. 2006. Quantification of 31 Volatile Organic Compounds in Whole Blood Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry. J. Chromatography B 832(2):292-301; doi:10.1016/j.jchromb.2006.01.019.

Appendix C: Analytes

Metals:

Urine

Antimony

Arsenic (total)

Arsenobetaine

Arsenochline

Trimethlyarsine

Dimethlyarsonic acid

Arsenous acid

Arsenic acid

Barium

Beryllium

Cadmium

Cesium

Chromium

Cobalt

Lead

Manganese

Mercury

Molybdenum

Nickel

Platinum

Thallium

Tungsten

Uranium

Blood

Cadmium

Copper

Lead

Mercury (inorganic)

Mercury (total)

Selenium

Persistent Pesticides

DDE, p,p-

DDT o,p-

DDT p,p-

Dieldrin

Aldrin

Heptachlor

Hexachlorobenzene

Hexachlorocyclohexane, beta

Hexachlorocyclohexane, gamma

Mirex

Oxychlordane

Transnonachlor

Endrin

Non Persistent Pesticides

Cholinesterase Inhibiting Pesticides

Acephate

Dimethoate

O-Methoate

5 chloro, 1,2-dihydro1-isopropyl-3[H]-1,2,4-triazol-3-one

Coumaphos

Diethyldithiophosphate (DEDP)

Diethlyphosphate (DEP)

Dimethylthiphosphate (DMTP)

Dimethyldithiophosphate (DMDTP)

Dimethylphosphate (DMP)

Dimethylthiophosphate (DMTP)

Diethylthiophosphate (DETP)

Malathion

Methamidophos

2-(diethylamino) -6-methylpyrimidol

2-Isopropyl-4-methyl-6-hydroxypryamine

p-nitrophenol

3,5,6-trichloro-2-pyridinol (metabolite of chlorpyrifos)

Herbicides

2,4, D

2,4,5-T

Atrazine mercapturate

Acetochlor mercapturate

Metachlor mercapturate

Pyrethroids

3-Phenoxybenzoic acid

Fungicides

Ethylene thiourea

Propylene thiourea

Repellants

DEET

Polychlorinated Biphenyl's (PCB's)

18
28
44
49
52
66
74
87
99
101
105
110
118
128
138/158
146
149
151
153
156
157
167
170
172
177
178
180
183
187
189
194

195

196/203

199

206

209

Volatile Organic Compounds

2,5-Dimethylfuran

Hexane

Heptane

Octane

Nonane

Decane

Undecane

Dodecane

1,2-dichloroethane

Tetrachloroethee

Bromodichloromethane

Benzene

Chloroform

Carbon tetrachloride

1,2-dibromoethane

Ethylbenzene

Methylene chloride

Ter butyl methyl ether

0-xylene

Styrene

Trichloroethene

Toluene

M,p-xylene

Appendix D: Statistical Methods

Statistical Methods for Analyzing Data Collected in the Sierra Vista Biosampling Study

Background and Introduction

The Arizona Department of Health Services and Cochise County Health Department requested assistance from the National Center for Environmental Health (NCEH), Centers for Disease Control and Prevention (CDC) to investigate the occurrence of excess childhood leukemia in Sierra Vista Arizona. After collaborative discussions and meetings, it was decided that the best scientific study under the circumstances would be to assess exposure to a variety of chemicals, active elements to see if there was any ongoing environmental exposure in the community. Families of children diagnosed with acute lymphocytic leukemia [ALL] and acute myelocytic leukemia [AML] and comparison children and their families would be sampled. The ten cases of childhood leukemia reported in Sierra Vista between 1997 and 2003 suggested that there might be a localized increase in the incidence of ALL/AML for this area.

The primary goal of this study is to assess whether known or probable carcinogens are present at previously unrecognized high levels in Sierra Vista, Arizona as evidenced by levels measured in biological samples from residents. . In total, 12 cases of childhood leukemia were detected in children living or formerly living in Sierra Vista between 1997 and 2006.

It was hypothesized that there might be known or suspected carcinogens present in the county that could be related to this disease.

Descriptive assessments and statistical analyses were performed on 128 analytes measured via laboratory analyses of biological samples taken from Sierra Vista study participants, indicative of environmental exposure, and, to the extent possible, these measurements were compared to national levels published in the Second and Third National Exposure Report [1] or from other NHANES samples. Data was also analyzed

using information collected from brief questionnaires and interviews that included questions on health, occupation, and military history.

Ten children with leukemia were identified in Sierra Vista between 1997 and 2003, and the time of the inception of the study. Of these, two case family declined to participate in the CDC investigation. Two families were not able to be contacted by the Cochise County Health Department. One child with leukemia was deceased, but the family agreed to participate in the study. Thus, four children met the case definition. All four had ALL. The comparison children were recruited through neighborhood searches of households inSierra Vista. They were required to be residents of the county at the time of enrollment. Children were excluded from the community based comparison group if they were siblings of a case child or if they had ever been diagnosed with any form of cancer. There were 9 comparison children enrolled from 9 households.

Statistical Methods

For each of the variables analyzed, univariate descriptive statistics provide an overall picture of the data. For continuous variables, sample sizes, geometric means, and selected percentiles are presented to summarize the range and distribution of the data for the various sub populations of interest. Geometric means (with 95% confidence intervals) are constructed under the assumption that the data approximates a log-normal distribution. The estimate of the mean and confidence interval is based on a statistical model that controls for the possible correlation of observations within a family (i.e. a variance components model), when appropriate. In some cases, comparison values (based on Second or Third National Exposure Report [1] and/or other published levels) are presented in order to give an indication of what constitutes a “large” or “small” value for a selected summary statistic. For categorical variables, frequency counts and percentages are presented as summary statistics for the subpopulations of interest. The denominator

for calculating percentages is related to all relevant study subjects with valid responses, and omits those with missing, invalid or incomplete information.

Questionnaire and Interview Responses

Categorical Variables

For each of the analyzed categorical variables from the questionnaire and interviews, summary statistics are computed and presented. A table displaying cell frequencies and percentages of total number of valid respondents (non-missing) for each of the possible question responses and for each subgroup of interest. The subgroups of the study population are characterized as case or control based on the status of the enrolled child and include members of the case or control family to whom the question applies.

- All Subjects – data reported here describe all children, regardless of case or control status.
- Case Subjects – data reported here describe the case children.
- Comparison Subjects – consists of all children recruited into the study as comparisons.

Continuous Variables

For each of the analyzed continuous variables from the questionnaire and interviews, summary statistics, figures are computed and presented. A table displaying summary statistics (arithmetic means, standard deviations and percentiles) for responses to the question of interest. The statistics are calculated for the same subgroups of the study population as described for the categorical variables in the questionnaire and interview datasets.

Biologic Samples

Statistical analyses of the biologic samples are broken into two analysis sections, one for the comparison subjects group, and one for the case subjects group. The comparison group summaries provide a snapshot of the potential exposure in the entire Churchill County population, while the case population summary helps summarizing the case group although it must be emphasized that there were only 4 case children in this study. With a sample of only 4, it is not prudent to draw conclusions about exposure of case children to environmental contaminants and any differences to comparison children in this regard. The study does not possess sufficient power to detect meaningful differences

An additional complexity in the analysis of biological and environmental samples is the inclusion of the limit of detection (LOD). The LOD is generally considered the lowest concentration of an analyte that the analytical process can reliably detect. Thus, concentration values that fall below the LOD may be considered below the range that a measurement is considered reliable. For purposes of analysis, if a large percentage of the measurements falls below the LOD, some of the reported summary statistics may be uninformative. Geometric means for biologic were computed when less than 40 percent of the data were observed below the detection limit. LODs for lipid-adjusted sample results were computed based on methods used and documented in the Second National Exposure Report [1].

Limitations and Caveats

Several limitations are present in the collected data and in the analyses presented. They are as follows:

1. Although the goal of the study was to match 2 community-based comparison children to each case child, this was not always achieved. Further, comparison children were enrolled for the deceased case child from whom no biologic samples were collected. These comparison children/families provide useful information for the primary goal of characterizing exposures among the Sierra Vista community.

2. Some of the chemicals measured have short half-lives, and are thus indicative of only a brief period prior to biosampling; the levels of these chemicals/analytes may not be indicative of levels in Sierra Vista prior to development of the leukemia cluster.
3. The laboratory findings in case children may have been affected by the treatment of their leukemia. It is not possible to determine how treatment will have altered the presence of analytes or infectious agents. Therefore, current measurements may not accurately reflect pre-treatment exposures.
4. We have insufficient statistical power to detect any differences between the cases and community-based comparisons due to the small sample size.