

**University of Michigan Dioxin Exposure Study:  
A Proposal for a Follow-up Study of Blood Outliers  
July, 2007**

**Study Goals and Objectives:**

The initial follow-up study of blood outliers was intended as an exploratory investigation to generate hypotheses as to why some UMDES subjects have unusually high levels of dioxins in their blood. The definition of 'outlier' in the initial study was based solely on the total TEQ (after adjustment for age and body mass index). The findings of the pilot study were summarized in a poster presented in Oslo (see below for more complete summary). The overall goals of the present proposal are to extend the findings of the pilot study to include outliers defined on the basis of specific congeners, and to confirm or refute the hypotheses that have evolved from the findings of the UMDES and the pilot study. We will achieve these goals via a case-control study design.

The specific objectives include the following:

- 1) Cases will be defined to include not only UMDES subjects with a 'high' total TEQ, but will also include UMDES subjects who were found to have 'high' blood levels of any of four specific congeners: 2378-TCDD, 123678-HxCDD, 23478-PeCDF, and PCB-126.
- 2) Controls will be UMDES subjects that are group-matched to the cases on age, and whose TEQ and congener results for 2378-TCDD, 123678-HxCDD, 23478-PeCDF, and PCB-126 are less than the age adjusted median for UMDES subjects from Jackson/Calhoun counties for each congener.
- 3) Each case and control will be invited to participate in a semi-structured interview that focuses on personal medical history, diet, occupation, and recreational activities.

No repeat blood sampling will be performed.

The main hypotheses to be tested are:

- 1) Cases with 'high' total TEQ are significantly more likely to report a history of frequent consumption of wild game and fish, including sport-caught fish and store-bought fish.
- 2) Cases with 'high' 2378-TCDD are significantly more likely to report a history of employment with opportunity for chemical exposures and/or residence in the Midland plume area.
- 3) Cases with 'high' 23478-PeCDF are significantly more likely to report a history of exposures to contaminated sediments and soil in the Tittabawassee River and the Tittabawassee River flood plain, or are more likely to report a history of eating wild game and fish, including sport caught fish from the

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Tittabawassee River. It is also possible that cases with 'high' 23478-PeCDF may have had occupational exposure to certain chemical processes, such as working at Dow.

4) Cases with 'high' PCB-126 are significantly more likely to report a history of frequent consumption of wild game and fish, particularly sport-caught fish.

5) Cases with 'high' 123678-HxCDD are significantly more likely to report opportunity for exposure to pentachlorophenol at work.

**Background:**

The University of Michigan Dioxin Exposure Study (UMDES) has collected information based on questionnaires and samples of blood, soil and/or house dust from over 1,300 randomly selected subjects in Midland, Saginaw, Bay, Jackson and Calhoun counties. A primary goal of the study is to determine whether dioxins, furans and polychlorinated biphenyls (hereinafter collectively referred to as 'dioxins') in soil and/or house dust are related to or explain blood levels of these contaminants, with adjustment for other known risk factors (i.e., diet, occupation, age, body mass index, etc.). More generally, the study seeks to understand sources of variation in the observed blood and environmental measurements. Not surprisingly, the distributions of blood results are skewed, with a small number of 'high' total TEQ values, after adjustment for age and BMI. This pattern of results is intriguing, and this follow-up study seeks to better understand these (and other) blood outlier values.

**Preliminary Studies:**

Over the last year we conducted a pilot study of 'high' serum outliers. To be eligible for the pilot follow-up study, a person must have: been a participant in the UMDES; completed an initial interview and provided a blood sample; been an 'outlier' as defined below; and, requested their serum dioxin results. Nine hundred and forty-six subjects completed initial interviews and also provided blood samples in the UMDES. Eight subjects were eligible for the pilot blood outlier study, and all participated and were interviewed.

For this pilot investigation an outlier was defined as a subject with a total serum TEQ that was more than 2.5 studentized residuals above the mean of the log-transformed serum TEQ results after adjustment for age, age<sup>2</sup> and BMI.

Eligible subjects were invited to be interviewed in-person. The follow-up interview was semi-structured, and included topics that were covered in the original questionnaire (e.g., residential history, occupational history, recreational activities (e.g., fishing and hunting, gardening and spraying pesticides), pregnancy history, basic demographic information, and diet). Interviews also included open-ended questions that sought more information about these topics, and also other topics that may potentially impact the levels of dioxins in serum, but were not covered in the original questionnaire. For example, we asked

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about hobbies or other activities performed in the home or on the property by the subject and also other occupants of the house, details about the construction of the home or other structures on the property, whether the house had been damaged by fire, fireplaces or other burning activities (e.g., trash burning), and whether fill dirt or other materials may have been deposited on the property. Subjects were also asked for their opinions as to why their serum dioxin levels might be high. No repeat serum measurements were sought for this follow-up investigation. Participants were paid \$50 as an incentive for participating in the follow-up interview. This follow-up study was reviewed and approved by the University of Michigan Institutional Review Board.

Table 1: Descriptive Information on the Eight Participants Contacted for Additional Interviews

Case No.	Age Range	Total TEQ	% TEQ Dioxins	% TEQ Furans	% TEQ PCBs	Region of Residence
1	70-79	238	64	13	23	FP
2	80-89	210	40	11	49	NFP
3	70-79	191	23	5	72	NFP
4	60-69	131	33	11	56	NFP
5	60-69	117	34	14	52	FP
6	60-69	115	25	19	56	FP
7	60-69	109	35	10	55	OMS
8	50-59	108	39	12	49	J/C

FP=Midland/Saginaw flood plain; NFP=near flood plain; OMS=other Midland/Saginaw/Bay; J/C=Jackson/Calhoun [TEQs based on 1998 TEFs]

Table 2: Summary of Results of Additional Interviews of the Eight Participants

Case No.	Relevant Interview Notes
1	<ul style="list-style-type: none"> <li>• No occupational exposure for self or spouse</li> <li>• No consumption of wild game since childhood</li> <li>• Consumed one fish meal per day, for two weeks every year from 1973-1993 of pike, perch or walleye from Canada</li> </ul>
2	<ul style="list-style-type: none"> <li>• Worked for 40 years as a tool and die maker, retired in the 1970s</li> <li>• Lifetime consumer of sport-caught perch (6 meals per year) from the SB; remote history of consumption of lake salmon and consumption of fish from SR</li> <li>• Lost 35 pounds one year ago</li> </ul>
3	<ul style="list-style-type: none"> <li>• No occupational exposure for self or spouse</li> <li>• Consumes one sport-caught perch meal from the SB every week, for the past 20 years</li> </ul>
4	<ul style="list-style-type: none"> <li>• Varied work history: military service, assembly line and office work</li> <li>• Consumes 15-20 deer meals from outside the floodplain every year for the last 10 years</li> <li>• Consumes 10-20 sport-caught walleye meals from outside TR/SR/SB</li> </ul>

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	<ul style="list-style-type: none"> <li>every year, for the past 20 years</li> <li>• In 1970s, purchased and consumed 50 pound bags of commercially-caught SB perch</li> </ul>
5	<ul style="list-style-type: none"> <li>• Varied work history: six years in the 1970s as an industrial tank cleaner followed by administrative work until retirement</li> <li>• Has been consuming venison from outside TR/SR/SB floodplain for the past 40 years (except for late 1970's): in recent years ~3 meals per month, prior to past few years ~1 meal per week</li> <li>• From 1961-1981 consumed one bear meal per year from the study areas</li> <li>• From 1990-1995 R consumed about three fish meals per year from the Tittabawassee River</li> </ul>
6	<ul style="list-style-type: none"> <li>• Varied work history including Vietnam service and chemical company work</li> <li>• Has consumed 1 deer from outside TR/SR/SB floodplain meal per week, for the past 20 years</li> <li>• Consumes 1-2 meals per week of game fowl from outside TR/SR/SB floodplain for the past 4 – 5 years</li> <li>• Consumes one meal per week of trout and salmon from the Great Lakes (not including SB)</li> </ul>
7	<ul style="list-style-type: none"> <li>• No occupational exposure for self or spouse</li> <li>• Consumes 1 – 2 deer meals every week for the past 43 years from outside TR/SR/SB floodplain</li> <li>• Consumes 1 muledeer meal from outside TR/SR/SB floodplain every week for past 17 years</li> <li>• Consumes 1 - 2 meals per week in every third year of elk from outside TR/SR/SB floodplain for the past 17 years</li> <li>• For the past two years P has had moose from outside TR/SR/SB floodplain: 2-3 meals per week for the first year, 1 meal every 2-3 weeks currently</li> <li>• In the 1980s P consumed beef and pork from outside TR/SR/SB floodplain as part of their regular diet</li> <li>• Has regularly consumed locally caught sport fish including walleye and perch from the SR, and steelhead trout and suckers from outside TR/SR/SB for the past 30 years</li> </ul>
8	<ul style="list-style-type: none"> <li>• No occupational exposure for self or spouse</li> <li>• From 1965 to present has consumed 1 -3 deer meals per week from outside TR/SR/SB floodplain</li> <li>• Consumes 5-6 wild turkey meals from outside TR/SR/SB floodplain every year</li> <li>• From 1977 to present has consumed 1 – 2 catfish meals per month from outside TR/SR/SB floodplain</li> <li>• Lost more than 100 pounds around year 2000</li> </ul>

Conclusions:

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- The congener pattern of serum outliers is usually low in furans and high in PCBs, which does not match the sediments in the floodplain
- Regular consumption of sport fish and wild game appear to be the dominant factors that explain most serum outliers who have ‘high’ total TEQ
- Outlier blood levels based on total TEQ do not appear to be due to occupation or activities in the TR/SR/SB floodplain
- Case 1 has a different congener pattern, which suggests that there are other sources of exposure in the community

**Proposed Study Design:**

The present study will consist of five separate, but linked, case-control studies that compare exposure histories of outlier cases to a common pool of non-outlier controls randomly selected from the UMDES study population. This study only focuses on statistically defined outliers at the high end of the distributions, and not those at the low end. The distributions of serum TEQ and all specific congener results are highly skewed (approximately log-normal) and an ‘outlier’ from the low end of these distributions would only differ from an ‘average’ value by a small absolute amount (in the original, untransformed scale). Given the small absolute differences involved, it is unlikely that much could be learned from pursuing such low outlier cases.

The initial follow-up study focused on individuals who were 2.5 studentized residuals above the mean of the age, age<sup>2</sup> and BMI adjusted log-transformed TEQ. Table 3 displays the number of subjects that are 2.0, 2.5 and 3.0 Studentized residuals above the mean for the TEQ and for each of the 7 congeners that account for the bulk of the TEQ in blood (based on 1998 TEFs). Three specific congeners, 2378-TCDD, 23478-PeCDF and PCB-126 appear to have a more skewed distribution, as reflected by a greater number of subjects in the upper tail of their respective distributions. The congener 123678-HxCDD is also of note because it can be a marker for occupational exposure to pentachlorophenol, such as among some current or former workers at Dow.

Table 3: Number of Serum Observations 2.0, 2.5 and 3.0 Studentized Residuals Above the Mean after adjustment for Age, Age<sup>2</sup> and BMI for the TEQ and 7 congeners that account for most of the TEQ (not accounting for overlaps among different congeners, and not accounting for subjects who did not request blood results – TEQ based on 1998 TEFs)

	<b>Studentized Residuals Above the Mean</b>		
	2.0	2.5	3.0
TEQ	31	12	3
2378-TCDD	39	19	8
12378 - PeCDD	23	8	2
123678 - HxCDD	20	6	1

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23478 – PeCDF	32	13	4
PCB 118	17	4	1
PCB 126	34	19	9
PCB 156	18	5	0

Table 4 displays the top 20 individual outlier subjects (after adjustment for age, age<sup>2</sup>, and BMI) for TEQ, 2378-TCDD, 123678-HxCDD, 23478-PeCDF and PCB-126. It is notable that 14 of the 20 top outlier subjects for PCB-126 overlap with the top 20 outliers for TEQ, and that both of these sets of outlier subjects are largely disjoint from the top 20 outliers for 123678-HxCDD, 23478-PeCDF and 2378-TCDD. A large fraction of top 20 outliers for 123678-HxCDD, 23478-PeCDF and 2378-TCDD are disjoint from each other, though there is also some overlap among these congeners. These patterns suggest that, in many cases, the sources of exposures likely overlap for TEQ and PCB-126, and, based on the pilot study results, appear to be linked to a diet high in wild game and sport-caught fish. In many, but not all cases the sources of exposure appear to be different for 2378-TCDD, 123678-HxCDD and 23478-PeCDF.

Table 4: Outlier Subjects for TEQ and each of the three main congeners

Case Number	Studentized Residuals Above the Mean (Rank)				
	TEQ	2378-TCDD	123678-HxCDD	23478-PeCDF	PCB 126
1	3.95(1)	4.59(1)	2.53(6)	3.76(2)	2.64(14)
2	3.37(2)			2.33(17)	4.13(1)
3	3.06(3)		2.91(2)	2.24(20)	2.99(10)
4	2.84(4)				3.33(4)
5	2.84(5)			2.62(7)	3.32(5)
6	2.79(6)	3.84(2)			
7	2.78(7)			2.47(14)	3.22(7)
8	2.76(8)				3.47(2)
9	2.71(9)	2.48(20)	2.08(14)	2.60(8)	2.57(17)
10	2.65(10)				3.17(8)
11	2.64(11)			2.36(16)	2.51(18)
12	2.62(12)		2.56(5)		
13	2.50(13)	3.27(4)	2.07(15)		
14	2.37(14)		3.05(1)		
15	2.36(15)				3.34(3)
16	2.34(16)			2.25(19)	3.28(6)
17	2.32(17)	2.89(11)			
18	2.31(18)		2.07(16)		2.51(19)
19	2.31(19)	3.04(7)		2.76(6)	
20	2.23(20)				3.04(9)
21		3.28(3)			
22		3.17(5)			

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23	3.11(6)	2.16(11)	
24	3.01(8)		
25	2.99(9)		
26	2.98(10)		
27	2.83(12)		
28	2.80(13)		
29	2.75(14)		
30	2.66(15)		
31	2.59(16)		
32	2.54(17)		
33	2.52(18)		
34	2.51(19)	3.16(3)	
35		2.60(3)	
36		2.58(4)	2.60(9)
37		2.45(7)	
38		2.38(8)	
39		2.21(9)	
40		2.20(10)	
41		2.14(12)	
42		2.11(13)	
43		2.06(17)	
44		2.04(18)	2.33(18)
45		2.04(19)	2.59(10)
46		2.01(20)	
47			4.18(1)
48			3.16(4)
49			2.89(5)
50			2.54(11)
51			2.54(12)
52			2.51(13)
53			2.45(15)
54			2.87(11)
55			2.72(12)
56			2.68(13)
57			2.64(15)
58			2.62(16)
59			2.45(20)

\*Rank order is decreasing by furthest from mean

There are a number of key observations and conclusions based on the pilot study, the information displayed in Tables 3 and 4, other results from the UMDES study, and other available information:

- 1) 23478-PeCDF is one of the two congeners that contribute to most of the contamination in the flood plain of the Tittabawassee River, this congener can

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also accumulate in human blood, and therefore this congener can serve as a potential serum ‘marker’ of consumption of fish or other exposures to contamination in the Tittabawassee River, and possibly the Saginaw River, and the Saginaw Bay. It is also possible that certain occupational exposures cause exposure to this congener.

- 2) 2378-TCDD was found to be relatively high in soil (and house dust) in the Midland Plume area, and it is also known from other studies that some workers at Dow have high serum levels of 2378-TCDD, and so serum 2378-TCDD may be a possible marker of exposure from either of these sources.
- 3) Based on our pilot results, and the information shown in Tables 3 and 4, we believe that the sources of environmental exposure of 2378-TCDD, 23478-PeCDF and PCB-126 in the UMDES study population are likely to be different.
- 4) Based on our pilot results and the information shown in Tables 3 and 4, we believe that most subjects who are outliers for serum TEQ and PCB-126 have a history of consumption of wild game and sport-caught fish.
- 5) As noted above, 123678-HxCDD can be a marker for occupational exposure to pentachlorophenol, such as among some current or former workers at Dow.
- 6) Given the more limited number of ‘high’ outliers for the other three congeners (12378-PeCDD, PCB-118, and PCB-156), it would be more difficult and less cost-effective to identify contrasting differences in routes of exposures for these congeners, and so further investigation of outliers of these specific congeners will not be pursued.

Based on these observations, the present study will focus on outliers defined on the basis of the total TEQ and the four specific congeners shown in Table 4 (2378-TCDD, 123678-HxCDD, 23478-PeCDF, and PCB-126). The five hypotheses for the study are also derived on these observations.

To be eligible for the follow-up study, a case must have: been a participant in the UMDES; completed an initial interview and provided a blood sample; been an ‘outlier’, as defined below; and, requested their serum dioxin results. We will seek to recruit a minimum of 15 outliers each based on TEQ, 2378-TCDD, 123678-HxCDD, 23478-PeCDF, and PCB-126 – i.e., there will be five separate groups of cases, each with a minimum of 15 subjects. We will recruit subjects in descending order of studentized residuals, as shown in Table 4. As shown in Tables 3 and 4, cases recruited to participate will be approximately 2.0-2.5 studentized residuals above the mean after adjustment for age, age<sup>2</sup> and BMI, although the exact cut-off cannot be predicted since it will depend on the pattern of recruitment. Based on our experience with the pilot study (8 out of 8 eligible were contacted and agreed to participate), we anticipate a similar high rate of participation in this study.

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As shown in Table 4, due to overlaps among the outlier groups, the actual total number of cases will be less than 75, most likely about 60, depending on the exact pattern of recruitment and participation of eligible subjects. We will only seek to recruit subjects to the follow-up study who requested and received their previous laboratory test results (e.g., if a subject had a 'high' blood result, but he chose to not receive his results, then we will not seek to recruit this person to be a subject in the follow-up study). Overall, 95% of 946 subjects who had blood samples analyzed as part of the UMDES requested their blood results. Cases will be recruited without regard to their geographic area of residence (i.e., all five counties – Midland, Saginaw, Bay, Jackson and Calhoun counties). We will attempt to recruit and interview subjects who have changed residences in the interval since their participation in the UMDES.

Controls will be group-matched on age to the overall case group. In addition, controls will meet the following eligibility criteria: total TEQ less than the median for Jackson/Calhoun counties, after adjustment for age and age<sup>2</sup>; each of the 4 congeners (2378-TCDD, 123678-HxCDD, 23478-PeCDF, and PCB-126) less than the median for Jackson/Calhoun counties, after adjustment for age and age<sup>2</sup>. We will seek to recruit 20 subjects to form the control group. For efficiency, the same control group will be used in comparisons to each of the five case groups defined above.

Potential subjects for this follow-up study will be identified based on examination of existing blood results from the UMDES as described above. No new blood samples will be sought.

Potential subjects will be contacted by telephone. If the subject expresses interest, then an appointment will be made for a home visit by members of the University of Michigan Dioxin Exposure Study research team. Subjects will be asked to sign a written informed consent at the time of the home visit. With slight modification, the consent document and IRB approval for the pilot study also cover this expanded study. Participation is voluntary; subjects can stop their participation at any time and can decline to answer any questions.

All study participants will be asked to complete a semi-structured open-ended interview with an interviewer from the University of Michigan. The interview will be similar to that used in the pilot study. The structured portion of the interview will review topics that were covered in the original questionnaire (e.g., questions about residential history, occupational history, recreational activities (e.g., fishing), pregnancy history (for women only), basic demographic information, and diet). Questions about diet will probe game and sport fish consumption by specific location where game or fish were caught (e.g., Tittabawassee River, Saginaw River, Saginaw Bay or elsewhere) to the extent possible. We will also inquire about store-bought fish and fish consumed in restaurants. The interview may also include open-ended questions that seek more information about these topics, and also other topics that may impact the levels of dioxins in blood, dust or soil, but were not covered in the original questionnaire. For example, we may ask about

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hobbies (e.g., ceramics) or other activities performed in the home or on the property, details about the construction of the home or other structures on the property, fireplaces or other burning activities, and whether fill dirt or other materials may have been deposited on the property. The exact nature of the open-ended questions will vary from subject to subject, depending on the pattern of responses on the previous questionnaire, laboratory results, and answers to questions in the interview. The interview will not include questions that might be considered potentially embarrassing (e.g., use of illegal drugs or other criminal behavior).

Participants who agree to participate in this study will be paid \$50 for completing the interview.

Confidentiality procedures for this follow-up study will be the same as for the UMDES, including protection via a Certificate of Confidentiality issued by the National Institutes of Health.

**Power, Data Analysis and Reporting of Results**

Table 5 displays a range of power estimates. These estimates are based on alpha = 0.05, and testing of one-sided confidence intervals for a comparison of proportions between cases and controls. In the pilot study we found that six or seven of the eight cases with high TEQ reported consuming a diet that was high in wild game and/or sport-caught fish. We therefore base our power estimates on proportions of 70%, 75% and 80% in the case groups, and 30%, 25%, and 20%, respectively, in the control group.

Table 5. Power Estimates

# of Cases	# of Controls	Proportion Among Cases	Proportion Among Controls	Power
15	15	.75	.25	.79
<b>15</b>	<b>20</b>	<b>.75</b>	<b>.25</b>	<b>.86</b>
20	20	.75	.25	.91
15	15	.80	.20	.94
15	20	.80	.20	.97
20	20	.80	.20	.98
15	15	.70	.30	.57
15	20	.70	.30	.65
20	20	.70	.30	.73

The proposed study design seeks to recruit 15 cases for each of the five case groups, and 20 controls. If the proportion of cases with the exposure of interest is 75% (as was found for dietary factors among persons with ‘high’ TEQ in the pilot study), and we assume that the proportion is only 25% among controls, then the resulting power is 86% (see bolded row in Table 5). Therefore, we believe that the study is reasonably powered for the hypotheses related to TEQ and PCB-126. It is harder to estimate the power for the remaining three hypotheses related to 2378-TCDD, 123678-HxCDD and 23478-PeCDF

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since the hypotheses related to these congeners were not addressed directly by the previous pilot study.

Statistical analyses will include descriptive statistics, tables, and plots. The primary outcomes will be comparisons of proportions of exposures among cases and controls via chi-square statistics and/or odds ratios.

**Time Line**

January –March, 2007

- Finalize questionnaire
- Submit any necessary amendments to IRB for review and approval
- Begin telephone recruitment

April-June, 2007

- Continue telephone recruitment
- Begin interviews

July-September, 2007

- Continue recruitment and interviews

October-December, 2007

- Complete interviews
- Clean and organize data files
- Perform analyses
- Begin manuscript preparation

January-March, 2008

- Complete final drafts of manuscript(s)
- Initial submission(s) to journals

April-June, 2008

- Respond to journal requests for revisions, or to paper rejections requiring submission(s) elsewhere