Michigan Dioxin Exposure Study: Planning Phase and Protocol Development

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Introduction

The University of Michigan has been commissioned to conduct one of the largest environmental epidemiology studies (700 residents) of dioxin exposure among the population of Michigan to describe the pattern of serum dioxin levels among adults and to understand the factors that explain variation in serum dioxin levels. The study is being undertaken (2004-2006) in response to concerns among the population of Midland and Saginaw Counties that dioxins from the Dow Chemical Company facilities in Midland have resulted in contamination of areas of the City of Midland and have contaminated the sediments in the Tittabawassee River flood plain (Figure 1). There is concern that body burdens of dioxins are elevated because of environmental contamination. The appropriate way to respond to these concerns is to measure the serum dioxin levels in a probability sample of the population in the region and to estimate each individual's past exposure to various factors that are believed to contribute to the body burden of dioxins. By measuring factors that reflect potential exposure to dioxins through air, water, soil, food intake, occupations, and various recreational activities, we can identify the factors that correlate with (and explain variation in) serum dioxin levels. The central goal of the study is to determine which factors explain variation in serum dioxin levels, and to quantify how much variation each factor explains. This paper provides information on the planning phase, study scope and objectives.

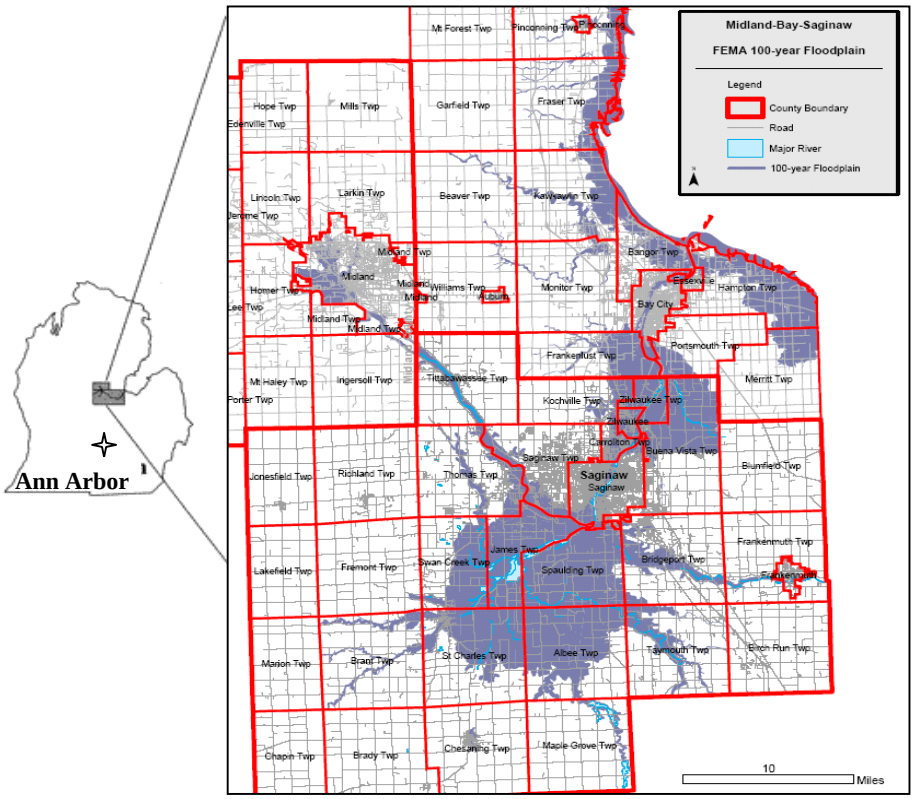

Figure 1. Topographical map of Michigan, indicating the Tittabawassee River floodplain and surrounding counties.

Materials and Methods

Study Population Selection: Three populations will be selected: (i) Residents of the Tittabawassee River floodplain between the Dow facility in Midland and the Center Street bridge (approx.) in Saginaw (350 individuals); (ii) Residents of Saginaw County, Midland County, and part of Bay County who do not reside in the floodplains of the Tittabawassee or Saginaw Rivers or the confluence floodplain of the Shiawassee River (175 individuals); (iii) Residents of Michigan outside of Saginaw and Midland counties (in Calhoun and Jackson counties west of Ann Arbor (175 residents). The population that resides in the Tittabawassee River floodplain between the Dow facility in Midland and the Center Street bridge in Saginaw is of interest due to elevated levels downstream from Midland. Upstream from Midland, the concentration levels in sediments are in the range of 2-9 ppt TEQ, a typical background seen in Michigan. Residential properties that are within census tracts that are wholly or partially within the Federal Emergency Management Administration (FEMA) 100-year flood plain will be eligible for inclusion.

Population Sampling: All three populations will be sampled using a two-stage area probability sample design. The first stage will sample U.S. 2000 Census blocks using probability proportionate to size selection. The second stage will select households using probabilities inversely

proportionate to size. This design yields an equal chance of selection for households across the two stages. Within each sample household, a roster of eligible household members will be prepared, and one eligible household member will be selected at random. In order to be eligible for participation in the survey, subjects must be age 18 or older and must have lived in the residence at least five years. They must also meet the eligibility criteria for having their blood drawn. Each subject who is selected will be asked to complete informed consent documents and will be asked to participate in an interview, blood draw, sampling of dust from their home, and sampling of soil from the property on which they reside.

Blood Sampling: Each participant will be asked to give an 80 mL sample of blood. Blood samples will be collected and handled by a mobile phlebotomy service. Blood will be allowed to clot, will be centrifuged and the serum will be decanted. Serum will be frozen at -70°C and will be shipped on dry ice to the analytic laboratory.

House Dust Sampling: Dust sampling of both hard surfaces and soft surfaces will be conducted in the home of each respondent. Soft surface vacuum dust sampling will be based, with minor modifications, on the US EPA/ERT Standard Operating Procedure. The household soft surface dust sample will consist of two stations taken from household locations that present the highest potential for human contact with household dust and dirt. One station will be located in a frequently occupied living or family room and the other station will be located in the respondent's bedroom. The sample from each station will be obtained from multiple side-by-side specific areas. A 1 m^2 template will be used to determine and mark each specific area. A minimum of 10 grams of total sieved dust will be needed to yield an analytical detection limit of 1 part per trillion (ppt).

Soil Sampling: Each property will be sampled in multiple locations using a push core sampler that will collect a core of soil from the surface to 6 inches depth. Surface vegetation at the site of the core will also be collected. Selection of locations for sampling will follow a protocol that will identify the house perimeter, property areas where skin contact is likely, and areas in the flood plain of the Tittabawassee River (Figure 2).

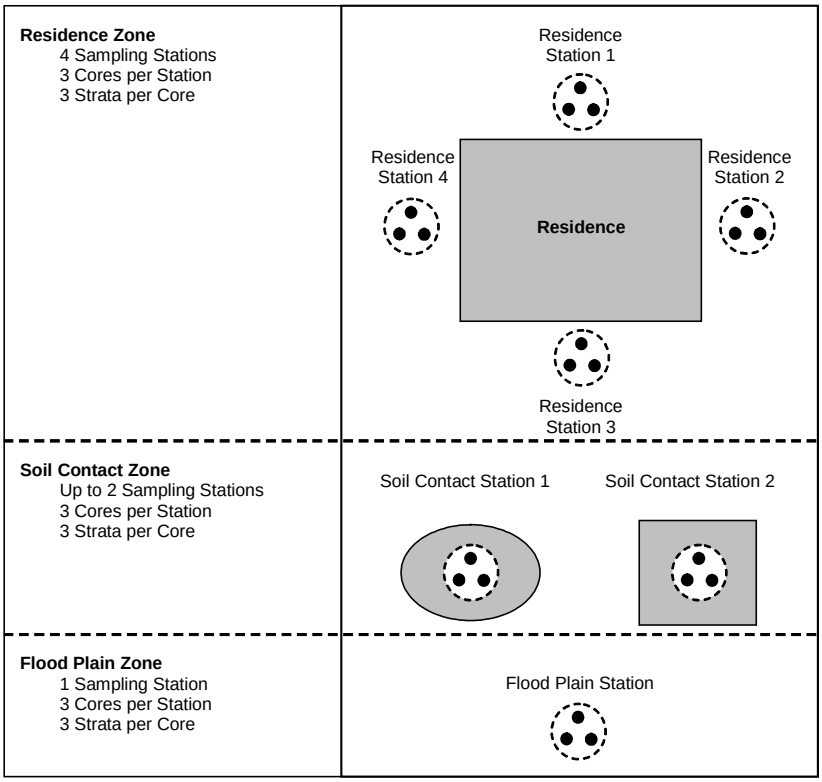

Figure 2. Soil Sampling Locations.

Each sampling station will be defined by laying out a 5' diameter sampling ring. Three equally spaced cores around the ring will be collected using single-use Lexan push samplers or stainless steel push samplers with butyrate liners, depending on the soil conditions. The samplers will allow for direct sample collection in the tube, in situ sealing of the tube, and minimization of cross-contamination between samples. All sealed sample cores will be stored on ice (4°C). The soil type, unusual characteristics of the sample location, and time of collection will be recorded and sample location coordinates will be established using global positioning system (GPS) procedures. The sealed Lexan tubes will be sliced open and the core will be separated into two strata: the top 2.5 cm and bottom 10 cm. If vegetation (grass) is evident, the leaf cover and roots will be separated from the top 1" stratum using a wrist-action mechanical shaker. The three strata (vegetation, top 1 inch, and bottom 5 inches) of each soil set (residence perimeter, soil contact, or flood plain) will be combined and homogenized.

Analytical Methods: Analyses of serum and house dust analytes will be performed for the 29 dioxins, furans, and PCBs for which the consensus toxic equivalency factors (TEF) have been published[1]. Soil analyses will follow a decision sequence that is designed to maximize the information gathered from samples that are above background dioxin concentration while minimizing the analysis of samples that are below background (Figure 3). It is believed that aerosol

deposition is the principal route of dioxin accumulation on properties in Calhoun and Jackson Counties and on most properties outside of the floodplain in Midland and Saginaw Counties. The dioxin content of the top 2.5 cm of soil will be used to characterize this deposition. Exposure to dioxin contaminated soils in these regions is also most likely to involve the surficial soils and it is therefore appropriate to characterize the dioxin content of the surface stratum from each property. If the residence is outside of the flood plain and the composite sample is below a trigger value of 8 ppt TEQ, then no further analyses will be done on the samples from that residence. If any part of the residence is inside the flood plain or the house perimeter composite sample is above 8 ppt TEQ, then each stratum in each soil set from that property will be assayed using the AhR in vitro bioassay. Based on these bioassays, the sample with the highest TEQ will be identified and will be analyzed using HRGC/HRMS to measure the congener specific concentrations. The 8 ppt value represents the 75th percentile of concentrations considered background in the Lower Peninsula of Michigan, and will allow for detection of a four-fold dilution of the maximum background level (35 ppt TEQ).

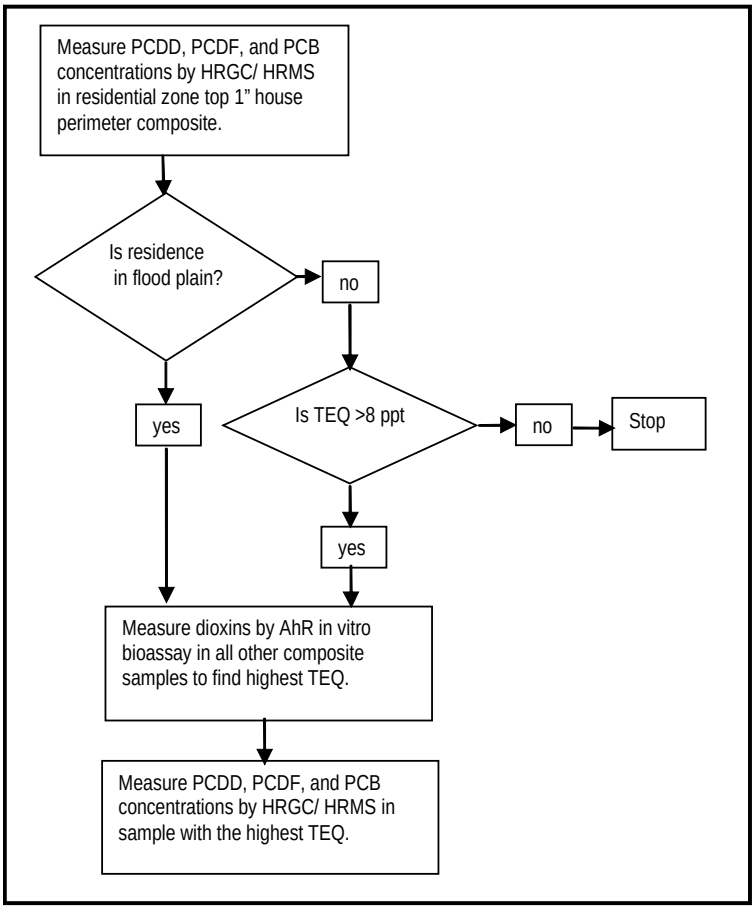

Figure 3. Analytical Soil Dioxin Protocol.

A screening assay will be used to identify samples that have dioxin TEQs above background, based on measuring AhR-mediated activity in in-vitro H4IIE-luc recombinant cells[2]. Limits of detection (LOD) are now typically 1 ± 0.5 pg TEQ/g dry sediment, and sample throughput is approximately two weeks or less[2]. This assay performs well in detecting dioxin-like activity in soils and sediments, and exhibits a high correlation (r2 = 0.94) with instrumentally-measured TEQs. Even though there is no current EPA approved protocol for immunoassay-based characterization of dioxins in sediments, a framework for application of the TEQ methodology for dioxins,furans, and PCBs in ecological risk assessment is currently in draft form[3]. It is anticipated that in excess of 10,000 soil cores will be collected during this study. After stratification and homogenization, it is expected that up to 4000 samples will be screened using the AhR-bioassay, and that up to 1800 soil samples will be analyzed isomer-specifically using HRGC-HRMS.

Statistical Analysis:
The analysis is aimed at exploring three objectives: (i) associations between serum dioxin levels and factors including soil, dust, occupation, and demographics; (ii) distribution of serum dioxin levels by region and risk profile; (iii) congener clustering using principal component analysis (PCA). Considering the prevalence of left-centered data (concentrations below LOD), SAS Proc Lifereg, a statistical software module for censored data, fits regression models with the option of seven different parametric error distributions (Weibull, lognormal, loglogistic, gamma, extreme value, normal, and logistic) will be used. It is likely that at least one of these distributions will provide a good fit to the data. This procedure will test continuous or categorical covariates, providing likelihood-based tests and estimates. For each continuous covariate (such as soil concentration), the functional form of the relationship with serum will be explored. Model fits will be examined using residual plots and influence diagnostics. In addition, adjustment for non-response will be performed to the extent possible using propensity weights, which are predicted probabilities of non-response based on a logistic regression model using limited demographic data from non-responders.

In the range of dioxin blood levels expected (0-20 ppt), the median blood level can be estimated within 3 ppt TEQ, and for a given dioxin level such as 50 ppt, P (a blood dioxin > 50) can be estimated with a 95% confidence interval of width +/- 0.03. The statistical analysis has over 90% power to detect a 1.8-fold difference in dioxin levels between exposed and referent groups. Furthermore, for testing the effect of any dichotomous covariate, such as fish consumption, on blood dioxin levels, if the proportion in each group is approximately 0.5, we will have 96% power to detect a 1.4-fold difference between groups.

Results and Discussion
Protocol Development and Stakeholder Outreach: The study protocol as detailed earlier is currently under review by all stakeholders involved in the project. The stakeholders include: The Dow Chemical Company, the Michigan Departments of Environmental Quality (M-DEQ) and Community Health (M-DCH), the Agency for Toxic Substances and Disease Registry (ATSDR) in Atlanta, as well as regional (Ecology Center) and local (Lone Tree Council) citizen groups. Residents and public health professionals in the Tittabawassee River area have a great interest in the design and execution of this study. The research team is committed to proactive community engagement in the design and implementation of the study. Communications with the population of Midland, Saginaw, and Bay Counties for the purposes of soliciting input on their concerns

regarding dioxin contamination in their environment, designing a scientific study that will help to address these concerns, providing reliable and valid scientific evidence that is responsive to their concerns, and explaining what the scientific evidence means and how it addresses the concerns of the affected population are central to the conduct of this research.

Development of Sample Populations and Survey

The first stage of the population sampling design using US 2000 census blocks has been completed for the floodplain residents and regional control. We have further selected the Michigan control population residing in Calhoun and Jackson counties based on demographics that are closely matched to those of Midland and Saginaw counties. For the regional control group (estimated at 175 residents), the following considerations were made in the power calculations to estimate the population sample size: Occupancy rate (estimated from ongoing surveys: 91% housing units); Screening cooperation rate (estimated from past surveys: 95% households); Eligibility rate (estimated from Census: 55% of persons); Response rate (estimated from past surveys: 75% of eligible persons); Serum collection rate (estimated from other surveys: 55% of eligible persons completing an interview); Replicate sample also being selected to allow further adjustment, in case assumptions are incorrect. The final sample size will be 1,070 households to obtain target completed interviews.

A pilot test has been conducted using the survey questions on 25 respondents, including (i) Workers with potential dioxin exposure; (ii) Residents of flood plain; (iii) Residents of non-flood plain areas. A "cognitive interviewing" approach was used to yield qualitative data about respondents' question comprehension, recall and reporting. This information was used to fine-tune the questionnaire and improve accuracy of responses.

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