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Family Clustering of PBB Exposure

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Family Clustering of PBB Exposure

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B.S., Shanghai Normal University, 2013

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An abstract of

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Abstract

Family Clustering of PBB Exposure

By Xiao Wang

Background: PBB contamination of food served as a major exposure source following an industrial accident in Michigan in the 1970s. People living in the same household at the time of the accident were expected to have similar serum PBB levels. Thus, husbands and wives may have similar PBB levels even 40 years later. PBB can also pass from mother to children, by means of breastfeeding or in utero. In addition, there may exist genetic factors that influence PBB absorption, distribution, metabolism and excretion which lead to correlated levels between parents and children.

Methods: Current PBB levels were collected and analyzed during 2012 and 2014. Paired t-tests, correlation statistics and the linear regression analyses were performed on the log-transformed current PBB levels in mother and child, father and child, as well as spouse pairs. Scatter plots presented logarithmically on un-transformed PBB levels and on log-transformed PBB levels, for the three types of family pairs were also generated. All of the analyses use $\alpha = 0.05$ as the significant level.

Results: There were 134 family groups in the study, including 63 spouse pairs, 84 mother and child pairs, 44 father and child pairs. Significant Pearson correlations coefficients using the log-transformed current PBB levels were found within each of the three types of family pairs. The spouse pairs have the strongest Pearson correlation, with a coefficient of 0.635 and a p-value of <0.001. The results of the paired t-tests show there were significant mean differences between husbands and wives, mothers and children, fathers and children’s current PBB levels.

Conclusion: Females’ current PBB levels are significantly lower than males in spouse pairs. Because PBB can be transported from mothers to children through breast milk and in utero, we would expect for the mothers’ PBB levels to have more impact on children’s PBB levels, compared to fathers’ PBB levels. However, the coefficients between mother and child pairs, and father and child pairs are almost identical (0.388 versus 0.399). Although the correlation between fathers and children's current PBB levels is significant, further investigation is needed to address the mothers’ PBB levels as a confounder.

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I really feel grateful for the faculty, advisors and staff of the Epidemiology Department at Rollins School of Public Health for the two years of training. I learnt a lot of things here and this thesis is only a part of the training and knowledge I have had during these two years. I would especially say thank you to Dr. Marcus and Mrs. Terrell for their time, efforts, and patience to guide me to complete this work. I also want to thank my parents for their enormous supports for my study and living aboard. Thank you very much!

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Introduction:

The accumulation and long-term persistence of organic pollutants in humans has arose increasing interest in the society. Many pollutants, for example, PCBs (polychlorinated biphenyls), DDE/DDT, etc. are resistant to environmental degradation through chemical, biological and photolytic processes. These exposures may cause developmental defects, chronic illnesses, and even death.

In the 1970's, the Michigan Chemical Company made two products at the same plant: polybrominated biphenyl (PBB), a fire-retardant chemical used in the manufacture of electrical appliances, and magnesium oxide, a nutritional supplement for livestock feed. In 1973, the company accidentally shipped PBB to the Farm Bureau instead of magnesium oxide. The PBB was mixed into livestock feed and consumed by cattle, pigs, and chickens. Contaminated milk, beef, and other farm products were sold throughout the state until the accident was discovered about a year later[1]. As a result, the contaminated food served as a major exposure source. People living in the same household were expected to have similar serum PBB levels[2]. In addition, PBB can also pass from mother to children, by means of breastfeeding or in utero.

The presence of PBB has been documented repeatedly in human blood, adipose tissue, and more importantly, in the breast milk of nursing mothers [3]. It is lipophilic and poorly metabolized. Therefore, PBB has a long half-life in the human body. An in-depth investigation conducted by Wolff, et al., between 1976 and 1978 found that on November 1976, less than 1% of 993 dairy farm residents and 55 chemical workers had no detectable PBB in serum, and 2% had levels at the limit of detection of the method used (0.2 parts per billion (ppb)). They re-examined 92 dairy farm residents and fourteen chemical workers again on April 1978. The measurements of serum and adipose tissue concentrations of PBB suggest that these people had apparently achieved equilibrium of their PBB body burdens [4].

Another cross-sectional study was also carried out in 1978 to evaluate residual burdens of PBB, of the population in Michigan [5]. PBB levels of 1681 people were measured in serum specimens, and 844 people in adipose samples. The results showed that ninety-seven percent of the adipose samples had detectable PBB concentrations. It already had been five years since the PBB contaminated the food supply, while the PBB concentrations in the adipose samples still had high detection rates.

PBB exposure is suspected to disrupt endocrine function [6]. The study of Blanck, et al., which was published in 2000 found that breastfed girls exposed to higher levels of PBB had an earlier age at menarche than the breastfed girls exposed to lower levels of PBB in utero. The perinatal PBB exposure as also associated with earlier pubic hair stage in breastfed girls.

PBB exposure may impact ovarian function by menstrual cycle length and bleed length [7]. The Michigan Female Health Study, which included 337 women with self-reported menstrual cycles of 20-35 days, showed that a there was a significant interaction between PBB exposure with past year weight loss. In addition, higher PBB exposure among women with past year weight loss was also associated with longer bleed length and shorter cycle length.

A nested case-control study conducted by Henderson, et al., indicates that women with serum PBB levels of 2.0-3.0 ppb or greater had a higher estimated risk for breast cancer than women with less than 2.0 ppb [8]. Women with mid-range and high PBB exposure had increased odds of spontaneous abortion compared to those with the lowest exposure [9].

In this study, we will examine how the PBB exposure levels cluster within families and households, as well as identify the types of relationships within families. We will look at the differences in PBB levels between spouse pairs, mother and child pairs, as well as father and child pairs.

By comparing at the current PBB levels in family units, we can characterize how this class of persistent organic pollutants cluster among family members. It will be an important clue to investigate what are the factors that account for the correlations of PBB levels between the family members, and what can be done to reduce the exposure of them, thus, minimize the adverse health effects caused by this class of chemicals.

Wolff, et al., conducted a similar study in 1976 [2]. They looked at the PBB levels between husbands and wives, mothers and daughters, and fathers and sons. Highly significant linear regression coefficients were observed between these three pairs. There are seldom other studies investigated the family clustering of chemical organic pollutants. However, we found another study carried out by John E, et al.[10], which was published in 1969, looked at the DDE levels in four kinds of populations, infants, 1-7 years old children, employees of the Dade County Health Department, and Food handlers. They found the DDE levels increases with ages, and confirmed the prenatal exposure of the insecticides can contribute to the pesticide body burdens of children.

There is rich data on the MI PBB cohort, with historic serum PBB measurements collected from 1976-1994.The parent study (2R01ES012014-06; PI Marcus) aimed to collect more recent blood samples at community meetings and site visits. Currently, there are 853 samples (for 817 individuals) collected and analyzed from 2012-2014.Over half of the individuals who have a current PBB level completed a General Health Questionnaire (GHQ), which asked questions regarding their historical family (parents, siblings, children, grandchildren, etc.). Based on the information provided in the GHQ. We matched family members to one another. This study includes the 134 family groups where at least two members of the same family had current PBB levels (N=440). The study population includes 184 males and 256 females, and is made up of 63 spouse pairs, 84 mother and child pairs, and 44 father and child pairs.

Methods:

The procedures to identify family group/households, as well as the family pairs are available in the Appendix (figure A1). It can be divided into the following steps:

Step 1, transform the record layout to assign everyone a unique ID: We exported the data from the GHQ for 647 people who had taken the interview. These people provided their personal information including name, date of birth, address, email etc., as well as family relationships and their corresponding contact information. We excluded those who did not fully complete the questionnaire (42 of 647). We then rearranged the data in a layout so that every person mentioned in the GHQ became a separate observation, for purposes of assigning everyone a unique ID. This was done regardless of whether the individual answered the questionnaire himself / herself, or were mentioned by someone else who completed the questionnaire, as a relative. Following this step, we had 3361 observations, including some duplicate observations for people mentioned multiple times. Of the 3361 observations, it included 605observations for people who completed the GHQ, and 2756 observations for people who were mentioned by a person who completed the GHQ.

Step 2, data clean the records for those who completed the GHQ (n=605):

Because a person who completed the GHQ could have been mentioned as a relative by someone else, we had to delete duplicate records. Among the 605 people who completed the GHQ, 30 duplicate records were found and deleted. We used the GHQ record ID as the unique ID for the 605 people who completed the questionnaire themselves.

Step 3, data clean the records for those who do not take the questionnaire but were mentioned as a relative (n=2756):

We used an Excel add-in, called the "fuzzy look up function" to identify duplicate records for those who did not take the questionnaire themselves. We matched on name, address, and date of birth, when available, and then reviewed the records where the similarity match was 60% or higher to determine if records were indeed duplicate records. We assigned duplicate records the same ID and were left with 2196unique people. We then assigned these individuals a unique ID beginning with the number 1000. We did a final check to compare names and ID numbers. We created a standard list of relationship types and added this uniform relationship type by each person. The possible relationship types were parent, child, grandchild, spouse, sibling, relatives, etc.

Step 4, Creating the family groups:

We used an R program to divide people into different family groups. Since each person had a unique ID, they also have a "record_linkage ID" which indicates which person mentioned them as a relative. People were considered to be in the same family group if they had arecord_linkage ID in common. We used R to transform the whole dataset to a 3350*3350 Matrix. If two people were related, there would be a 1 to indicate that these two people were in the same family. After all of the people in the same family were identified, this family would be ignored and R would begin picking out the next family. As a result, we found320 family groups in total. Then we removed the families in which less than two family members had PBB levels. At last, 134 family groups remained for the study.

Step 5, creating the family pairs:

We created four kinds of family pairs: mother and child pair, father and child pair, spouse pair and sibling pair. If people indicated each other as spouse, or they didn't do so, but have a child in common, we considered them to be a spouse pair. Anyone without PBB level would not be included in a pair. At last, we had84 mother and child pairs, 44 father and child pairs, 63 spouse pairs and 74 sibling pairs. For those parents who have more than one child, the average of the log-transformed PBB level of the children would be used as an aggregate PBB exposure level for the children.

PBB Measurements:

Blood samples were collected via venipuncture and processed to isolate the blood serum, during 2012 and 2014. Samples were stored at -80C until analysis. For analysis of

PBB153, a 1-mL aliquot of each serum sample was spiked with a known concentration of isotopically labeled PBB-153, deproteinated with the addition of formic acid, and homogenized. The serum samples were extracted twice with hexane, passed through activated silica to remove residual fats, and concentrated to 50 µL in isooctane. Samples were analyzed using gas chromatography-tandem mass spectrometry (GC-MS/MS) with electron impact ionization in the multiple reaction monitoring mode with one quantification and one confirmation parent-to-product ion pair for each analyte and internal standard. The method limit of detection(LOD) was 0.005ng/mL (=0.005 part per billion or pbb) with an accuracy of 100±20% and a relative standard deviation of <15%. Each analytical run included a full calibration curve and two spiked QC samples (1 high level, 1 low level), and a laboratory blank that were prepared and analyzed concurrently with unknown samples. For an analyte to be considered detectable, it had to co-elute with its isotopically labeled internal standard (differentiated by mass), have ions within 20% of the theoretical ion ratio for naturally occurring isotopes of bromine, and have the correct quantification and confirmation ions.

The current PBB levels in the study are not normally distributed, so we log transformed them for data analyses. The PBB levels which are lower than the LOD, were treated as zero in the exposure dataset. For the convenience of log-transformation, the zero values were converted using the formula: LOD / sqrt 2, (for the actual results the LOD = 0.001 ng/mL, 0.001 ppb).

Data and Statistical Analyses:

The data analyses for this study were performed using SAS 9.4 (Cary, NC, USA). Significant level $\alpha = 0.05$ were used during the analyses. Summary tables for family groups, family pairs and

PBB levels were created. The paired t-tests, correlation statistics and the regression analyses were performed for the log-transformed PBB levels in mother and child, father and child, as well as spouse pairs. Scatter plots presented logarithmically on log-transformed PBB levels, for the three types of family pairs were also generated.

People who didn't indicate each other as spouses, but have a child in common, are also considered as a spouse pair. We have 127 mother and child pairs, and 66 father and child pairs. Since some parents have more than one child,three IDs were used to indicate their relationships: the motherID, childID, and pairID. For example, record_id 1153 is the mother of record_id 27, 301 and 624. So for these four people (one mother and three children), they have a same motherID, that is 1153. Each child has a childID which is the same as their record_id. The pairID is the combination of the motherID and the childID. In this family, there are three pairs of mother and child pairs. The pairIDs are 1153_27, 1153_301 and 1153_624. So the mother has three pairIDs for her three children. If the record_ID is equal to the motherID, then this person is the mother. If it equals to the childID, then this person is the child. In this study, 30 mothers and 15 fathers have more than one child identified. When we perform the paired t-tests, it would be biased if parent's PBB levels are used more than one time. Thus, we log-transformed the children's PBB levels, and used the mean of the levels to create a child exposure level representative of all the children to that parent. Making this adjustment satisfies the independence assumption for paired t-tests. As a result, the total number of parent child pairs would be based on the number of parents. So we have 84 mother and child pairs and 44 father and child pairs.

Results:

The total number of family groups in the study dataset is 320, with a mean family size of 6.83. The minimum family size is one, whereas the maximum is 32. Among these 320 family groups, only the families with at least two member have PBB level would be eligible to be included in further analyses. There remains 134 family groups in the study, with a mean family size 10. The minimum family size is two, whereas the maximum is 32. Table 1 shows the summary of all the family groups.

Table 1. The Summary Table for the Whole Family Groups

Family Group	# of family group in total	Mean family size	Min family size	Max family size
Original Family Groups	320	6.83	1	32
Family Groups After Adjustment*	134	10	2	32

* Adjustment: Removed the families in which less than two family members have current PBB levels.

The Table 2 shows the summary information of the family groups by family size. The family sizes are in the range of 2 to 32. Almost half (66/134) of the family sizes are in the range of 4 to 9 family members. Three families have a family size of two and all six members of these families have PBB levels (represented by the 100% with current PBB level in the family), which was the minimum requirement for inclusion in the study. For family sizes of three or four, there are still more than half of the family members that have current PBB level (66.7% and 55.0%, respectively). The percentage of family members with current PBB levels is less than 50% in all other families, whose family sizes are larger than four.

Table 2. The Summary Table for Family Groups of Different Family Sizes

Family size	Total # of families	Total # of family members	Total # of individuals in the families with a current PBB level	% with current PBB level in Family
2	3	6	6	100.0%
3	7	21	14	66.7%
4	10	40	22	55.0%
5	11	55	26	47.3%
6	12	72	28	38.9%
7	11	77	26	33.8%
8	12	96	31	32.3%
9	10	90	30	33.3%
10	6	60	16	26.7%
11	6	66	24	36.4%
12	8	96	33	34.4%
13	9	117	30	25.6%
14	5	70	18	25.7%
15	3	45	7	15.6%
16	7	112	31	27.7%
17	2	34	9	26.5%
19	2	38	8	21.1%
21	2	42	12	28.6%
22	1	22	12	54.5%
23	4	92	21	22.8%
27	1	27	12	44.4%
30	1	30	13	43.3%
32	1	32	11	34.4%
Totals:	134	1340	440	32.8%

Table 3 shows the summary information a different way, by looking at the count of family members that have current PBB levels. We require at least two family members to have current PBB levels to be in the study. There were 63 families that had two family members with current PBB levels (n=126 individuals). Almost half (63/134) of the families have the minimum PBB count (of at least two members with PBB levels). Thirty-three families have three family members with current PBB levels. Seventeen families have four family members with current PBB levels, and nine families have five family members with current PBB levels. The maximum number of family members with PBB levels, within a family is 13. There is only one family that has this maximum number of family members with PBB levels.

The distribution of log-transformed PBB levels of family members is more normal than the un-transformed PBB levels (Figure 1).

Table 3. The Summary Table for Families with Members that Have Current PBB Levels

Number of Family Members Per Family Group with PBB Levels	How Many Families Represented	Total Number of Family Members with PBB Level
2	63	126
3	33	99
4	17	68
5	9	45
6	3	18
7	4	28
8	1	8
11	1	11
12	2	24
13	1	13
Totals	134	440

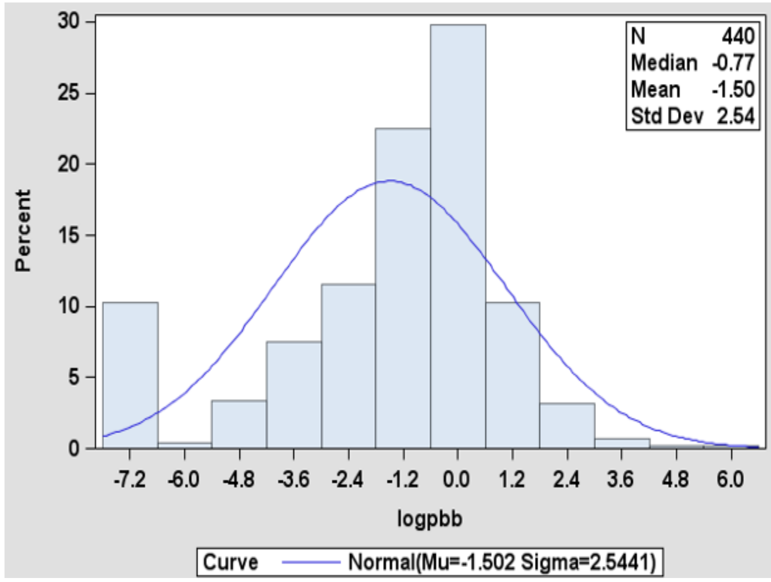

Figure 1, The Distribution of Log-Transformed PBB Level of Family Members

The Figures 2.1-2.4 show examples of family trees for family sizes of 6, 11, 24, and 32 correspondingly. Each number represents the unique ID for an individual person. The direction of the arrows indicates which person reported the other person. If an arrow goes from person A to person B, that means person A mentioned person B in the GHQ. The relationship between them is also noted. The shadowed box means this person has a current PBB level.

Figure 2.1 shows the family tree of family group 15, whose family size is six. There are two people (Record_ID 22 and Record_ID=23) that have current PBB levels, and their relationship is ex-spouses. So in this family, we have identified one spouse pair. Although there are children and siblings in this family, they lack PBB levels and were excluded from further analyses.

Figure 2.2 shows the family tree of family group 10, whose family size is 11. There are four people (Record_ID=15, 16, 21 and 493) that have current PBB levels. In this family, we identified one spouse pair (16 with 493), two mother and child pairs, two father and child pairs (16 and 493 are the parents, 15 and 21 are the children), and one sibling pair (15 and 21). The information on the gender of the parents was also used to confirm relationships.

Figure 2.3 shows the family tree of family group 109, whose family size is 24. There are 13 people (Record_ID=393,232,230,209,210,226,409,394, 407, 215,218,441 and 469) that have current PBB levels. In this family, we identified two spouse pairs (393 with 409, 210 with 230), eight mother and child pairs (210 with 209, 210 with 226, 409 with 210, 409 with 215, 409 with218, 409 with 394, 407 with 441, 407 with 469), five father and child pairs (230 with 209, 393 with 210, 393 with 215, 393 with 218, 393 with 394), and three sibling pairs (209 and 226, 441 with 469, 210,215,218 with 394). For the parents who have more than one child, it will be biased if we use the parent’s PBB level several times in one analysis. Thus, in order to combine the multiple pairs into one pair, we used the mean of the log-transformed PBB levels for the children against the single parent's PBB level.

Figure 2.4 shows the family tree of family group 230, whose family size is 32 and is the largest family in the study. There are 11 persons (Record_ID=438, 436, 448, 460, 582, 450, 437, 2467, 2499, 2520 and 1023) that have current PBB levels. In this family, we identified two spouse pairs (436 with 450, 460 with 582), four mother and child pairs (460 with 2467, 460 with 2499, 460 with 2520, 46 with 1023), one father and child pair (438 with 1023), and two sibling pairs (1023, 2467, 499 with 2520; 436 with 438). Although 460 and 438 are the parents of 1023, we would not consider them as a spouse pair. They didn't mention each other at all, and 582 mentioned 460 as spouse. So we would consider 582 and 460 a spouse pair, rather than 460 and 438.

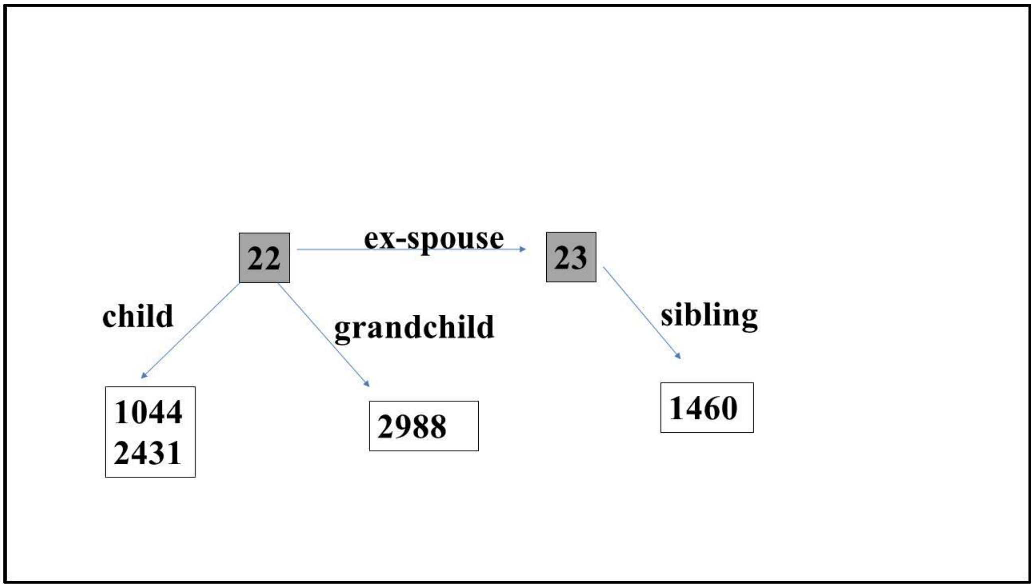

Figure 2.1, Family Tree of Family Group 15 (family size=6)

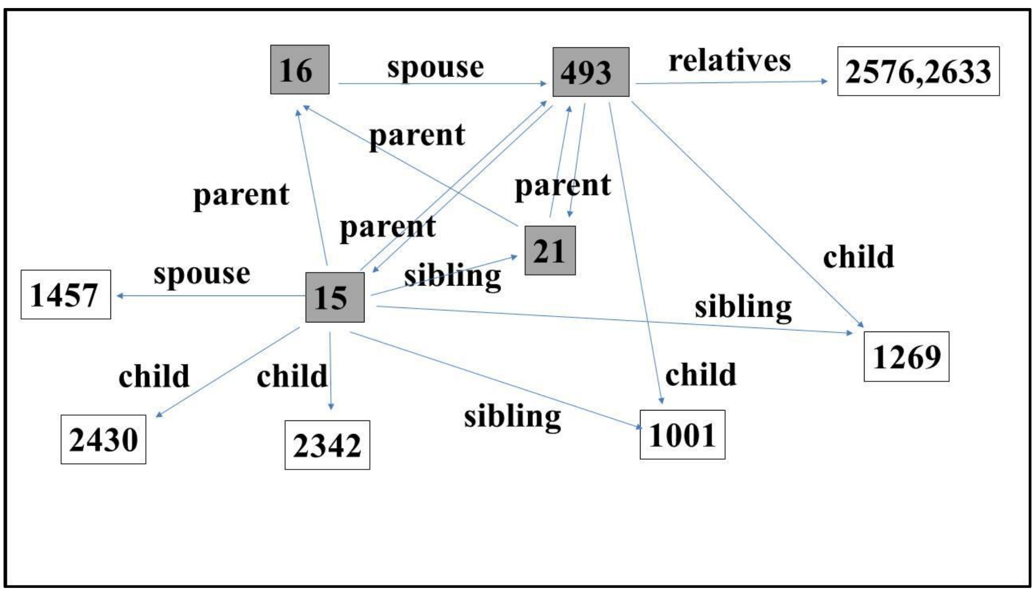

Figure 2.2, Family Tree of Family Group 10 (family size=11)

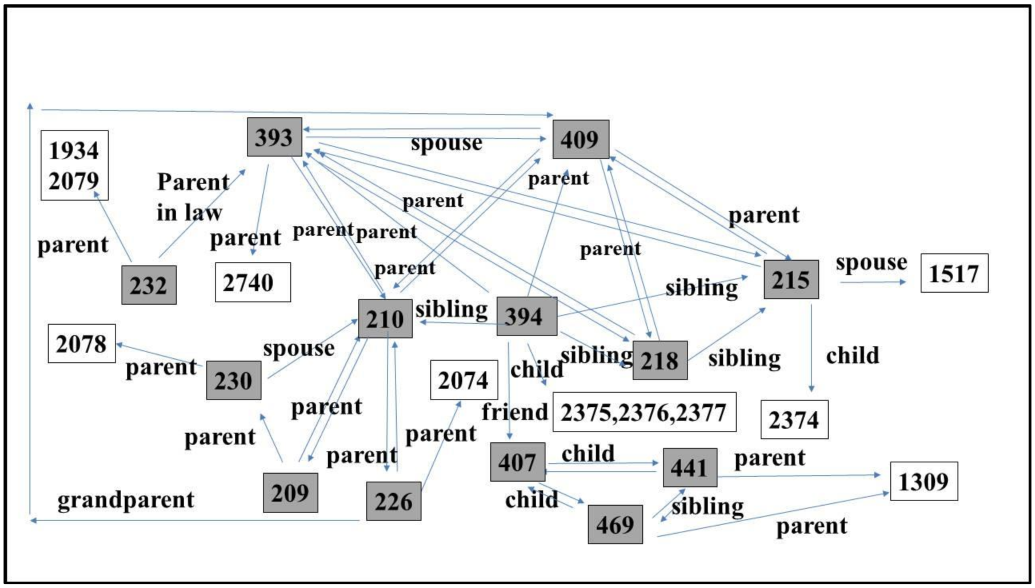

Figure 2.3, Family Tree of Family Group 109 (family size=24)

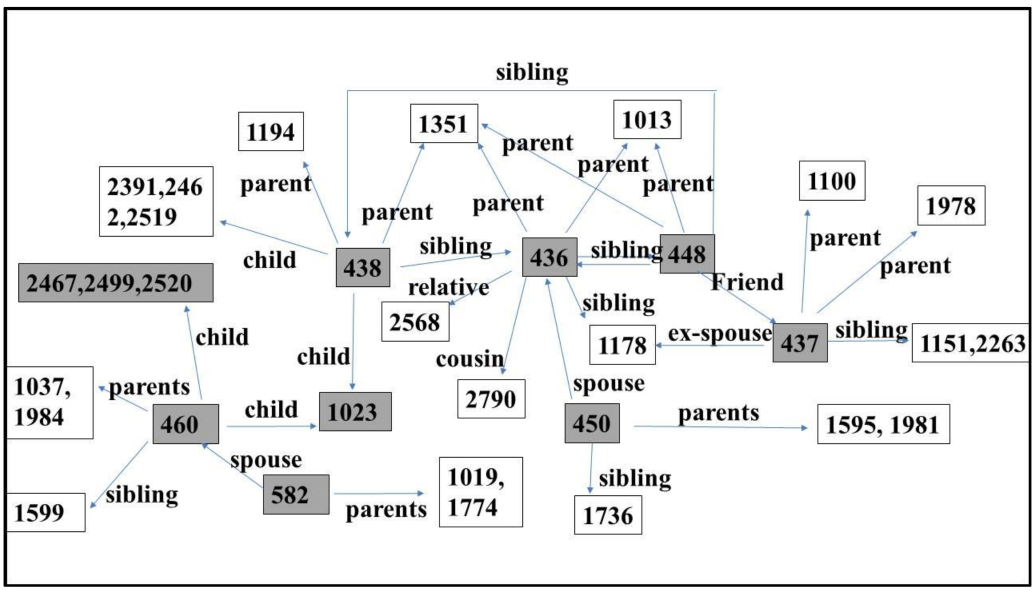

Figure 2.4, Family Tree of Family Group 230 (family size=32)

Table 4 shows the age of the parents and the children in the study at the time of the blood draw. There are 170parents in total. 74 are fathers and 96are mothers. The minimum age is 23. The maximum is 88.4. The median and the mean are 59.2 and 58 respectively. For fathers, the minimum and maximum ages are 26 and 88.4, respectively, the median is 62.3, and the mean is 59.5. For mothers, both the minimum and the maximum ages are slightly lower than the fathers. There are 126 children in total. Forty-five are males and 82 are females. The minimum age is 7.2. The maximum is age 75.6. The median and the mean ages for male and female children are 34.6 and 36.1, respectively. For males, the minimum and maximum ages are also 7.2and 75.6, respectively, the median is 36, and the mean is 37.2. For females, the minimum and the maximum ages are 8 and 63 respectively. The Median and the mean ages of the female children are less than the male children.

Table 4. The Ages of Parents and Children

	Parents			Children		
	Total	**Male**	**Female**	**Total**	**Male**	**Female**
N	170	74	96	126*	45	82
MIN	23	26	23	7.2	7.2	8
MAX	88.4	88.4	86.6	75.6	75.6	63
MEDIAN	59.2	62.3	54.9	34.6	36	32.2
MEAN	58	59.5	56.8	36.1	37.2	35.5

* One female child didn't have the date of birth on record. She was excluded for this table.

Table 5 shows the summary information for family pairs. There are 63 spouse pairs, and 74 sibling pairs. The original number of mother and child pairs was 127, and for the father and child pairs was 66.After combining multiple children's current PBB levels into one to make a new child PBB level, the number of mother and child pairs is 84, and the number of father and the child pairs is 44.

The distributions of log-transformed PBB levels of mother, father and child are shown in Figure 3 to Figure 5 respectively. The distribution of the mother's and the father's PBB levels are more normally distributed, compared to the distribution of the children's PBB levels. Mainly this is because there are more children with PBB levels below the LOD than mothers and fathers.

Table 5. The Summary Table for the Family Pairs

	# of Pairs	# of Parents
Spouse Pair	63	NA
Sibling Pair	74	NA
Mother and Child Pair	127	84
Father and Child Pair	66	44

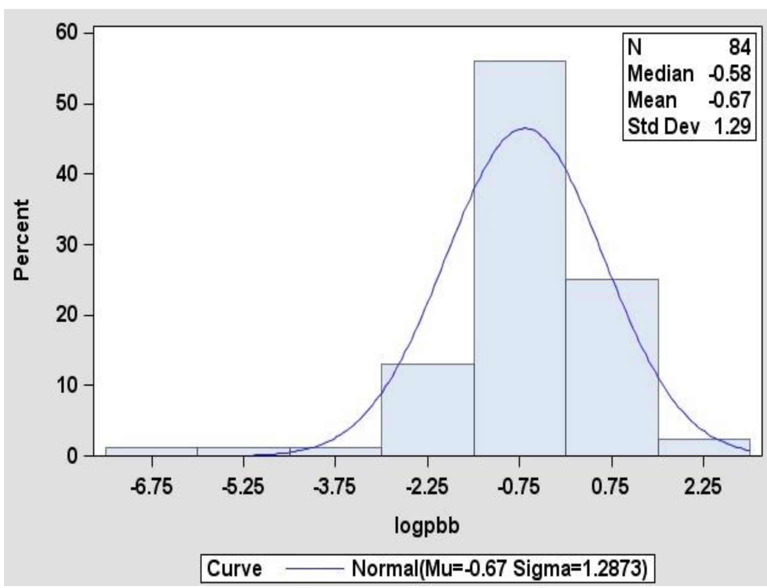

Figure 3. The Distribution of Log-Transformed PBB levels of Mothers

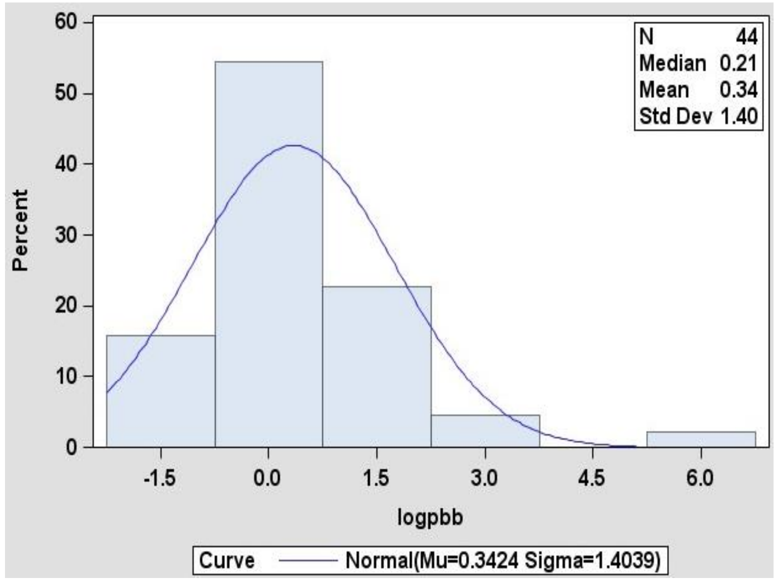

Figure 4. The Distribution of Log-Transformed PBB levels of Fathers

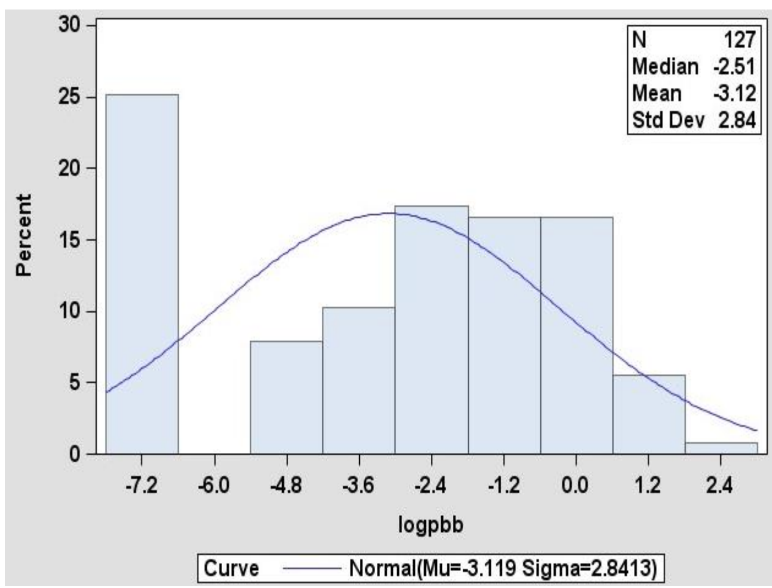

Figure 5. The Distribution of Log-Transformed PBB levels of Children

Table 6.1 shows the current PBB levels of family pairs. There are 63 husbands and 63 wives. 1.59% of their current PBB levels are below the limit of detection (LOD). All of the quantiles of the husbands are higher than that of wives. The mean difference of their log transformed PBB levels is 0.475, with a p-value of 0.018 (Table 6.2). A highly significant Pearson correlation coefficient was also observed. It is 0.635, with a p-value less than 0.001 (Table 6.2). The R-square from the regression analyses is 0.404 (Table 6.2). The relationship for them is shown in Figure 6.

There are 84 mother and child pairs. 1.19% of the mothers' current PBB levels are below the LOD. The percentage of the children with PBB levels below the LOD is 23.81%. All of the quantiles of the mothers are higher than that of children. The mean difference of their log transformed PBB levels is -2.678, with a p-value less than 0.001 (Table 6.2). A highly significant Pearson correlation coefficient was also observed. It is 0.388, with a p-value of 0.0003 (Table 6.2). The R-square from the regression analyses is 0.151 (Table 6.2). The relationship for them is shown in Figure 7.

There are 44 father and child pairs. All of the fathers' current PBB levels are higher than the LOD, while 13.64% of the children's current PBB levels are below the LOD. Similarly as the mother and child pairs, all of the quantiles of the fathers are higher than that of children. The mean difference of their log-transformed PBB levels is -2.728, with a p-value less than 0.001 (Table 6.2). A highly significant Pearson correlation coefficient was also observed. It is 0.399, with a p-value of 0.0072 (Table 6.2). The R-square from the regression analyses is 0.160 (Table 6.2). The relationship for them is shown in Figure 8.

Table 6.1 Current un-transformed PBB Levels of Family Pairs

	Husbands	Wives	Mothers	Mothers' Children Group	Fathers	Fathers' Children Group
N	63	63	84	84	44	44
% Below LOD*	1.59%	1.59%	1.19%	23.81%	0	13.64%
25% Quantile	0.299	0.263	0.329	0.002	0.641	0.014
Median	0.809	0.49	0.559	0.070	1.238	0.233
75% Quantile	2.101	1.059	1.055	0.455	2.403	0.849
90% Quantile	3.979	2.426	1.690	1.186	5.758	2.036

*LOD: The limit of detection.

Table 6.2 Analyses of the Log-Transformed PBB Levels of Family Pairs

Pair	Number of Pairs	Pearson Correlation Test		T-Test			Regression Analyses
		Coefficient	p-value	Mean Difference	Test Statistic	p-value	R-square
Spouses	63	0.635	<0.001	0.475	2.44	0.018	0.404
Mother Child	84	0.388	0.0003	-2.679	-9.63	<0.001	0.151
Father Child	44	0.399	0.0072	-2.728	-6.84	<0.001	0.160

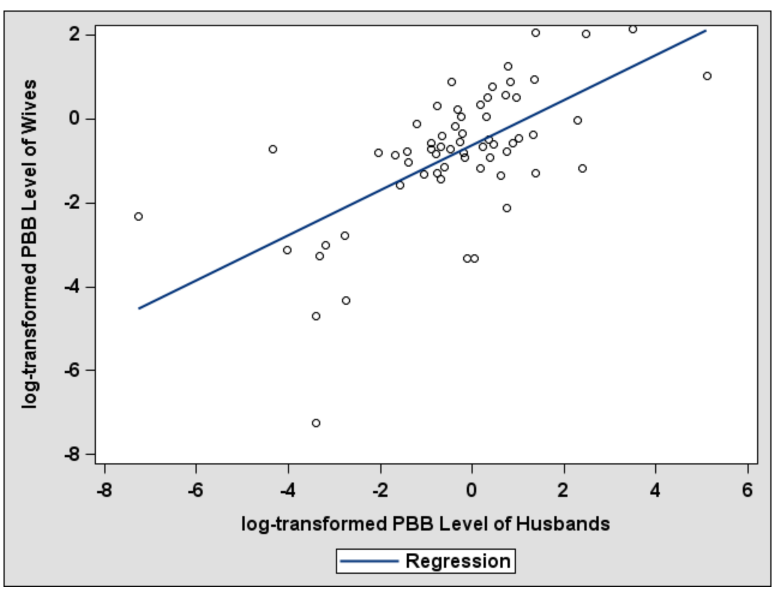

Figure 6, Relation of Log-Transformed PBB Levels for Husbands with Wives

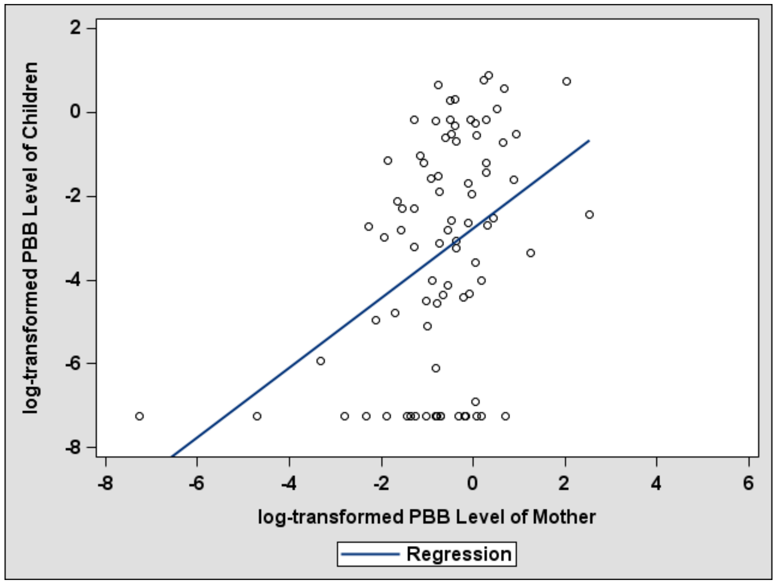

Figure 7, Relation of Log-Transformed PBB Levels for Mothers with Children

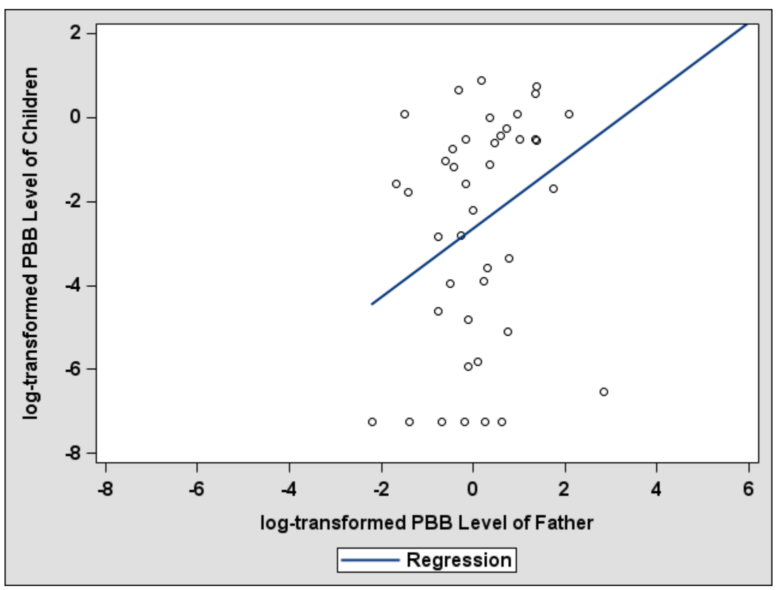

Figure 8, Relation of Log-Transformed PBB Levels for Fathers with Children

Discussion:

The mean family size of the family groups after adjustment seems to be much larger than that of the original family groups (6.83 versus 10). It is because that the larger family groups would be more likely to have at least two family members with current PBB levels. The mean family size increases after excluding the families where only one family member had a PBB level, which are also the families with smaller family sizes.

The mean of current PBB levels of parents are significantly higher than the children. On one hand, this reflects PBB levels are correlated with age because the old people were likely to eat contaminated food. One the other hand, since PBB can pass from one generation to the other, it explains why parents have higher PBB levels than their children, and why their current PBB levels are significantly correlated.

In John E's study [10], although they did not look at the exposure of DDE in family units, they found the DDE levels were increasing with age. In the infant group, no zero value of DDE levels was observed, confirming the contribution of prenatal exposure of DDE to the pesticide body burdens of these children.

The result of the t-tests in this study shows that the mean difference between fathers and children is larger than that between mothers and children. Compared to fathers, mothers' PBB levels are more likely to affect the children's PBB levels, by means of breastfeeding or in utero. Although fathers may still have effects on children's PBB levels, which may be related to genetic factors, the correlation between fathers and children may be confounded by mothers PBB levels.

The major route of exposure to PBB was probably related to consuming contaminated food for those born before the exposure period [2]. Thus people living in the same household tend to have similar PBB levels. We did not have the household information during the exposure periods for participants to test this. However, interestingly, we found a significant correlation between spouse pairs that was higher than that of parent-child pairs, and found that the mean difference between spouses is significant. This may be due to the fact that the spouses were both residents of Michigan during the exposure period, although we cannot confirm this. PBB exposure was state-wide across Michigan in the 1970s. Because females can pass some amount of the PBB to her child through breastfeeding and in utero and females have more adiposity, this may explain why the females' current PBB levels are significantly lower than the males' current PBB levels in spouse pairs.

In Wolff's study[2], they used log-transformed PBB levels, to perform the regression analyses between spouse pairs, mother and daughter pairs, and father and son pairs. A highly significant linear regression coefficient was observed for husbands versus wives ($r^2 = 0.93$, n=60), sons versus fathers ($r^2 = 0.97$, n=73), and daughters versus mothers ($r^2 = 0.71$, n=64). This study was conducted in 1976. Those husbands and wives were more likely to be in the same house hold at exposure, since the study was done closer to the end of the exposure period versus ours, which was done so many years out. Thus, their PBB levels were closer to the time of exposure. In our study, we also plotted the log-transformed PBB levels, between spouse pairs, mother and child pairs, father and child pairs. The PBB levels tended to cluster in a certain range in both studies. Increases in husbands' PBB levels seems to be related to increases in wives' PBB levels. Same relationships are also observed in mother and child pairs, and father and child pairs.

The R-squares of log-transformed PBB levels from the three types of pairs in our study are much smaller than that in Wolff's study. One of the reasons is that a large amount of children's PBB levels are below the LOD in our study. Besides, the population and the time to when the data were collected were many years apart.

In conclusion, females tended to have lower PBB levels than males in spouse pairs, and have almost identical impact on children's PBB levels as males. Breastfeeding can be reduced to protect children from more exposure for this class of chemicals. The correlation of PBB levels between fathers and children pairs needs to address the mothers' PBB levels as a confounder in a future study.

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Appendix:

Layout 1: The original layout of data

Record_id	Name	……	Mother	Father	Child	spouse	……
1	A		B		C	D	
2	B				A		
3	C		A	D			
4	D				C	A	
……							
647	XX		……	……	……	……	

Layout 2: The layout after assign every one an unique record_id

Record_id	Record_linkage	Name	Relationship	……
1	1	A	self	
1	2	A	mother	
1	3	A	child	
1	4	A	spouse	

Layout 3: The layout after assign everyone an unique record_id, and family group number

Record_id	Record_linkage	Name	Relationship	Familygroup NO	……
1	1	A	self	1	
1	2	A	mother	1	
1	3	A	child	1	
1	4	A	spouse	1	
2	1	B	child	1	
2	2	B	self	1	
3	1	C	mother	1	
3	3	C	self	1	
3	4	C	father	1	
4	1	D	spouse	1	
4	3	D	child	1	
4	4	D	self	1	
……	……	……	……	……	

Layout 4: The final layout with family pair ID

Record _id	Record_ linkage	Name	Relationship	…	Sp_pairID	Mc_PairID	Fc_PairID
1	1	A	self				
1	2	A	mother			2_1	
1	3	A	child			1_3	
1	4	A	spouse		1		
2	1	B	child			2_1	
2	2	B	self				
3	1	C	mother			1_3	
3	3	C	self				
3	4	C	father				4_3
4	1	D	spouse		1		
4	3	D	child				4_3
4	4	D	self				
……	……	……	……	…	……	……	……

Figure 1, The Process to Get The Family Group Number, And The Family Pair ID