

National Children's Study Methods Development Pilot Study Progress Reports, 2003

Study Design Issues

Feasibility of Primary Care Practice Sites for Subject Observation and Data Collection – A Pilot Study for the National Children's Study

Project Managers: Michele Kiely and Marion Balsam (NICHD/NIH), David Lanier (AHRQ/NIH)

Brief Summary:

Primary care practices in research networks are being considered as a model for potential sites of National Children's Study (Study) participation. The Practice-Based Research Network (PBRN) Resource Center was requested to assess the relevant characteristics of PBRNs and their enrolled practices, their levels of interest in Study participation, and the relevant strengths and potential barriers for such participation. The Pilot Study has 2 phases: 1) to assess PBRN characteristics and interest in Study participation; and 2) to assess capability of PBRN practices to carry out a reasonable facsimile of the Study protocol. Phase 1 is complete, revealing significant interest along with relevant concerns. Phase 2 is currently being planned.

Focus Group Phase I: Eliciting Community Involvement, Recruitment, and Retention of Subjects

Project Managers: Pauline Mendola and Danelle Lobdell (NHEERL/EPA)

Brief Summary:

In February of 2003, a small pilot study was conducted examining recruitment and retention issues for the National Children's Study (Study). Briefly, we conducted a series of 18 focus groups across the continental U.S. among five identified stakeholder groups – expectant parents, parents of disabled children, parents of non-disabled children, health care professionals, and community organization leaders. The groups focused on three main themes: 1.) getting you interested, 2.) time commitment and data collection activities, and 3.) keeping you interested. From the discussions, many great suggestions and ideas were generated that will help the planning process for the Study.

Focus Group Phase II: Follow Up on Recruitment and Retention of Subjects

Project Managers: Danelle Lobdell and Pauline Mendola (NHEERL/EPA)

Brief Summary:

In February of 2003, a small pilot study was conducted examining recruitment and retention issues for the National Children's Study (Study). From the discussions, many great suggestions and ideas were generated that will help the planning process for the Study. Because the Study intends to represent all of America's children, we are planning to conduct a second round of focus groups to improve our understanding of recruitment and retention issues for groups that were not well represented in Phase I as well as expand on ideas generated from the first set of focus groups. All of the following stakeholder groups will encompass both pregnant mothers and parents of school-aged children: racial/ethnic groups (i.e., Mexican American, other Hispanic, African American, other black, Native American, and Asian) teenagers and biospecimen groups. Couples attempting pregnancy will also be included. One of the themes that emerged in Phase I was the mistrust some of the minority participants had toward research and the federal government. Because of the under-representation of minority participants in the first pilot study, the follow-up focus groups for the racial/ethnic groups will include questions to address the theme of mistrust in research/federal government as well as institutionalized racism in the context of recruitment and retention for the Study.

Identification of Emerging and Innovative Technologies for Use in the National Children's Study – Evaluation of the State of the Science

Project Manager: Sherry Selevan (NCEA/EPA)

Brief Summary:

A longitudinal study of 100,000 children presents a number of challenges for data collection and management. Current technological innovations and those possible during the course of the Study may make data collection and management more efficient, accurate, and consequently, more cost effective. This first effort resulted in a white paper of the current and emerging technologies of potential use to the longitudinal cohort study, which was completed in 2002. The second phase of this project was a workshop, entitled "Workshop on Innovative Technologies for Remote Collection of Data for the National Children's Study," and was completed this year. The workshop summary is available online at www.nationalchildrensstudy.gov

Health-Related Outcome Projects

Use of Biomarkers of Response for Assessing Potential Sensitivity of Children to Adverse Health Outcomes from Exposure to Environmental Chemicals

Project Managers: Carl Blackman and John Rockett (NHEERL/EPA)

Brief Summary of Subproject 1:

Principal Investigators:

R. Julian Preston (NHEERL, ECD), James Allen (NHEERL, ECD), Carl Blackman (NHEERL, ECD), Russell Owen (NHEERL, ECD), Don Delker (NHEERL, ECD), Andy Kligerman (NHEERL, ECD), Sheau-Fung Thai (NHEERL, ECD), Witold Winnik (NHEERL, ECD), David Dix (NHEERL, RTD), John Rockett (NHEERL, RTD), John Rogers (NHEERL, RTD)

The general approach for the development of biomarkers of response is to use animals and animal and human cellular models to identify changes in expression or activity of cellular housekeeping genes. The housekeeping pathways are selected for initial study because there are indications that their effectiveness can change with age. Such differential sensitivity could result in an increased susceptibility to mutation induction and thus lead to concomitant differences in the probability of adverse health outcome in different age groups. Increased mutation frequency is a risk factor for a range of adverse health outcomes including cancer, and reproductive and developmental disorders. This research will be accomplished by comparing very young, young, mature and older animals or cells obtained from such animals or from humans. Biomarkers so identified will then be utilized in coordinated studies with research conducted elsewhere in NHEERL of human children and adults to determine if these markers of response can demonstrate the existence of differential sensitivity of cellular housekeeping processes to environmental challenge. Similarly, animal models or primary cell cultures or cell lines derived from animals or humans with particular genetic polymorphisms or knockouts for housekeeping genes can be used as being representative of susceptible individuals for assessing magnitude of response from exposure to environmental chemicals.

Brief Summary of Subproject 2:

Principal Investigators:

John Rockett (NHEERL/RTD), David Dix (NHEERL/RTD)

Genomics technology has made it possible to define molecular physiology in exquisite detail, when tissues are accessible for sampling. However, many target tissues are not accessible for human diagnostic evaluations or experimental studies, creating the need for accessible surrogates that afford insight into exposures and effects in the target tissues. Surrogate tissue analysis (STA) incorporating contemporary genomic technologies may be useful in determining toxicant exposure and effect, or disease state, in target tissues at the pre- or early clinical stage. We have proposed that gene expression changes in accessible tissues such as blood or hair follicles will often reflect those in inaccessible

tissues, thus offering a convenient biomonitoring method to provide insight into the effects of environmental toxicants on target tissues and reveal early biomarkers of adverse effects prior to clinical manifestation. In a laboratory study using a rodent model, we were able to correlate gene expression changes between blood and uterus following estrogen exposure. Results from this animal study were used to develop plans for a human study to test the feasibility for gene expression profiling in adult surrogate tissues and potential applicability to the National Children's Study (Study). Methods were developed to extract good quality RNA from blood and hair follicles.

Biomarker Database (Phase I and II)

Project Manager: Pauline Mendola, (NHEERL/EPA)

Brief Summary:

Advances in biological markers (biomarkers) have facilitated the assessment of associations between environmental exposures and disease with greater accuracy and precision than ever before. Biomarkers can be measures of exposure, measures of susceptibility to disease (showing either increased or decreased susceptibility to negative health outcomes), or measures of the presence of disease itself. The measurement of all three types of biomarkers is useful in the characterization of the exposure-disease relationship. This project produced a searchable user friendly database of over 2000 published review articles focused on areas of interest to the National Children's Study (Study), including asthma, neurodevelopment, and markers associated with childhood cancer. The database is intended to facilitate evaluation of this information to assure that the most appropriate biomarkers are identified and considered for planning this study.

Pilot Study to Validate the Extraction and Analysis of DNA from Non-Invasive DNA Sources for Application in Environmental Epidemiology Studies

Project Manager: Pauline Mendola, (NHEERL/EPA)

Brief Summary:

Buccal washes and fingernails have been investigated as alternative specimen types for epidemiological studies that offer cost-effective, non-invasive options to whole blood collection. To date, determination of most genetic polymorphism associated with disease susceptibility has been accomplished utilizing DNA from ideal tissues such as whole blood or buffy coats. We collected buccal and fingernail and blood and analyzed DNA from 25 individuals for eight metabolism, activation and repair genotypes by matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS). A subset of individuals was re-consented and nail and blood-mercury levels determined on the same nail samples used to conduct the genetic analyses. Collection of human nails for exposure and genetic assessment represents an important contribution to the study of gene-environment interactions in the larger National Children's Study (Study).

Assessment of Neurobehavioral Toxicity in Human Infants and Laboratory Animals

Project Manager: Suzanne McMaster (NHEERL/EPA)

Brief Summary:

The goal of this research is to provide methods to directly assess neurobehavioral effects in human infants and experimental animals on parallel tasks. Exposure scenarios that are suspected to affect development in human infants (for example, exposure to mixtures of pesticides) can be replicated, amplified and/or disaggregated in the laboratory. Animals can be exposed to the same environmental conditions observed in homes and day care settings. This will provide the bridge needed to link environmental exposures to clinical outcomes. Progress to date suggests that the most promising behavioral endpoints to pursue in the development of these methods are spontaneous motor activity and attention.

Methods Advancement for Milk Analysis (MAMA)

Project Manager: Sue Fenton (NHEERL/EPA)

Brief Summary:

In the MAMA (Methods Advancement for Milk Analysis) study, directed by Sue Fenton and in collaboration with Larry Needham, Rick Wang, and Andreas Sjodin at the CDC, lactating women will donate breast milk, blood, urine, and saliva samples at two specified intervals during early lactation. Samples will be analyzed for two classes of components - endogenous (EPA) and exogenous (CDC). As this study is mainly focused on method development and proof of concept, there are several goals. The first is to define a source for reliable assays for the several endogenous endpoints defined in this study. The funds have been committed, through a competitive search, to a contractor that can provide valid measurement of triglycerides, hormones (estradiol, prolactin), immunoglobulins (IgG, IgA), and other components such as leptin, interleukin 6 (IL-6), and tumor necrosis factor alpha in serum and milk. The validation efforts necessary for these assays are supported through the recent National Children's Study (Study) funding of this project. Milk reactive oxygen species, IgA, and glucose assay validation has begun within EPA. Samples will be evaluated either before or after freezing for the above listed endogenous components, all of which have some link to a change in adolescent and/or adult health. As the Study may generate only frozen milk samples, it is important to compare the concentrations of these compounds in both fresh and frozen milk to assure the accuracy of the values in frozen milk (is the value in frozen milk equal to the actual amount the child ingested). Finally, to evaluate partitioning and time course of clearance of exogenous milk components, the collaborators at the Center for Disease Control (CDC) in Atlanta have proposed use of breast milk, blood, saliva, and urine samples to measure and compare the following environmental toxicants: polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFOS and PFOA), pesticides (organophosphates,

carbamates, pyrethroids, chlorotriazines), polychlorinated biphenyls (PCBs), dioxins, dibenzofurans, bis/alkylphenols, and phthalates. The CDC has assays available for all of these toxicants (and many of their isoforms or metabolites) in serum, urine, or saliva, but not necessarily milk. The Study funding they have acquired through this project will allow development of separation/extraction methods and validation of their assays for use in milk. Within both phases of this study is careful collection and storage of the samples to avoid contamination with exogenous factors, evaluation of the plastics used in the study for exogenous contaminants, and evaluation of the definition of “fresh” milk. Sample collection for this study should begin in late 2003.

Reliability and Validity of Injury Reporting for the NCS

Project Manager: Peter Scheidt (NICHD)

Brief Summary:

Given the incidence and the consequences of injuries in children, study of modifiable environmental factors contributing to injuries and of currently unrecognized effects of injuries is planned for inclusion in the NCS. To develop appropriate criteria for the definition and inclusion of injury events in the NCS, this pilot study evaluates the reliability of parental reporting of injuries in relation to criteria for inclusion of injuries and how injuries impair activities and function of the child victim and those who care for the victim. Specifically, the study will measure the parental reporting accuracy and recall bias for both medically attended injuries and non-medically attended injuries with limitation of activity and how recall and accuracy vary with the setting of medical care and other factors.

The study is carried out in the population of children who are members of Group Health Cooperative of Puget Sound, a large HMO that provides comprehensive health care and enables nearly all health care utilization to be tracked through a unique medical history number. Parents of children who had an injury that involved a medical visit to a Group Health facility are interviewed regarding the injury event and the accuracy and reliability of the report is to be compared to the medical record of the injury. In addition parents of children without medically attended injuries are interviewed to determine the frequency and pattern of injuries that result in some limitation of activity without requiring medical care. Analyses will determine how reporting of injuries varies by age, gender, birth order of child, size of family, age of parent, nature of injury and source of medical care. This information can be used to determine the most efficient frequency or interval of contact for ascertainment of injuries and help the NCS to determine whether to include injuries that do not involve medical treatment. This information will also enable estimates of child injury rates for the purposes of determining study design and sampling strategies.

Exposure Projects

Demonstration of Low Cost, Low Burden Exposure Monitoring Strategies – Birth Cohort

Project Manager: Roy Fortmann (NERL/EPA)

Brief Summary:

This study was designed to develop and demonstrate relevant, low cost, low burden monitoring strategies that can be used in a longitudinal epidemiological study that focuses on pregnant women and young children. The focus of this study was on (1) recruiting and retaining participants (children and their caretakers) in a longitudinal exposure study and (2) demonstrating the feasibility of measurement strategies that use remote employment of readily available, easy to use, state-of-the-art methods, instruments, and/or techniques for assessing human exposures to environmental contaminants. The monitoring strategy involved mailing sampling kits to study participants with instructions for collecting biological samples (e.g., urine, breast milk, and hair) and environmental samples, such as water, house dust, surface wipes, and personal air samples (badges worn by participating children). After collection, participants packaged and shipped the samples back to the laboratory. Participants also completed an on-line questionnaire that collected information on contaminant sources and participant activities at the time of sample collection. The study has been completed. Results of the study demonstrated high retention rates over the one-year sample collection period. Compliance with sample collection protocols was very good and return rates were high for most sample types. Return of urine samples collected with diapers or as first morning voids was very good. As expected, participants had difficulty collecting samples using more complex methods, such as a badge sampler for volatile organic compounds. Considerable information was generated that favorably supports the strategy of remote sample collection by study participants. Lessons learned in the study will be important in developing the sample collection protocols for the National Children's Study.

Evaluation of Disposable Diapers for Quantitative Measurements of Pesticide Metabolites and Creatinine in Urine Samples

Project Manager: Roy Fortmann (NERL/EPA)

Brief Summary:

This project consists of a laboratory study to evaluate an extraction and analysis method for quantifying biomarkers of pesticide exposure and creatinine in urine samples collected with commercially-available disposable diapers. For large exposure studies, such as the National Children's Study (Study), it is highly desirable to identify a low burden, low-cost method for collection of urine from infants and very young children.

Disposable diapers that contain polyacrylate fibers or granules can be used to collect the urine samples if the pesticide metabolites can be quantitatively recovered from the diaper materials. An initial set of tests will be performed to evaluate the feasibility of the proposed solvent extraction and gas chromatography/mass spectrometry (GC/MS) analysis method. If the initial tests demonstrate that the method should provide acceptable performance, a more comprehensive set of tests will be performed to quantify the method performance for metabolites of the synthetic pyrethroid pesticides that are currently used indoors.

Alternative Exposure Measurement Designs to Improve Epidemiologic Study Designs

Project Managers: James Quackenboss and Thomas McCurdy (NERL/EPA)

Brief Summary:

The general plan for the exposure monitoring component of the planned National Children's Study (Study) is to measure indoor and outdoor concentrations and personal exposures for a variety of pollutants, including combustion products and pesticides. Due to the size of the Study, it will be infeasible to measure every possible exposure for every child. Hence, an important statistical challenge is to develop an appropriate design that will allow adequate power to detect exposure effects, yet at the same time maintaining feasibility in terms of the number of children to be assessed for various exposure and biomonitoring measurements. Since the Study has a longitudinal design, a related challenge will be deciding a) which exposure-related measurements need to be assessed repeatedly over time; and b) the timing of such repeated exposure assessments. At the present time, the methods available in the statistical literature to address the design challenge outlined here are limited. However, there are methodologies available that have the potential to be extended to apply in the present context. This EPA/ORD/NERL pilot study will involve technical assistance from EPA funded contractors who possess strong expertise in statistical and environmental data analysis that is required to investigate the issue of an efficient exposure measurement design for a longitudinal birth cohort study, such as the planned Study. The pilot project will consist of three key components in order to achieve the goals of this pilot study: 1) Use of human exposure data and questions to identify exposure questions for use in the National Children's Study; 2) Statistical Analysis of Longitudinal NHEXAS-Maryland Data to Investigate Relationships between Long-term Activity and Dietary Patterns and Changes in Environmental Concentrations, Exposures, and Biomarkers; and 3) Developing and Efficient Exposure Measurement Design for the National Children Study.

Time-Integrated Exposure Measures to Improve the Predictive Power of Exposure Classification for Epidemiologic Studies

Project Manager: Gary Robertson (NERL/EPA)

Brief Summary:

Accurate exposure classification tools are required to link exposure with health effects in epidemiological studies. Exposure classification for occupational studies is relatively easy compared to predicting residential childhood exposures. Long-term, time-integrated exposure measures would help address the problem of developing appropriate residential childhood exposure classifications. Although long-term integrated exposure measurements are a critical component of exposure assessment, the ability to include these measurements into epidemiological studies is often limited by time, budget, and compliance issues. Another problem that arises when determining the sources, routes, and pathways of exposure to pesticides and other chemicals is the lag time between collection of a sample and the receipt of the results. Current methods that use laboratory analysis may take 30–60 days, or longer, to obtain results. This means that the source of exposure may be gone by the time it is discovered that the child has been exposed, given the relatively short half-lives of many compounds. Screening techniques could direct attention to the most highly exposed (to particular indicator compounds) population of children for which multiroute, multimedia monitoring would be of value. In addition, stratification of the study population (i.e., the majority of the environmental/biological samples collected from the “highly exposed population” of children) often is required, given the expense of multiroute, multimedia monitoring (numerous non-detect results are counterproductive in determining sources). This project will be conducted as four concurrent subprojects. Three subprojects will be directed at (1) demonstrating field performance of a semipermeable membrane device (SMPD) to collect long-term integrated samples for semivolatile organics in air, (2) developing simple rapid methods for analyzing the SMPDs, and (3) developing rapid field or near field methods for analyzing chemical metabolites in urine. The fourth subproject will be a literature review to identify available but not currently used techniques for long-term integrated exposure measurements.

Tampa Asthmatic Children’s Study (TACS)

Project Manager: Ronald W. Williams (NERL/EPA)

Brief Summary:

The Tampa Asthmatic Children’s Study (TACS) was a pilot research study to assess methodologies and research instruments needed for including asthma as a health outcome in the National Children’s Study. This was one of a series of pilot studies focusing on (a) simple, cost effective methods for assessing environmental exposures relevant to asthma and (b) techniques for the early assessment of asthma-related health outcomes. This research project focused on the development of methods for understanding the factors that contribute to indoor concentrations and exposures to combustion related products (CRPs), particulate matter (PM) and select air toxics. The research has helped develop and assess simple questionnaires and measurements for classifying children’s exposures. The questionnaires and methods were evaluated in a field monitoring study in Tampa, Florida. These research tools will be used to identify and quantify the microenvironmental factors associated with children’s exposures in a real-world

environment. Exposure findings will be used to aid in further development of simple models for estimating children's exposure in large epidemiological studies, such as the National Children's Study.

Exposures and Health of Farm Worker Children in California

Project Manager: Don Whitaker

Brief Summary:

The EPA STAR Program Center of Excellence in Children's Environmental Health and Disease Prevention Research at the University of California at Berkeley is currently conducting exposure and health studies for children of farm workers in the Salinas Valley of California. NERL Human Exposure Analysis Branch has provided additional funding to monitor pesticide exposures of twenty children ages 6 to 24 months in their home environment. Samples collected include indoor and outdoor air, house dust, surface wipes, toy wipes, urine, duplicate diet foods, and leftover handled foods. Estimates of dermal exposures will be estimated from analysis of union suits and socks worn by the children. Time and activity data for the 24-hour monitoring period are collected for each participant. Questionnaires designed to obtain general information on environmental exposures as well as food related exposures are also administered.

Evaluation of Exposure Measurement Methods and Approaches for the National Children's Study

Project Manager: Kent Thomas (NERL/EPA)

Brief Summary:

Several hypotheses have been proposed for the National Children's Study to examine the role of environmental exposures to chemical pollutants in health outcomes including asthma and impaired neurobehavioral or neurocognitive function. To address these hypotheses, it will be necessary to assess exposures to a range of chemical pollutants at different life stages through the use of survey tools and biological and environmental measurement methods. The purpose of this work is to survey the literature to obtain and synthesize existing information about personal and environmental measurement methods and approaches, exposure questionnaire and diary methods, and to provide exposure data from previous human exposure studies that are most relevant to the National Children's Study. This work will also examine the relative strengths and weaknesses of existing methodology for chemical exposure measurement in a longitudinal study of children.