The University of Michigan Dioxin Exposure Study

Study Protocol

David H. Garabrant, MD, MPH
University of Michigan School of Public Health

Alfred Franzblau, MD
University of Michigan School of Public Health

Brenda Gillespie, PhD
University of Michigan School of Public Health and
Center for Statistical Consulting and Research

Xihong Lin, PhD
University of Michigan School of Public Health

James Lepkowski, PhD
University of Michigan Institute for Social Research

Peter Adriaens, PhD
University of Michigan College of Engineering

Avery Demond, PhD
University of Michigan College of Engineering

January, 2005 REVISED

Table of Contents

1.0 Introduction and Study Goals

This is the protocol for a study of dioxin, furan and coplanar PCB exposure among the population of Michigan to describe the pattern of serum dioxin, furan and PCB levels among adults and to understand the factors that explain variation in serum dioxin, furan and PCB levels. The study is being undertaken in response to concerns among the population of Midland and Saginaw Counties that dioxin-like compounds 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. There is concern that people's body burdens of dioxins, furans and PCBs are elevated because of environmental contamination. The appropriate way to respond to these concerns is to measure the serum levels in a random 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 dioxin-like compounds. By measuring factors that reflect potential exposure to dioxins, furans and co-planar PCBs through air, water, soil, food intake, occupations, and various recreational activities, we can identify the factors that correlate with (and explain variation in) serum congener levels. A central goal of the study is to determine which factors explain variation in serum congener levels, and to quantify how much variation each factor explains.

The study will include populations who live in Midland County, Saginaw County, and part of Bay County, both in and out of the Tittabawassee River flood plain; and who live in a region of Michigan (Jackson and Calhoun Counties) that has no known industrial sources of dioxins (see Appendix 1 for maps of the state of Michigan and of Midland, Saginaw, and Bay Counties). By studying these two populations we believe it will be possible to understand whether the serum dioxin, furan and PCB levels among people who live in the Tittabawassee River flood plain are different than levels among similar people who live in the same region of Michigan, and whether they are different than levels among people who live in other parts of Michigan.

An additional central goal of the study is to communicate the results and the implications of the results in an effective manner to the population in the Saginaw and Midland region. The study is intended to solicit input from the affected population, to be responsive to their questions, to provide reliable and valid answers, and to explain those answers in a manner that is desired by the population. The findings of the study will be communicated to the public in a series of open forums, mailing, and a website. Additionally, public comment will be sought through a Community Advisory Board.

The goals of the study are:

1 To communicate with the population of Midland, Saginaw, and Bay Counties for the purposes of
   - Soliciting input on their concerns regarding dioxin, furan and PCB contamination in their environment
   - Designing a scientific study that will help to address these concerns
   - Providing reliable scientific evidence that is responsive to their concerns

- Explaining what the scientific evidence means and how it addresses the concerns of the affected population

2 To select random samples of four populations:
   - Residents of Midland and Saginaw counties who reside in the flood plain of the Tittabawassee between the Dow Chemical plant in Midland and the confluence of the Tittabawassee and Shiawassee Rivers in Saginaw.
   - Residents of Midland, Saginaw, and Bay counties who reside in census blocks adjacent to the flood plain of the Tittabawassee River between the Dow Chemical plant in Midland and the confluence of the Tittabawassee and Shiawassee Rivers in Saginaw.
   - Residents of Midland, Saginaw and Bay counties who do not reside in the flood plain of the Tittabawassee or Saginaw Rivers or in the confluence flood plain of the Shiawassee River.
   - Residents of Jackson and Calhoun counties, Michigan.

3 To collect the following data from each participant:
   - Responses to a personal interview
   - Measurements of 29 specific congeners of dioxins, furans, and co-planar PCBs, in
     - Serum
     - House dust
     - Soil

4 To explain the variability in serum dioxins, furans and PCBs (both specific congeners and TEQ) as a function of:
   - Soil congener concentrations and measures of soil contact
   - House dust congener concentrations
   - Proximity and duration of residence to
     - The Tittabawassee River
     - The Dow facilities in Midland
   - Consumption of fish and game from the Tittabawassee River and flood plain
   - Consumption of food grown or raised in the Tittabawassee River flood plain and region
   - Past occupations
   - Other factors (age, sex, race, diet, etc.)

This study is not intended to address potential adverse health effects of exposures to dioxin-like chemicals. We believe that studies of health effects should not be done unless there is evidence of excessive exposure that has resulted in elevated tissue burdens in the exposed population. This study is also not intended to provide information on the geographic distribution of dioxins, furans and PCBs in Midland and Saginaw Counties or address potential economic consequences of dioxin exposures.

2.0 Scientific Advisory Board

The investigators will report to a Scientific Advisory Board (SAB) which will oversee all aspects of the study conduct. The University of Michigan investigators will have no duty to report study data or results to the Dow Chemical Company. No study data or results will be provided to the Dow Chemical Company in any form unless it is made publicly available in the same form. The SAB will report on the study progress to the Dow Chemical Company. It is anticipated that the SAB will:

- Review and comment on the draft study design. This review was completed in July 2004. The investigators responded to comments and recommendations by the SAB prior to the start of the data collection in October 2004.
- Convene in person in Michigan (either Ann Arbor or in the Midland/Saginaw area) twice yearly for 1-2 days each time. It is anticipated that each meeting will include a session with the UMDES investigators, a session with the Michigan Department of Community Health and the entities that petitioned the ATSDR for a health consultation on the dioxin issue in Midland county, a session with a representative(s) of the Community Advisory Panel, an executive session, and other sessions the SAB deems necessary. Minutes of these meetings will be kept and made public. The executive session may be exempted from keeping minutes.
- Monitor the conduct of the UMDES. The SAB may request periodic written progress reports from the investigators.
- Provide feedback to the investigators regarding the conduct of the UMDES. It is critical to the public perception of the UMDES that the SAB provide a rigorous and fair assessment of the conduct of the research.
- Decide under what circumstances and with what frequency it will meet with involved parties such as local, state and federal agencies, community groups, the Dow Chemical Company, and other stakeholders. It is important that there will be a forum where stakeholders' questions and concerns regarding the scientific conduct of the UMDES can be addressed.
- Review and comment on draft reports from the investigators before they are released to the public.
- Release final reports on the UMDES to the public.

The University of Michigan appointed the Scientific Advisory Board, with membership based on independence, qualifications in research relevant to the dioxin issues, and scientific stature. The investigators solicited nominations to the SAB from interested stakeholders, recognized scientific organizations, and from colleagues who are knowledgeable in areas relevant to dioxin exposure studies.

As is customary with academic research, the University of Michigan retains the right to conduct this research and report the results in the open scientific literature. The SAB will function in an advisory capacity to the investigators. The SAB has the right to comment on and issue reports regarding the UMDES, including dissenting opinions and criticisms. In instances where the SAB recommends that modifications to the conduct, analyses, or reporting of the UMDES is needed, the investigators will either adopt the recommendations of the SAB or will respond in writing to the SAB, indicating their reasons for disagreement. The goal is to encourage open dialogue between the SAB and the UMDES investigators.

3.0 Communications Plan and Community Advisory Panel

Fundamentally, potential exposure to toxins such as dioxins is a public health concern. 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 and the population of Jackson and Calhoun Counties for the purposes of soliciting input on their concerns regarding dioxin, furan and PCB 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. There are five key areas of the research team's community outreach efforts.

- We will conduct research designed to clarify the concerns of the community and to identify key community resources and leaders. Data collection will include focus groups and key-man interviews. This research will solicit information on concerns within the community and the prevalence of those concerns. This will allow us to identify areas that can be addressed by the study team and will guide us in our interactions with the community.
- We will develop a broad audience outreach/educational campaign to describe the efforts of the research team and provide critical information to the public. The campaign will involve media resources, direct mailing, and web site development.
- We will perform outreach to targeted groups. Targeted audiences include area physicians; elected officials; public health officials and key community leaders.
- We will develop effective messages to all audiences. These messages are anticipated to include descriptions of the research study, periodic updates on study progress, findings from the study as they are available for release, and interpretation of the findings. The investigators will meet with community members to discuss results and answer questions.
- We will form a Community Advisory Panel (CAP) for Midland, Saginaw, and Bay Counties and for Jackson and Calhoun Counties with membership based on independence, representation of community groups, and stature and respect in the community. Each Community Advisory Panel will provide feedback to the investigators regarding the concerns of the community and inform the community about the conduct and progress of the study.

We also plan to report the results of the UMDES to participants. Individual participants will be given the results of their tests (if they wish to receive them) by mail. Aggregate data will be presented in scientific reports which will be peer-reviewed by the SAB. Scientific reports that have been reviewed by the SAB will be posted to a website which will be publicly available.

4.0 Research Plan

4.1 Study Population

Four populations will be identified for study and a random sample of approximately 175 adults from each of these populations who have lived in their household for at least five years will be selected. The four populations are:

- Residents of Midland and Saginaw Counties who reside in the flood plain of the Tittabawassee River between the Dow Chemical plant in Midland and the confluence of the Tittabawassee and Shiawassee rivers in Saginaw.
- Residents of Midland, Saginaw, and Bay Counties who reside in census blocks adjacent to the flood plain of the Tittabawassee River between the Dow Chemical plant in Midland and the confluence of the Tittabawassee and Shiawassee Rivers in Saginaw.
- Residents of Midland, Saginaw and Bay Counties who do not reside in the flood plain of the Tittabawassee or Saginaw Rivers or the confluence flood plain of the Shiawassee River.
- Residents of Jackson and Calhoun Counties, Michigan.

The population that resides in the Tittabawassee River flood plain between the Dow Chemical plant in Midland and the confluence of the Tittabawassee and Saginaw rivers in Saginaw is of interest because it is believed that contamination of the Tittabawassee River flood plain by dioxin-like compounds is appreciable downstream and is not appreciable upstream of the Dow plant in Midland. Soil samples collected by the Michigan Department of Environmental Quality (MDEQ) in the sediments of the Chippewa River, Pine River, and Tittabawassee River above Midland, Michigan are in the range of 2-9 ppt TEQ. These levels are believed to be in the background range typically seen in many areas of Michigan. Sediment samples below Midland have highly variable dioxin, furan and PCB levels indicating contamination from Midland downstream to Saginaw. The choice of the confluence of the Tittabawassee and Shiawassee rivers in Saginaw as the lower end of the flood plain is based on information from the MDEQ that flood waters from the Shiawassee River do not travel upstream in the Tittabawassee River beyond this point. In addition, the congener profiles of soils from the north side of the Tittabawassee River at the confluence are similar to those found on the Tittabawassee River upstream of the confluence. Thus, sediments upstream of this point are believed to represent contaminants that have come downstream and are unlikely to be combined with contaminants that have originated from other sources. The boundaries of the eligible population north of the Tittabawassee River will be the FEMA 100-year flood plain from the Dow plant in Midland to the confluence of the Tittabawassee and Shiawassee rivers. The boundaries of the eligible population south of the Tittabawassee River will be the area bounded by South Center Road, Stroebel Road, River Road, Gratiot Road, and the FEMA 100-year flood plain from Gratiot Road to Midland, ending at the Dow plant. Residential properties that are within census blocks that are wholly or partially within the FEMA 100-year flood plain will be eligible for inclusion.

The population that resides in census blocks adjacent to the Tittabawassee River flood plain is of interest because it is believed that soils from the flood plain have been transported and used as fill in areas near the Tittabawassee River. In addition, it is believed that residents who live near the flood plain are likely to use and be exposed to soils and sediments from the flood

plain. Residential properties that are in census blocks adjacent to the FEMA 100-year flood plain will be eligible for inclusion.

The sub-population that resides in Saginaw County, Midland County, and part of Bay County who do not reside in the flood plains of the Tittabawassee or Saginaw Rivers or the confluence flood plain of the Shiawassee River was chosen to provide a comparison group that is believed to have had the opportunity for dioxin exposure that is typical for residents of this region of Michigan. This population is believed to represent a range of dioxin, furan and PCB exposures including those who have background exposures that are typical of Michigan residents, those whose properties may have received fill dirt from the flood plain, those who have worked at Dow, those who recreate in the flood plains of the local rivers, those who eat sport fish from the local rivers, and those who live in areas downwind of Dow operations. The eligible population will be residents of Saginaw County and Midland County who reside on properties that are outside of the FEMA 100-year flood plain of the Tittabawassee River (below the point where the Chippewa River joins), the Saginaw River and its tributaries (Shiawassee, Flint, and Cass Rivers), and Saginaw Bay. In addition, the southwest corner of Bay County (Williams Township and City of Auburn) will also be included in this population because these areas are geographically close to the Tittabawassee River (see Appendix 1). It is anticipated that 10% of the participants will reside in the City of Midland, 18% in the City of Saginaw, and the remaining 72% in the surrounding areas of Saginaw County, Midland County, and the eligible part of Bay County.

The population of Michigan outside of Midland and Saginaw Counties will be chosen from Jackson and Calhoun Counties. These counties are similar to Midland and Saginaw Counties in terms of age, sex, and race distribution, proportion employed in manufacturing industries, a comparable mix of urban and rural communities, and access to rivers and lakes for recreational use (see Appendix 2). The purpose of including this population is to provide a referent group that is believed to have had background dioxin, furan and PCB exposures that are typical for residents of Michigan who live in areas that are not believed to be contaminated by dioxin-like compounds from Dow. There are no advisories against consuming sport fish related to dioxin contamination in these counties (see Appendix 3).

All four populations will be sampled using a two-stage area probability household sample design. In the first stage, U.S. 2000 Census blocks will be selected using probabilities proportionate to the number of households in the block. 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. The random selection of a single eligible adult in each household will yield a sample that over-represents persons living in households with fewer eligible persons. This over-representation will be compensated in analysis using survey design weights. In order to be eligible for participation in the survey, subjects must be age 18 years or older and must have lived in the residence at least five years.

Each participant will be compensated $100 for participation, to be paid in parts. Sixty dollars will be paid upon completion of the interview and agreement to the blood collection, $20 upon completion of the dust collection, and $20 upon completion of the soil collection.

4.2 Interviews

Each subject who is selected will be asked to complete informed consent documents and will be asked to participate in an interview and blood draw. If the respondent is the owner or co-owner of the residence, they will also be asked to permit sampling of dust from their home and sampling of soil from the property on which they reside. The interview questionnaire is in Appendix 4. The interview will be on average one hour long, conducted in-person, and solicit information regarding

- Residential history
- Activities involving soil contact including gardening
- Household pets
- Activities on the Tittabawassee River, Saginaw River, Saginaw Bay, and Kalamazoo River below the Morrow Pond dam
- Activities in the flood plain
- Work at Dow Chemical (self and persons they have lived with)
- Occupations with exposure to dioxins, furans and PCBs as well as other exposure opportunities
- Consumption of fish
  - From the Tittabawassee River, Saginaw River, Saginaw Bay, and Kalamazoo River below the Morrow Pond dam
  - Store bought
  - From other areas
- Consumption of meat and other foods
  - Game from the Tittabawassee River, Saginaw River, Saginaw Bay, and Kalamazoo River below the Morrow Pond dam
  - Store bought
  - From other areas
- Consumption of vegetables and fruits raised on contaminated properties
- Pregnancy and nursing history
- Remediation activities on the property and in the home

Interviews will be conducted in-person, using pre-printed interview forms or computer-assisted interviews (CAI). Interview responses from preprinted forms will be computerized on a batch basis. Interview responses from computer assisted interviews will be computerized automatically at the time of the interview. Interviewers will transmit respondent information and consent completion information via a secured method to the University of Michigan daily, and will meet or speak weekly with data collection supervisors to review contact patterns, data collection progress and edit completed hard copy interviews. Data collected by interviewers will also be used to determine the patterns of non-response and their resulting biases (see Appendix 5).

4.3 Blood Collection and Analysis

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 -20°C and will be shipped on dry ice to the analytic laboratory (see Appendix 6 for protocol). Analyses will be performed for the 29 dioxins, furans, and PCBs for which the consensus toxic equivalency factors (TEF) have been published (Van den Berg et al, 1998). These are listed in Table 1.

Table 1. World Health Organization toxic equivalency factors for humans and Alta Analytical Laboratory's expected limits of detection for blood and serum[1], soil, and house dust

Congener	TEF	LOD, Serum: Whole Weight (pg/g)[2]	LOD, Serum: Lipid Adjusted (pg/g-lipid)[3]	LOD, Dust and Soil (pg/g)
2,3,7,8-TCDD	1	0.004	0.80	0.50
1,2,3,7,8-PentaCDD	1	0.004	0.80	2.5
1,2,3,4,7,8-HexaCDD	0.1	0.02	4.0	2.5
1,2,3,6,7,8-HexaCDD	0.1	0.02	4.0	2.5
1,2,3,7,8,9-HexaCDD	0.1	0.02	4.0	2.5
1,2,3,4,6,7,8-HeptaCDD	0.01	0.02	4.0	2.5
OctaCDD	0.0001	0.04	8.0	5.0
2,3,7,8-TetraCDF	0.1	0.004	0.80	0.50
1,2,3,7,8-PentaCDF	0.05	0.004	0.80	2.5
2,3,4,7,8-PentaCDF	0.5	0.004	0.80	2.5
1,2,3,4,7,8-HexaCDF	0.1	0.02	4.0	2.5
1,2,3,6,7,8-HexaCDF	0.1	0.02	4.0	2.5
1,2,3,7,8,9-HexaCDF	0.1	0.02	4.0	2.5
2,3,4,6,7,8-HexaCDF	0.1	0.02	4.0	2.5
1,2,3,4,6,7,8-HeptaCDF	0.01	0.02	4.0	2.5
1,2,3,4,7,8,9-HeptaCDF	0.01	0.02	4.0	2.5
OctaCDF	0.0001	0.04	8.0	5.0
3,4,4′,5-TetraCB (81)	0.0001	0.016	3.2	0.50
3,3′,4,4′-TetraCB (77)	0.0001	0.016	3.2	0.50
3,3′,4,4′,5-PentaCB (126)	0.1	0.016	3.2	0.50
3,3′,4,4′,5,5′-HexaCB (169)	0.01	0.016	3.2	0.50
2,3,3′,4,4′-PentaCB (105)	0.0001	0.016	3.2	0.50
2,3,4,4′,5-PentaCB (114)	0.0005	0.016	3.2	0.50
2,3′,4,4′,5-PentaCB (118)	0.0001	0.016	3.2	0.50
2′,3,4,4′,5-PentaCB (123)	0.0001	0.016	3.2	0.50
2,3,3′,4,4′,5-HexaCB (156)	0.0005	0.016	3.2	0.50
2,3,3′,4,4′,5′-HexaCB (157)	0.0005	0.016	3.2	0.50
2,3′,4,4′,5,5′-HexaCB (167)	0.00001	0.016	3.2	0.50
2,3,3′,4,4′,5,5′-HeptaCB (189)	0.0001	0.016	3.2	0.50

Abbreviations: TEF, toxic equivalency factor; CDD, chlorinated dibenzodioxins; CDF, chlorinated dibenzofurans; CB, chlorinated biphenyls; LOD, limit of detection.
[1]Assumes a 25 g starting sample weight.
[2]pg/g = picograms/gram, or parts per trillion (ppt).
[3]Assumes 0.5% lipids.

Serum dioxin, furan and coplanar PCB concentrations will be measured by Alta Analytical Laboratory of El Dorado Hills, CA using modified US EPA protocols 8290 (US EPA, 1994) and 1668 (US EPA, 1999) (see Appendix 10 for analytic protocols). Congeners will be extracted from serum, cleaned following a multi-column protocol and quantified using High

Resolution Mass Spectrometry (HRMS). Final analyte concentrations will be provided on both a whole weight and lipid-adjusted weight basis. Expected limits of detection, both for whole weight and lipid-adjusted weight congeners, are in Table 1. A turn-around time of three weeks is expected for results.

4.4 Dust Sampling and Analysis

Vacuum sampling will be conducted in the home of each respondent, following consent of the respondent, if the respondent is an owner of the residence. The sampling protocol will be based, with minor modifications, on the American Society for Testing and Materials (ASTM) method "Standard Practice for Collection of Floor Dust for Chemical Analysis" (ASTM, 2000; see Appendix 7). The household vacuum dust sample will be taken from two sampling locations that present the highest potential for human contact with household dust and dirt. The locations will be a frequently occupied living space (e.g., living or family room) and a high traffic hallway or pathway. Samples will be taken from both hard and soft surfaces and will not be taken of undisturbed dust in generally inaccessible areas.

The sample will be obtained from designated sampling areas within each sampling location. Two measuring tapes will be placed and taped down so that they are parallel to each other and on either side of each sampling area. A High Volume Small Surface Sampler (HVS3) will be used to collect the sample. The HVS3 is a vacuum cleaner equipped with a cyclone and a fine-particle filter capable of capturing 99.95% of particles above 0.3 μm aerodynamic mean diameter. The dust sampling technicians will attempt to collect a minimum of 10 grams of total dust in order to yield an analytical detection limit of 1 part per trillion (ppt). If the amount of dust collected from the initial sampling area within each location is not sufficient, secondary areas will be marked and sampled as needed. The total surface area of all of the sampling areas that make up each sampling location will be recorded on a pre-printed field data sheet (see Appendix 7), as well as the surface types from which the sample was taken. Samples will be transported on ice to a dedicated 4° C cooler until delivery to Alta Analytical Laboratory for analysis.

Analyses of the vacuum samples will be performed by Alta Analytical Laboratory for the 29 dioxin congeners (see Table 1) using US EPA methods 8290 (US EPA, 1994) and 1668 (US EPA, 1999) (see Appendix 10).

4.5 Soil Sampling and Analysis

Soil sampling will be conducted at each respondent's residence following the consent of the respondent, if the respondent is an owner of the residence. Each property will be sampled in multiple locations using a push core sampler that will collect a core of soil from the surface to about 6 inches depth. Surface vegetation at the site of the core will also be collected except in situations where garden plants may be damaged. 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 or near the flood plain of the Tittabawassee River. The location of sampling stations is portrayed in Figure 1.

The sampling stations will be located around the perimeter of the house (up to 4 stations around the residence) and where activities occur that are likely to result in skin contact with soil. These latter locations will be determined from the interview responses that indicate the soil contact activities of the participants and are anticipated to include vegetable gardens and flower gardens (2 stations maximum). For residences located in the flood plain, one additional station in the flood plain will be sampled. The flood plain station will be placed at the lowest, safely accessible location on the respondent's property in the direction of the river. There will be a maximum of seven sampling stations at each residence (4 house perimeter, 2 soil contact, 1 flood plain).

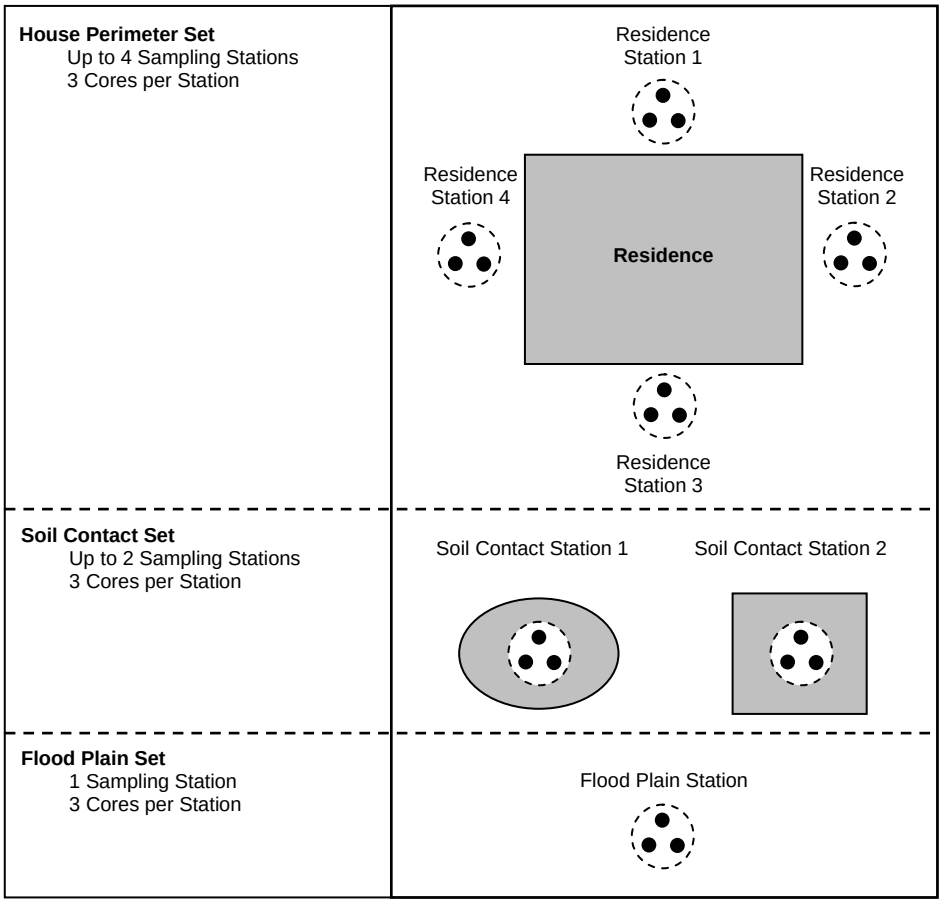


Figure 1. Soil sample locations

Each sampling station will be defined by laying out a 3-foot diameter sampling ring. Three equally spaced cores around the ring will be collected using single-use Lexan push samplers or stainless steel push samplers, depending on the soil conditions. The samplers will allow for direct sample collection in the tube, sealing of the tube, and minimization of cross-contamination between samples. All sealed sample cores will be stored on ice (4° C) before transport to the University of Michigan Environmental and Water Resources Engineering (EWRE) laboratories. The coring locations will be brought back to grade using commercial top soil. Time and date of collection, location of samples and location of combustion areas, for example, will be recorded as field notes (see Appendix 8). All sample location coordinates will

be established using global positioning system (GPS) procedures, for mapping purposes, and to relocate sample sites, if necessary.

The sealed Lexan or stainless steel tubes will be brought to a staging area in the EWRE laboratories, where they will be extruded. The cores from the house perimeter and the floodplain stations will be separated into two strata: the 0-1 inch and 1-6 inch. The cores from the soil contact stations will not be separated into strata. If vegetation (grass) is evident, the leaf cover and roots will be separated from the 0-1 inch stratum. The strata from each station will be first combined and homogenized and then the stratum composites will be combined to produce a set composite using the procedures described in Appendix 8. The collected vegetation will be composited separately from the soil. Ultimately, each residence will yield the following composite samples for analysis:

- House perimeter set 0-1 inch composite;
- House perimeter set 1-6 inch composite; and
- House perimeter set surface vegetation composite.

If there is a soil contact station or stations, the residence will yield the following additional samples:

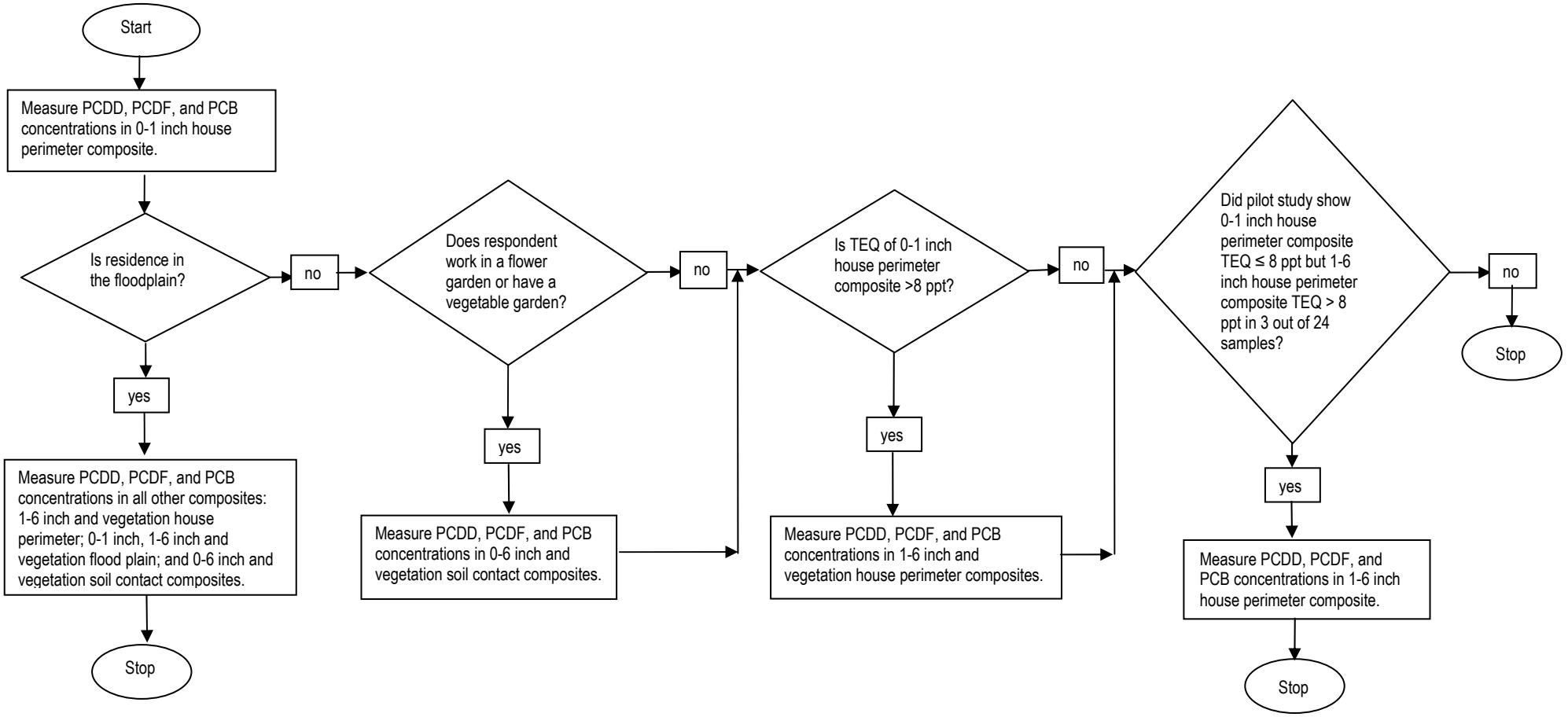
- Soil contact set 0-6 inch composite; and
- Soil contact set surface vegetation composite (if available).

In addition, residences in the Tittabawassee River flood plain will yield the following samples:

- Flood plain set 0-1 inch composite;
- Flood plain set 1-6 inch composite; and
- Flood plain set surface vegetation composite.

The soil samples will be archived in 4 oz dioxin-grade amber glass jars to avoid photolytic degradation, and stored in dedicated 4° C cold rooms prior to analysis. All samples that are subjected to analysis will be analyzed for the WHO 29 PCDD, PCDF and PCB congeners by Alta Analytical Laboratory using internal modifications of USEPA methods 8290 (US EPA, 1994) and 1668 (US EPA, 1999). The decision sequence of which samples will be analyzed is shown in Figure 2. The 0-1 inch house perimeter composite sample will be analyzed for all eligible and consented properties. If any part of the property is in the floodplain, then all remaining composites (1-6 inch and vegetation house perimeter; 0-1, 1-6 inch and vegetation floodplain; and 0-6 inch and vegetation soil contact) will also be submitted for analysis. If the respondent does not live in the flood plain, but has a vegetable garden, or works in a flower garden, the 0-6 inch and vegetation soil contact composites will be analyzed. If the TEQ of the 0-1 inch house perimeter composite for any property outside the floodplain is > 8 ppt, then the 1-6 inch and vegetation house perimeter composites will be analyzed. The trigger value of 8 ppt TEQ represent the 75^{th} percentile of the background distribution for the lower peninsula of Michigan (i.e., 25% of soil samples are expected to be above 8 ppt).

Figure 2: Soil and vegetation analytic sequence



It is assumed that the majority of the presence of dioxin-like compounds in the soil outside of the flood plain is due to atmospheric deposition. Therefore, it is assumed that on any property outside the flood plain where the 1-6 inch stratum contains an elevated level of dioxin-like compounds, the 0-1 inch stratum will be at least above the 8 ppt TEQ trigger value. To verify the assumption that if the 1-6 inch stratum contains elevated levels of dioxin-like compounds then the 0-1 inch stratum will also contain elevated levels of dioxin, a pilot study will be conducted on 24 residences in the Midland/Saginaw area outside of the flood plain. The following procedures will be employed in conducting the pilot study:

1. From the residences in the Midland/Saginaw area in which the 0-1 inch house perimeter composite yields a TEQ below the 8 ppt trigger:
   a) Twelve residences will be randomly selected where obvious fill activity has taken place.
   b) Twelve residences will be randomly selected where no obvious fill activity has taken place.
2. The 1-6 inch house perimeter composite will be submitted for congener specific chemical analysis from these 24 selected residences.

The residences in the Midland/Saginaw area will be targeted as there may be a greater likelihood of elevated subsurface concentrations of dioxin-like compounds in those areas because of the reputed use of Tittabawassee and Saginaw River flood plain sediment as fill material.

If more than 3 of the 24 1-6 inch composite samples yield a TEQ above the 8 ppt trigger, the 1-6 inch house perimeter composite from all the properties in the Midland/Saginaw area will be analyzed.

4.6 Incinerator Plume Dispersion Modeling

Dispersion modeling will be conducted using a probabilistic approach with the aim to predict census block-averaged soil concentrations of dioxin TEQ that may have resulted from atmospheric deposition of dioxins from incinerators in the study areas. These averaged concentrations will be generated based on spatial correlations between wet and dry deposition of particle concentration isopleths generated using a publicly available dispersion model. Since the available datasets for the Dow Chemical Plant incinerator are from the last two decades only, dispersion modeling for earlier incineration will be evaluated using model sensitivity analysis. For the incinerator located near Jackson, input information is available pertaining to operational characteristics, as well as stack-averaged TCDD-equivalent fluxes for 1988 and for 2004. However, no soil data are available. Dispersion models will test the influence of plume-deposition correlations to predict extent and census block averaged concentrations of dioxins (see Appendix 12).

4.7 Statistical Analyses Plan

4.7.1 Introduction

The primary goal of the study is to explain the variability in serum dioxins, furans and PCBs (both specific congeners and TEQ) as a function of soil congener concentrations; house dust congener concentrations; proximity and duration of residence to the Tittabawassee River and to the Dow facilities in Midland; consumption of fish, game, vegetables, and fruits from the Tittabawassee River and flood plain, past occupations, and other factors (age, sex, race, diet, etc.). A principal focus of the study is investigating whether known dioxin, furan and PCB contamination in the Tittabawassee River sediments downstream from the Dow Chemical plant are associated with elevated blood dioxin, furan and PCB levels in the regional population. Several statistical approaches, including descriptive measures and statistical modeling, will be used to address this question.

The sample being drawn is a probability sample of a well-defined population from five counties in Michigan. The eligibility criteria and geographic regions sampled have been precisely defined in previous sections of the protocol. Inference can only be made to the eligible population in the defined geographic regions. Both sampling weights and non-response weights will be available to adjust the sample results to represent the entire eligible population or any geographic subpopulation. When possible and appropriate, analyses will be performed using these weights. However, there are reasons to favor either weighted or unweighted analyses in different situations, and in some cases, both analyses will be performed. For example, estimating the proportion of the population with elevated blood levels will require sampling weights. These estimates will be computed for specific geographic regions as well as overall. In contrast, a sample-weighted regression analysis representing the entire population area, including the two reference counties, may have a relatively small proportion of the population with elevated blood levels. Because the weights are based on the number of people in the population that each sampled person represents, the sampling weights for the (over-sampled) flood plain area will be small compared to the sampling weights for the (under-sampled) group from Jackson or Calhoun Counties. In this case, using sampling weights could tend to diminish the estimated effects of covariates on blood dioxin levels in the overall population. For this reason, we will consider unweighted regression analyses in addition to the standard weighted analyses.

As discussed in the Power Calculation section below, we will sample 350 people from census blocks in or partially in the flood plain. Of these, we estimate that 175 households will be on property that contains land in the flood plain and 175 will be on property near but not in the flood plain. In addition, we will sample 175 people from households in Midland/Saginaw/Bay Counties outside of the flood plain census blocks, as described previously. If the samples do not include at least 30 participants in the region of the plume from the Dow incinerator (based on an estimated contour that includes ground concentrations of 75 ppt and higher), then additional random samples in this region will be drawn to yield approximately 30 participants. Finally, we will sample 175 people from Jackson/Calhoun Counties to serve as a reference population. We refer to these four subgroups respectively as Flood Plain (FP), NEAR FP, REGIONAL, and REFERENT. For comparisons made based on geographic region alone, we will consider the FP versus REFERENT to best represent the effect of elevated dioxin, furan or PCB levels from the Tittabawassee River. We will also compare NEAR FP versus REFERENT and REGIONAL versus REFERENT to investigate whether the contamination has affected the population beyond the immediate flood plain area.

Blood levels of dioxins, furans and PCBs, even in a referent population, tend to have a skewed distribution (Wittsiepe, 2000; Fingerhut, 1991). Skewness exists for congener-specific data as well as toxic equivalent dose (TEQ) (Wittsiepe, 2000). Based on this literature, we assume that blood levels in parts per trillion (ppt) (or picograms/gram, pg/g) will be expressed on the natural log scale to eliminate skewness. In addition, although standard statistical methods for testing differences between subgroups consider the central tendency of the distributions (using means or medians), we are also interested in estimating the upper percentiles of the blood level distribution in all regions. Thus, the analysis plan described below considers shifts in the mean or median congener and TEQ levels as well as estimation of upper percentiles.

All analyses will be performed for the dioxin TEQ as well as for each of the 29 specific congeners. However, primary interest will be in the TEQ and the specific congeners 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,4,7,8-PentaCDF, and PCB 126, which are the most likely to be related to local exposure.

4.7.2 Estimation of population distributions of blood congener levels

We will first estimate the distribution of blood congener levels in each subgroup (FP, NEAR FP, REGIONAL, and REFERENT) using the sample-weighted empirical cumulative distribution function. Although the lower end of the distribution may not be estimable due to the limit of detection (LOD), our major interest lies in the upper tail of the distribution. We have planned a large enough blood volume that the LOD for serum levels will be quite small, approximately 1 ppt for the dioxin and furan congeners of greatest interest. If blood volumes from different people vary, such that the LOD varies through the sample, then the distribution of blood levels will be estimated using a Kaplan-Meier type estimator for the case of left-censored data. When exposure measurements are below the LOD, such measurements are left-censored because the true measurement is less than the LOD (observed value).

Using the estimated cumulative distribution of blood congener levels, the population median and upper percentiles can be estimated. For each geographic subgroup, we will estimate the median blood level and the 80^{th}, 90^{th}, and 95^{th} percentiles with point estimates and 95% confidence intervals. We can still estimate the median if up to 50% of the sampled values are below the LOD. If we have sufficient data above the LOD to generate a nonparametric 95% confidence interval for the true median, we will prefer this method of estimation. Alternatively, we will use a parametric estimator of the median, assuming a lognormal distribution or other distribution that fits the data well. Although parametric estimation can be robust to model misspecification near the center of a distribution, it may be less reliable for estimating upper percentiles.

Because of the large number of distributions to be checked, we will summarize the results graphically with a "congener profile" for each geographic region. The x-axis will list the 29 congeners, and the y-axis will show the concentration. The profile could include the median, upper percentiles, and maximum. Comparing the "profile" for each population would show differences in the congener levels by location. A log scale will be used if skewness in the concentrations is encountered.

Confidence intervals (or bounds) for the true population percentiles will also be obtained, either non-parametrically or parametrically. For example, we can calculate an upper confidence limit (B) for the 90th percentile of the population, such that we are 95% confident that at least 90% of the population has blood dioxin-like compound levels less than B. A nonparametric confidence bound can be constructed based on an inversion of the point-wise bounds for the cumulative probability. That is, the upper confidence bound for $t_{(0.90)}$ is the value of t such that the lower confidence bound for the cumulative distribution function, F(t), equals 0.95. A parametric bound based on the normal distribution (after log transformation) will be of the form exp(mean+k*s), where "exp" is the exponentiation of a bound computed on the natural log (ln) scale, "mean" and "s" are the sample mean and standard deviation on the ln scale, and "k" is the scaling constant that gives the appropriate coverage probability (Odeh and Owen, 1980). For example, for the FP sample (n=175), the scaling constant for a 95% one-sided confidence bound for the 90^{th} percentile would be 1.463 (Odeh and Owen, Table 1.4.3, p. 32). A similar but philosophically different goal will be to calculate a 95% confidence bound on the proportion of people above a certain blood level, such as 25 ppt. We will use Table 7 from Odeh and Owen, 1980, for this purpose. All confidence bound procedures described above can be modified for the situation of survey sampling weights.

To test for unadjusted differences in blood levels between any two subgroups, we will employ graphical methods as well as both nonparametric and parametric tests. Graphical methods may include side-by-side sample-weighted histograms or box plots, and sample-weighted Q-Q plots of the distribution for one geographic subgroup versus another. Parametric tests will employ a left-censored version of a large-sample 2-group test, assuming an appropriate error distribution. A non-sample-weighted version of this test is carried out in the SAS Lifereg procedure (SAS Institute, 1999) with four different parametric distributions available (Weibull, lognormal, loglogistic, and gamma). Stata software will be used to perform a sample-weighted version using the svyintreg procedure, assuming a normal distribution of log-transformed values. Nonparametric tests will overcome any difficulties in finding an appropriate distribution, and can test for differences in the tails of the distributions as well as for location shifts between groups. To non-parametrically test for a location shift, we will employ a sample-weighted, left-censored version of the logrank test in Stata using the stcox procedure. To test for tail differences, we will employ the non-sample-weighted version of the Kolmogorov-Smirnov test.

4.7.3 Estimating the distributions of exposure variables

A descriptive analysis of exposure variables, such as household dust and soil dioxin/furan/PCB levels, will be provided. In particular, the mean, standard deviation and range of exposure values will be given for each major type of exposure for each of the four geographic regions. Tests for equality of these levels among the geographic regions will also be performed. Upper quantiles will be compared among regions in addition to comparisons of mean levels. These analyses will primarily be performed for the summary measures of each exposure category, although descriptions for individual components may be performed as warranted. In addition, correlation coefficients between summary exposure categories will be computed, and scatter plots will be checked for linearity of relationships. The correlations and scatter plots will

help assess the degree of collinearity among the exposure variables, which will be useful for interpreting the models discussed below.

4.7.4 Modeling of potential predictors of blood congener levels

Model choice. To test for potential effects of variables such as age, fish consumption, vegetable consumption, soil dioxin/furan/PCB levels, duration of residence in the flood plain, and gender on blood levels, regression models for left-censored data, both with and without survey sample weights, will be employed using the entire dataset and all geographic regions. Stata software will be used for the weighted models, and both Stata and SAS software for the unweighted models. One option to handle the left-censoring is to use Cox regression on reverse-scaled data, although care would be required in interpreting such models. Discrete-time survival models or ordinal logistic models would also be appropriate. However, if the data reasonably fit one of the four parametric distributions described above, parametric regression will provide the most powerful option. STATA software can fit normal or lognormal parametric, Cox, or discrete-scale models, using sampling weights to reflect the cluster sampling and non-response probabilities. These procedures will test continuous or categorical covariates, providing likelihood-based tests and estimates. In all cases, model fit will be evaluated. Although we prefer non-parametric methods above for estimating the median and upper percentiles of the distributions, for modeling we prefer parametric distributions if an appropriate fit can be found.

Goals and scope of modeling. The model chosen as described above will provide the framework for testing the primary hypothesis of this study: whether dioxin, furan and PCB blood levels in the population are related to elevated dioxin, furan and PCB levels in the Tittabawassee River sediments downstream from the Dow Chemical plant. In particular, one aim is to identify behaviors or environmental conditions associated with risk of blood level elevation. With this information, we could advise the public regarding ways to reduce risk, and we could identify sub-populations at potential risk for elevated blood levels. In either case, our goal is to identify individual covariate effects. Because of the large number of potential explanatory variables and the potential for confounding, collinearity, and interaction (effect modification), our modeling strategy will be described in some detail. A model will be developed for each congener separately, and also for the TEQ. It is possible that different activities impact different congener blood levels. Therefore, it will be important to consider each congener model as a unique and independent modeling effort. However, a large table will be prepared that summarizes the results from all congener models and the TEQ model. This summary table will list the 29 congeners plus the TEQ across the top (continuing across several pages), with the rows being the primary exposure variables. The entries will be the regression coefficients and standard errors, with significance indicated with the asterisk notation. We describe below the considerations and analysis steps for a single model.

Covariate adjustment. It is well known that blood levels of dioxin-like compounds are affected by a person's age, body mass index (BMI), and possibly weight loss or gain in the recent past. We will use the control population of Jackson/Calhoun counties to establish a model for background blood levels as a function of these known covariates. We will check the linearity of all continuous covariates, and include transformed or polynomial terms as needed, such as logarithms or squared terms. Gender, while not implicated in other studies, will also be tested.

The final model will be our best estimate of predicted blood levels of dioxin-like compounds in the presence of only background levels of such compounds. As shown in Figure 3, this model will form the basis for testing exposure variables in the Midland/Saginaw area, and all subsequent models will be adjusted in the same way for these variables.

Univariate and bivariate exploration. We first consider each potential covariate, its number of levels (if categorical) or range (if continuous). A small number in a category (such as race) may be pooled with other categories, and skewed distributions in continuous variables may be transformed, with the intent to improve linearity and reduce the influence of a few large values. To get a preliminary assessment of collinearity among independent variables, both a correlation matrix and a scatter plot matrix will be generated. As continuous variables are added to the model, we will assess whether the relationship with blood levels is linear or nonlinear. Any evidence for nonlinearity will be followed by attempts to linearize the relationship using transformation or otherwise fit an appropriate predictive function.

Model-building. As discussed above and shown in Figure 3, we will begin with the model that includes only demographic variables, built using the control population. Next, as a crude measure of exposure, we will test for blood level differences based on current residence in one of four regional subgroups (FP, NEAR FP, REGIONAL, and REFERENT). We will then construct sets of individual exposure variables using data from the life-history calendar to estimate overall past exposure in several exposure categories. We will consider the association between blood dioxin/furan/PCB levels and all of the following potential exposures (a) soil dioxin/furan/PCB levels in the yard, garden, and (if applicable) proximal flood plain (these will be included as individual variables and also as a property maximum value; when no soil is available, e.g., for apartment dwellers, the soil exposure level will be considered to be zero), (b) dioxin/furan/PCB levels in household dust, (c) whether the subject gardens/has soil contact activities (included as an interaction with garden soil congener levels), (d) whether the subject eats vegetables from the property (included as an interaction with garden soil congener levels), (e) whether the subject eats fish of specific varieties from the Tittabawassee River, (f) whether the subject consumes game killed in the local area, (g) whether the resident has owned pets that regularly went both inside and outside of the house, (h) whether the residence is in the plume from the former Dow incinerator, and (i) whether remediation activities have occurred within the past year in the home or on the property.

In general, the model building will use a strategy of hierarchical, backward elimination that considers variables carefully in a non-automated way. For exposure categories addressed in multiple questions from the questionnaire, we will begin with summary exposure variables, such as the total number of years of exposure, or the number of exposure opportunities, or a combination of the time period and frequency/intensity of exposure. In constructing summary exposure variables, the half-life of the target congener will be considered; we will weight past exposures in proportion to their expected contribution to a current blood level. After testing the summary exposure measures, the individual exposure components will also be tested. Because of the large number of potential individual exposure variables, we will test variables in small groups, eliminating non-significant variables. If it appears that elevated blood levels are due to a few specific exposures, we will be able to identify those variables. However, if it appears that

many exposures have modest effects, then a summary exposure variable may provide the best way to model the cumulative exposure.

We will also compile residential histories, and create a historical residential exposure variable based on number of years lived in proximity to the flood plain. This residential exposure variable will be constructed in at least two ways: first, considering both the elevation and the linear distance of each property to the Tittabawassee River, and second, dichotomizing the property based on whether it is in the 100-year FEMA flood plain, or whether it was flooded during the large floods of 1986 and/or 2003. During the course of review or analysis, additional ways to investigate this question may be suggested and included.

Interactions (effect modification) between covariates will be considered, and some have already been mentioned above. Other interactions of interest may be between exposures and gender, age, and residence time in the flood plain area. Most interactions will follow the hierarchical principle, with main effects included in the model corresponding to any interaction tested. However, an interaction such as that between gardening and the garden soil congener level may be included without the corresponding main effects. It is hard to imagine that gardening will have an effect on blood congener levels in the absence of congeners in the soil, and hard to imagine that garden soil congeners will have much effect on blood levels in the absence of gardening. These main effects will be tested, but non-significant main effects may be removed from the model while leaving the interaction.

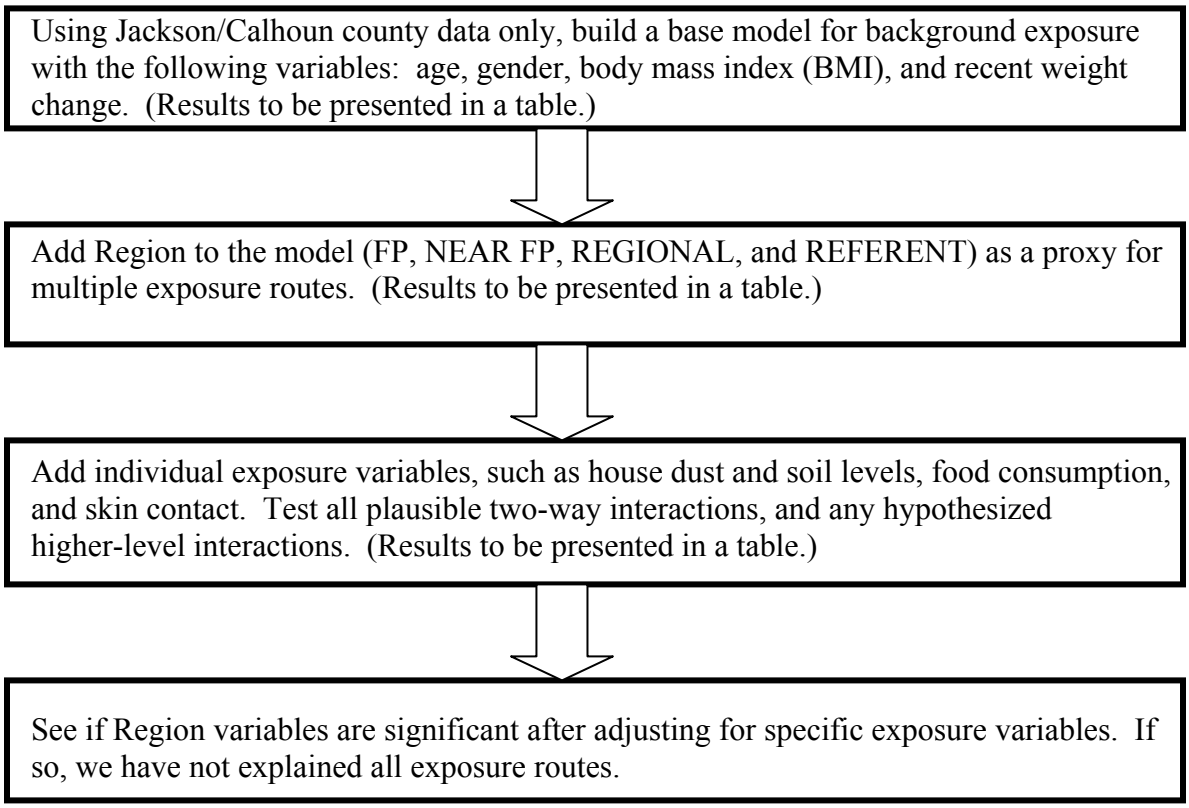


Figure 3. An outline of the modeling strategy is shown here. These steps will be performed separately for each congener and for the TEQ.

Collinearity, influence, and outliers. The existence of collinearity among exposure routes may be a problem for the modeling effort. For example, those who fish are likely to eat their catch. Separating the effects of soil exposure from fishing and ingestion of fish may be difficult. However, the inclusion of people who eat fish but do not catch them, and people who catch them but do not eat them, will help to separate these effects. At the least, we can identify groups of variables that have collinear effects. Collinearity will be assessed using the eigenvalues of the X'X matrix, the variance inflation factor for each covariate, and tolerance statistics. We will also assess any observations found to have large influence on the regression model estimates. When one or a few observations have an undue influence on the regression model, it is often due to values at the extremes of the covariate distributions that do not follow the same pattern as the other values. Influential values will be assessed using the hat diagonals and the DF-beta statistics. We will be particularly cautious in interpreting influential values, because they may arise from a small number of highly exposed individuals that exhibit the true functional form. Model outliers that are identified from plots of residuals versus predicted values, or plots of residuals versus individual covariates, and meeting predetermined criteria, will be similarly examined.

Interpretation of effects. For each significant effect, an interpretation will be made based on the point estimate and a 95% confidence interval of the regression coefficient. For dichotomous covariates, the effect will be interpreted as the increase in blood level attributed to the presence versus absence of the exposure. For continuous covariates, linear effects will be interpreted as the increase in blood level attributed to an increase in one unit (or a suitably scaled unit) in the exposure variable. If analyses are performed on the logarithm scale, then covariate effects on blood levels will be represented as percentage increases. For all models, the generalized R-squared will be calculated and used as a measure of explained variation.

Multiplicity. Because of the large number of statistical tests to be performed, the probability of a Type I error will be elevated far above 0.05. However, formal procedures for protection against Type I errors, such as the Bonferroni correction, are quite conservative, meaning that our ability to detect effects would be unduly diminished. We will implement the multiple comparison methods of Westfall and Young (available in SAS), which take into account the covariance among tests. However, because the study goal is to investigate effects, we plan for initial statistical tests to be liberal (i.e., unadjusted for multiple comparisons). However, the study results will be interpreted as a whole, and isolated statistically significant results that conflict with other results will not be considered conclusive. In our interpretation, we will primarily consider the congeners mentioned above as being potentially related to river sediment exposure. Second, we will consider the magnitude of effects. Third, we will consider the patterns of association with similar routes of exposure. For example, skin absorption could occur through soil contact (e.g., gardening), household dust, or pets tracking dirt into the home. Food consumption exposure could occur through eating garden vegetables, fish or game. A pattern of effects in the same direction would give more support to a hypothesis than an isolated significant value.

Finally, we will consider the distribution of residuals from the model(s) discussed above. We will not have the statistical power to thoroughly investigate outlying values, but any values well beyond the referent distribution will be reported.

4.7.5 Data management

The questionnaire data will be computerized by the Institute for Social Research (ISR) and saved as a SAS dataset. The laboratory data will be transferred as Microsoft Access datasets (separately for soil, dust, and blood), and will be converted to SAS datasets. The field notes datasets from the soil and dust samplers will be maintained in Access datasets (separately for soil and dust), and later imported into SAS. All data will be range-checked, and variable cross-checking will be performed as appropriate. Data will be merged on the participant ID number, which will appear in all records. Participant names will only be available in interviewer and sample tracking databases, and will not be included with the study data available for statistical analysis.

4.8 Sample Size Considerations

Assuming that estimates from the literature of blood 2,3,7,8 TCDD levels in referent populations range from 0 to 6 ppt (or 0 to 2 on the ln(x+1) scale), we use range/4 on the ln(x+1) scale to estimate a standard deviation of 0.5. These estimates are based on the German sample reported by Wittsiepe et al. (Wittsiepe et al, 2000) (median 1996 value 2.2 ppt, maximum 5.5 ppt, out of n=95), and the NHANES 1999-2000 data (the 95th percentile below detection, which averaged 4.8 ppt). Assuming a sample size of n = 175 in each subgroup and a normal distribution of ln-transformed blood TCDD levels, a 95% confidence interval (CI) for the true median(ln(x+1)) blood level will have width +/- $1.96*\sqrt{(\pi\sigma^2/2n)}$ = +/- 0.093. Thus, for example, if the estimated median blood TCDD level were 5 ppt (and ln(5+1)=1.79), then the 95% CI on the ln(x+1) scale would be 1.79 +/- 0.093, or (1.70,1.88). On the original ppt scale, the 95% confidence interval for the median blood level would be (4.47,5.55). A sample size of 175 people allows us to be 95% confident that the true median is between 4.47 and 5.55 if the sample median is 5.0 ppt.

The TEQ values, being a sum of components, may be more normally distributed than the individual congeners (by the Central Limit Theorem), although high correlations among the individual congener values could reduce the normalizing effect. The descriptive TEQ statistics presented in Wittsiepe et al. (Wittsiepe et al, 2000) does indicate less skewness. Thus, we consider confidence intervals for the median in each subgroup based directly on the normal distribution. Wittsiepe et al. present NATO/CCMS TEQ values in the German population of 6.1 to 41.5, mean=20.7, median=19.2. Based on range/4, we estimate a standard deviation of 35.4/4=8.85. A 95% CI for the TEQ would have length +/- $1.96*\sqrt{(\pi(8.85)^2/2*175)}$ =1.64. Given an estimated referent TEQ median of 19.2, the 95% CI for the TEQ would be (17.5,20.8), which is reasonably narrow.

We will use the cumulative distribution of congener blood levels to estimate various sample percentiles. The presence of values below detection will not be a problem for estimating the upper percentiles. The proposed sample size of n=175 in each subgroup will be sufficient to

display a cumulative histogram with reasonably high resolution. A bound for an upper percentile based on the normal distribution (of log-transformed values) will be of the form exp(mean+k*s). If the sample mean and standard deviation of the log-transformed data are 1.79 and 0.5 respectively, then the point estimate for the 90^{th} percentile is exp(1.79+1.282*0.5)=11.4 ppt. Using Table 1.4.3 of Odeh and Owen to obtain the k-factor, k=1.463, a 95% upper confidence bound for the 90^{th} percentile is exp(1.79 + 1.463*0.5) = 12.4 ppt. In this example, we would be 95% confident that at least 90% of the population had blood levels below 12.4 ppt. A confidence bound that is only one ppt higher than the point estimate for the 90^{th} percentile indicates reasonable precision in our ability to estimate upper percentiles. Precision will be reduced somewhat with the use of sampling weights, and may be further reduced if a larger sample variance is obtained, but even a bound that is within a several ppt of the point estimate would be useful.

To test for a difference in blood congener levels between two populations we will first base power calculations on a t-test comparing means of two independent groups. A significance level of 0.05 and one-sided test will be used. We employ a one-sided test to achieve maximum power to detect an increase in congener levels in the exposed group. We find it extremely unlikely that significantly *lower* levels would be found in the flood plain, and do not think it is worth wasting the statistical power to test this possibility. When the sample size in each group is 175, a 0.05 level one-sided t-test for equality of distributions will have 90% power to detect a difference between ln(x+1) means of 0.157. If the referent sample mean of log-transformed values were 1.79 (corresponding to an untransformed mean of approximately 5 ppt, then with 90% power we could detect a mean in the exposed group of 1.79+0.157, or an untransformed mean of approximately 6.02 ppt. Thus, for comparing means, we have high power to detect quite small effects, since a difference of about 1 ppt or larger would be considered significantly increased.

Next we consider testing for a difference between two populations in the proportion of subjects beyond a particular ppt value, such as 10 ppt. Assuming a 0.05 level one-sided Fisher's exact test for equality of proportions, n = 175 in each group, and a control group proportion of 0.01, we have 87% power to detect a proportion of 0.07 or greater in the exposed group.

For testing the effect of any dichotomous covariate such as gender on blood dioxin, furan or PCB levels, the power will depend on the proportion in each group. To increase power, we consider the pooled FP and NEAR FP samples, so n=350. Assuming a significance level of 0.05 and two-sided testing, if the proportion in each group is approximately 0.5, we will have 96% power to detect a 1.4-fold difference between groups. If the split is as uneven as 300 versus 50, we still have 88% power to detect a 1.5-fold difference. Given the anticipated small congener blood levels in the referent group, a fold-change of 1.5 will represent a modest increase, such as 7.5 versus 5.0 ppt. Testing for potential effects of continuous variables such as age on blood levels, a correlation as small as 0.17 (R^2=0.03) can be detected with 90% power. We conclude that the statistical power for all but the rare exposures (less than 10%) will be adequate to detect modestly small effects with high power.

5.0 Use of Human Subjects in Research

This study will be performed in compliance with University of Michigan policies and procedures governing the use of human subjects in research. Copies of all informed consent forms and communications with potential subjects are included in Appendix 11. The study has received a Certificate of Confidentiality from the National Institutes of Health (NIH), which provides legal protection for confidential data in this study from attempts to obtain it through subpoenas, Freedom of Information Act (FOIA) requests, and other mechanisms. A copy of the Certificate of Confidentiality is included in Appendix 13.

6.0 References

American Public Health Association (APHA). 1996. P450 Reporter Gene response to dioxin-like organics, Method 8070. In: *Standard Methods for the Examination of Water and Wastewater, 19th Edition, Supplement*. American Public Health Association, Washington, DC. pp. 24-25.

American Society for Testing and Materials (ASTM). 1997. E 1853-96 Standard Guide for measuring the presence of planar organic compounds which induce CYP1A, Reporter Gene Test Systems. In: *Biological Effects and Environmental Fate; Biotechnology; Pesticides, 1997 Annual Book of ASTM Standards, Volume 11.05 – Water and Environmental Technology*. American Society for Testing and Materials, Philadelphia, PA. pp. 1392-1397.

American Society for Testing and Materials (ASTM), Standard Practice for Collection of Floor Dust for Chemical Analysis, Designation D 5438-00, Reprinted from the Annual Book of ASTM Standards, Philadelphia, PA.

American Society for Testing and Materials (ASTM), Standard Test Method for Particle-Size Analysis of Soils, Designation D 422-63, Reprinted from the Annual Book of ASTM Standards, Philadelphia, PA.

Fingerhut MA, Halperin WE, Marlow DA, et al. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. New England Journal of Medicine 1991;324:212-8.

Hilscherova K, Kannan K, Nakata N, et al. Polychlorinated dibenzo-p-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. Environmental Science & Technology 2003;37:468-74.

Iglewicz B, Hoaglin DC. How to Detect and Handle Outliers. ASQC Quality Press, Milwaukee, WI, 1993.

McFarland VA, Inouye LS, Lutz CH, and Ang CY. Arlington-Garrows Bend Channel Sediment Characterization Using P450RGS Cell-Based Assays. Report to U.S. Army Engineer District, Mobile. Mobile, AL (2001).

Odeh RE, Owen DB. Tables for normal tolerance limits, sampling plans, and screening. New York: Marcel Dekker, Inc., 1980.

SAS Institute. SAS/STAT User's Guide Version 8. Cary, NC: SAS Institute Inc., 1999.

StataCorp. Stata Reference Manual Release 8. College Station, TX: Stata Press, 2003.

United States Environmental Protection Agency (US EPA). Method 1668, Revision A: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS. Washington, DC: Office of Water, 1999.

United States Environmental Protection Agency (US EPA). Method 3545, Revision 0: Pressurized Fluid Extraction (PFE). Washington, DC: Office of Solid Waste, 1996.

United States Environmental Protection Agency (US EPA). Method 4425, Revision 0: Screening extracts of environmental samples for planar organic compounds (PAHs, PCBs, PCDDs/PCDFs) by a reporter gene on a human cell line. Washington, DC: Office of Solid Waste, 2000.

United States Environmental Protection Agency (US EPA). Method 8290: Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Washington, DC: Office of Solid Waste and Emergency Response, 1994.

United States Environmental Protection Agency (US EPA). 2003. Framework for application of the toxicity equivalence methodology for polychlorinated dioxins, furans and biphenyls in ecological risk assessment. EPA/630/P-03/002A.

Van den Berg M et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect. 1998;106:775-92.

Wittsiepe J, Schrey P, Ewers U et al. Decrease of PCDD/F levels in human blood from Germany over the past ten years (1989-1998). Chemosphere 2000; 40:1103-1109.