Analysis of Environmental Contaminants in Dried Blood Spots: a pilot study

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SUMMARY

We propose to determine the feasibility of measuring the environmental contaminants PBB 153, p,p'-DDT or p,p'-DDE in archived blood spots in the state of Michigan, an area of widespread environmental contamination to persistent organic pollutants. Samples will be anonymyzed and double-blinded thus a waiver of informed consent is being sought.

INTRODUCTION

Background and significance

Biomonitoring is the measurement of a chemical, its metabolite or reaction product in biological media, usually blood or urine (Pirkle et al. 1995). Biomonitoring has become a useful tool for evaluating exposures to environmental chemicals, especially bioaccumulative chemicals such as lead, mercury, polychlorinated biphenyls (PCBs), and organochlorine pesticides like p,p'-DDT. Bioaccumulative chemicals usually persist in

the environment and have long biological half-lives and, thus, are often called persistent chemicals. Because of their long residence time in the body, biomonitoring of these chemicals is relatively straightforward, usually involving measurement of the chemical in blood up to many years after the exposure has occurred.

Dried blood spots have been routinely collected from newborns in all states in the United States to perform routine genetic testing to detect potentially damaging diseases such as phenylketonuria (PKU). After such testing has occurred, the state health departments typically retain the dried blood spots for a large amount of time, usually in excess of 20 years. These bloods spots are archived in various ways, some in an orderly fashion and others stored stacked in non-temperature controlled warehouses. Typically after about 21.5 years, archived blood spots are discarded.

In a best case scenario, each blood spot contains about 100 μL of blood which is roughly equivalent to 40 μL of serum, the lipid-rich matrix typically used to measure persistent organic pollutants. This volume is about $1/100^{th}$ of the volume typically required to measure PBB 153 in humans. Thus, measurement in and of itself will be a challenging aspect of the study. However, Burse et al. (1997) published a short paper in which they described the detection and quantification of p,p'-DDE, the major environmental degradate of p,p'-DDT and its potential metabolites, in residual dried blood spots. The technology used by Burse et al. included a series of extractions from blood spots followed by analysis by gas chromatography-electron capture detection (ECD). Using this method, Burse et al. (1997) were able to adequately measure p,p'-DDE in all blood spots tested.

Although ECD is extremely sensitive for detecting halogenated chemicals, it lacks the confirmatory selectivity of more modern equipment such as mass spectrometry. Many methods used today, including those used at the Centers for Disease Control and Prevention, use mass spectrometry (MS)-based methods which allow unequivocal identification of the target analytes. However, in some instances, the sensitivity of MS based methods may not achieve that of ECDs.

The Pine River Valley area of Michigan was contaminated with a mixure of polybrominated biphenyls (PBBs) in a mixture known as Firemaster in the late 1970s and early 1980s. In addition, the organochlorine pesticide p,p'-DDT was widely used during this time period and manufacturing emissions close to the local river contaminated it with the pesticide. Thus, Pine River Valley residents have been long concerned with their potential exposures to p,p'-DDT and PBBs and the potential health impact on their community. Michigan's archived blood spots have been stored for a great period of time in non-ideal conditions leading to speculation as to whether the samples could have been contaminated by PBB or p,p'-DDT (or its degradate).

This pilot study will seek to determine whether the PBB congener 153 and p,p'-DDT or p,p'-DDE are present in dried blood spots. In addition, we will determine whether improperly stored blood spots were subjected to contamination that would preclude the accurate quantification of these chemicals in the blood spots.

Justification for study and intended use of study findings

This study will allow us to identify whether archived blood spots are viable matrices for measuring environmental chemicals. Because blood spots are routinely

collected at birth for genetic testing, residual blood spots would allow an ideal, unobtrusive mechanism for evaluating environmental exposures in newborns. In addition, in instances where overt exposures occurred in the past, analyses of archived dried blood spots might allow the state to determine if exposures were excessive (compared to population reference values) and allow followup of those most highly exposed, or if exposure levels were low, and may help alleviate community concerns about potential exposures.

Roles and responsibilities of investigators

CDC/NCEH

- Dana B. Barr, Ph.D., Primary investigator in charge of protocol design, protocol preparation, data analysis, manuscript preparation, and serves as the liaison with the Michigan Department of Community Health and the Pine River Task Force.
- Andreas Sjödin, Ph.D., Co-investigator in charge of analytical development and feasibility testing, testing of blood spots, protocol design, data analysis, and manuscript preparation (lead author).
- Joanne Mei, Ph.D., Co-investigator in charge of developing test blood spots for feasibility testing, protocol design, data analysis and manuscript preparation.
- W. Harry Hannon, Ph.D., Co-investigator in charge of developing and providing blood spot information, serves as a liaison with the Michigan Department of Community Health Blood Spot Program, protocol design and manuscript preparation.
- Larry L. Needham, Ph.D., Co-investigator assisting with program design, protocol

preparation, data analysis and manuscript preparation

Objectives

(1) Develop methods to determine whether PBB 153 and p,p'-DDT or p,p-DDE can be detected in dried blood spots and at what level of sensitivity;

(2) To determine whether archived or older blood spots can be used to determine these environmental chemicals;

(3) To determine whether 10-20 anonymyzed blood spots from the Michigan Department of Community Health contain detectable levels of PBB 153 and p,p'-DDT or p,p-DDE ;

(4) To determine whether the archived blood spot cards from Michigan are contaminated by measuring PBB 153 and p,p'-DDT or p,p-DDE in another portion of the card that does not contain blood;

The success of each objective is dependent upon the success of the previous objective. Thus, if objective 1 is not successfully accomplished, then objectives 2-4 could not be completed.

Hypotheses

(1) PBB 153 and p,p'-DDT or p,p-DDE can be measured at low levels in freshly prepared blood spots.

(2) PBB 153 and p,p'-DDT or p,p-DDE can be measured at low levels in older

blood spots with different matrix properties.

(3) PBB 153 and p,p'-DDT or p,p-DDE can be measured at low levels in archived blood spots stored under uncontrolled conditions in Michigan. Questionnaire data and biomonitoring data will weakly agree.

(4) PBB 153 and p,p'-DDT or p,p-DDE will not be measured in other parts of the blood spot card indicating no internal contamination..

General approach

New blood spots will be developed in-house containing the compounds of interest at various levels. Newer technology (Sjodin et al 2004) will be used to determine whether the target chemicals can be measured at low levels in these blood spots. The analytic limit of detection will be determined.

Older bloods spots (archived QA/QC materials) developed at CDC and stored under controlled conditions will be measured to determine if matrix differences expected from blood spot age will interfere with the analysis.

We will request 10-20 totally anonymyzed blood spots from the Michigan Department of Community Health to test the feasilibity of detecting the target chemicals and whether the blood spot cards are contaminated because of uncontrolled storage conditions.

After these objectives have been met, we will discuss any future research activities, costs, and analytical capacity with the Michigan Department of Community Health and the Pine River Valley Task Force.

PROCEDURES AND METHODS

Design

This study is designed as a retrospective cross sectional feasibility study. Study participants will not be recruited. Older archived blood spots that will be stripped of any identifiers will be used to assess the feasibility of this project

Sample Size

Because this is a feasilibity study only, we are requesting 10-20 archived blood spots.

Program population and recruitment

Blood spots will be obtained from Michigan residents, not necessarily residing in the Pine River Valley area, but some will likely be residents of the area.

Inclusion Criteria:

1. Blood spots cards are anonymyzed; AND
2. The blood spot card should contain at minimum one complete unused spot; AND
3. The blood spot cards should be from Michigan residents.

Exclusion Criteria:

1. Blood spot cards with less than one complete spot
2. Blood spot cards with identifiers attached.

Questionnaires

Not applicable

Specimen collection, handling, and analysis

The Michigan Department of Community Health will select cards to be sent to CDC and will ensure no identifiers are present. The samples will be shipped via overnight delivery to CDC without ice or dry ice. CDC personnel will keep samples in -20C freezers until analyzed. Samples will be analyzed using a modification of the method of Sjodin et al (2004). No genetic testing will be performed.

Human Subject Protection

Because the samples are old and archived, the study cannot be practically carried out if we must seek informed consent for the feasibility study. In addition, since the samples will be anonymized and double-blinded, no breach of confidentiality is expected. In fact, if the participants were reconsented, the consent documents would be the only records linking the participants with the study data thus increasing the risk of breach of confidentiality. Furthermore, since the samples have already been collected, the study poses no more than minimal risk to the participants. Thus, we seek a waiver of informed consent according to sections 46.117 (c) (1) of 45 CFR.

Disposition of Samples

Following satisfactory analysis of chemical metabolites specified in the protocol, samples will be returned to Michigan Department of Community Health or discarded at their request.

Data Security

No identifiers will be present to identify the participant. To ensure that Michigan does not retain identifiers to later link to participants, we will randomize and relabel the samples upon receipt at CDC so that Michican Department of Community Health cannot relink the samples. Data will be kept in password-protected computers.

Data handling and analysis

All data, including lab results, will be maintained by CDC. Statistical data analysis and interpretation will be provided by CDC using SAS 9.0. These data will be shared collectively and individually with the Michigan Department of Community Health and, if the Michigan Department of Community Health deems necessary, with the Pine River Task Force.

Handling of unexpected or adverse events

Because samples are anonymized, we expect no adverse events. The only foreseen adverse event would be the unintentional loss of a sample in the analytical process. In this instance, we may request an additional sample or use the remaining

samples for our feasibility study. We have taken sufficient precautions to avoid breech of confidentiality.

Dissemination, notification, and reporting of results

These data will be shared collectively and individually with the Michigan Department of Community Health and, if Michigan Department of Community Health deems necessary, with the Pine River Task Force. If the study is successful, we intend to publish the information obtained from these analyses in a peer-reviewed journal. The information may also be presented at professional conferences. We anticipate that the results of this study may also garner local media attention in Michigan.

Reference List

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