Version Date: January 17, 2012

Title of Study: Analysis of Environmental Chemicals in Dried Blood Spots

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I. Background and purpose

The National Children's Study (NCS) is a 21-year prospective study of 100,000 children across the United States conducted by the National Institute of Child Health and Human Development under the Children's Health Act of 2000. The overall objective of the NCS is to examine the effects of environmental influences on the health and development of children. The Greater Chicago Study Center (GCSC) was charged with implementing the Study in Cook County, DuPage County, and Will County (not yet funded).

The NCS GCSC Dried Blood Spots project is a sub-study led by Dr. Thomas McDade, the Director of the Laboratory for Human Biology Research at Northwestern University, and Dr. William Funk of Northwestern University. This study aims to develop a method for directly measuring children's exposure to environmental toxins using biological markers of health found in samples of dried blood spots (DBS) from children. Biomarkers, rather than environmental observations, can provide a more precise estimate of a person's internal toxin makeup. They are also a better predictor of a person's overall health risks than observational markers are.

In other studies, biomarkers have been collected using mostly venous blood samples, the invasiveness and costs of which lessen the likelihood of their use in population-based studies, especially amongst infants and children. This study's motivation in using dried blood spots is to both explore their effectiveness in lieu of taking venous blood draws, as well as examine their success in measuring toxins in the blood.

The first toxins of particular interest to the above researchers are heavy metals, which include lead, mercury, cadmium, and arsenic, and are present in drinking water, air, and soil. While exposure to heavy metals is detrimental to everyone, children are most at risk, as they are both most likely to be exposed to, and are more susceptible to, their toxic effects than adults are. Results of high exposure to heavy metals include learning disabilities and developmental delays. Moreover, methods to analyze heavy metals using dried blood spots have been limited in scope due to the potential contamination of samples from the filter paper.

The second group of toxins to be studied are PAH-protein adducts, which include polycyclic aromatic hydrocarbons, chemical pollutants caused by hydrocarbon fuel, wood, incense, and cigarette smoke. Because PAH-proteins are difficult to measure biologically due to their short life spans, adducts of these toxins are used with abundant blood proteins as biomarkers of exposure.

Thus, the overall purpose of this study is twofold: 1) to examine, and potentially improve analysis of, the described toxins in children's blood, and 2) to explore the effectiveness of the dried blood spots method for analyzing toxicants present in the blood compared to venipuncture.

Several investigations have been conducted on both heavy metals and PAH-protein adducts that have informed this study. Dr. William Funk has investigated heavy metals in dried blood spots in a pilot study examining the ease of finding lead, cadmium, mercury, and arsenic in neonatal dried blood spots (Funk, W., Waidyanatha, S., Chaing, S., Rappaport, S. Hemoglobin Adducts of Benzene Oxide in Neonatal and Adult Dried Blood Spots. *Cancer Epidemiology Biomarkers & Prevention*, 17(8), 1896 – 1901). This study also assessed the effectiveness of using filter paper to collect dried blood spots. This study was part of the North Carolina Center for Birth Defects Research and Prevention, in collaboration with the US Environmental Protection Agency. It found simple methods to purely isolate the hemoglobin from the dried blood spot, when adding ethanol to aqueous dried blood spot extracts. However, only nine subjects participated in this study. More research needs to be conducted, on higher numbers of subjects, to examine best practices for collecting dried blood spots.

Additionally, Dr. Funk has conducted a study measuring protein adducts in dried blood spots (Sobus, J., Pleil, J., Madden, M., Funk, Wl, Hubbard, H., Rappaport, S. Identification of Surrogate Measures of Diesel Exhaust Exposure in a Controlled Chamber Study, *Environmental Science & Technology*, 42(23), 8822 – 8828). Through this study, Dr. Funk and his associates found that a single dried blood spot can provide adequate protein levels to determine levels of protein adducts in a given population, and this method will be used in the upcoming dried blood spots study. The previous study measured protein levels in adults only, so this study will be the first time this method will be used on children.

II. Summary of procedures

Two types of procedures will be performed: 1) a finger or heel stick of approximately five drops of blood and 2) the collection of an extra tube of blood on a current blood draw. This study will recruit 100 children, from infancy to age 21, from the Children's Outpatient Center Laboratory. The number 100 was selected to generate a comparison to previous study findings, while remaining small enough to be collected quickly and as conveniently as possible. GCSC staff from Northwestern University will recruit and formally consent participants for this study. Families who present for a previously scheduled blood draw will be approached for participation. CMH staff will verify the volumes of blood that have already been drawn from each child to ensure that no monthly blood draw limits are approached. Once consented, a phlebotomist will draw one 10 mL lavender top tube of blood, as well as collect five dried blood spots dropped on a single Whatman 903 Protein Savor Card from each participant. Each participant will fill out a 2-question survey asking for their age and sex. The CMH SSPC lab staff will process and store the specimens. Please see the attached detailed protocol, which describes the exact blood processing and laboratory procedures. Once collection is finished, all specimens will be shipped on dry ice to Evanston using a private courier.

III. Risks

This study poses minimal risks. The procedures performed on each child are a finger prick or heel stick for the dried blood spots and the capture of an extra tube of blood from a current draw. The volume of blood that has already been drawn from each child in the last month will be verified to ensure that too much blood is never drawn from any participant. No identifying information will be connected to the blood sample from any child. The only personally identifiable information collected will be the participant's name on the signed consent forms, and only the Principal Investigator will have access to the forms.

IV. Potential Benefits

The overarching benefits of this study are twofold. First, the study proposes exploring a less invasive, more cost-efficient method to examine biomarkers of toxin exposure using DBS compared to venous blood draws. Second, the study hopes to understand the benefits of recently developed methods of measuring quantities of heavy metals and PAH-protein adducts in blood.

V. Inclusion and Exclusion Criteria

- **Inclusion Criteria**

Any child from infancy through age 21 who presents at the Outpatient Center Laboratory for a currently scheduled venipuncture is eligible to participate in this study.

- **Exclusion Criteria**

Anyone older than age 21 is excluded from participating.

VI. Informed Consent Process

GCSC staff from Northwestern University will approach families who present for previously scheduled blood draws at the outpatient laboratory to recruit and formally consent participants for this study. Blood volumes previously drawn from each child will be verified to ensure that no blood draw limits are approached for any child. The study will be explained to the parents of children from infancy through age 11, and they will be given parental consent form to sign, confirming that they understand the parameters of the study. Children ages 12 – 17, of appropriate ability to understand the research being conducted, will be given assent forms to sign, and individuals aged 18 – 21 will be given adult consent forms to sign. In all cases, participants will be notified that their names or signatures will not be recorded or attached to their blood samples in any way.

Other Protocol Elements to Consider

Participants will be given a unique identifier when they consent that will be used to identify their blood specimens. The only identifying information is their name on the consent form.

Two demographic questions of age and sex will be asked while the blood samples are taken. Specimens will be stored in locked freezers at the Children's SSPC lab until they are transported in bulk via private

courier to the Anthropology lab in Evanston. In Evanston the specimens will also be stored in locked freezers.

Summaries and other reports produced from these analyses will report in aggregate and contain no identifiers. Specimens will be stored indefinitely so that it can potentially be used for future studies. All data transmitted to the National Children's Study Program Office at the NIH will be shared in secure transmission modalities that comply with the Federal Information Security Management Act (FISMA). Only the Children's Memorial Hospital IRB and the Principal Investigator will have access to the signed consent forms.