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A decay model to assess paternal levels of polybrominated biphenyls among men in the Michigan long-term PBB study at the time of their sons' conception.

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By

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B.S., Georgia Institute of Technology, 2010

Thesis Committee Chair: Michele Marcus, MPH, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2013

Abstract

A decay model to assess paternal levels of polybrominated biphenyls among men in the Michigan long-term PBB study at the time of their sons' conception.

By Michelle I. Leon

The continual use of synthetic chemicals with hormonal or anti-hormonal properties in consumer products has raised concerns for human health in the scientific community. The Michigan Long-Term PBB Study was established in 1976, after more than 4,000 Michigan residents consumed polybrominated biphenyl (PBB)-contaminated farm products in the early 1970s. Between 1976 and 1993, serum samples were collected from cohort members and analyzed for PBB. Measurable levels of PBB were detectable even 20 years after the industrial incident. The creation of this cohort provides an unprecedented opportunity to study the elimination and long-term effects of PBBs and their congeners. This study investigates the effect of covariates on the rates of serum PBB decay among 904 male participants. A general linear mixed model approach was used to develop the decay model which was ultimately used to estimate *in utero* PBB levels of offspring born to father's in the study. Time-independent covariates retained in the decay model were BMI at enrollment and having had a weight change of greater than 10lbs from 1973 (exposure) to enrollment. Time since exposure, an interaction variable between BMI and time and a time-squared ($Time^2$) variable were the time-dependent covariates retained in the decay model. Higher initial BMI was associated with a slower rate of decay; having had a weight change of greater than 10lbs since 1973 (exposure) to enrollment was associated with a faster rate of decay compared to those who did not have this fluctuation in weight since exposure. The predictive performance of the decay model was evaluated by comparing results derived from this decay model to observed participant serum PBB levels collected in 1991-1993. There was a strong association between the predicted and observed log(PBB) levels (Spearman Correlation Coefficient, $\rho_s = 0.80$). Serum PBB concentration levels were predicted for 134 offspring at the time of their conception. The median estimated *in utero* PBB concentration level was predicted to be 6.7 ppb and ranged from 1.0 ppb to 1535.5ppb.

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Acknowledgements

I am very grateful to Dr. Michele Marcus, lead scientist of the Michigan PBB Registry, for the opportunity to have carried out my thesis under her guidance. A special thanks to Metrecia Terrell, Biostatistician and Data Manager for the Michigan PBB Registry, for all of her patience, support and instruction on this project.

This thesis is dedicated to Mami, Papi, Michael, Lilita, Rosita and Ha. Thank you for all of your encouragement and love during this adventure.

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Introduction

Synthetic chemicals with hormonal or anti-hormonal properties are frequently used in consumer products. Their continual use has raised concerns for human health in the scientific community (Birnbaum and Staskal 2004, Sjodin, Jones et al. 2004). One classification of such chemicals are the halogenated organic flame retardants, such as brominated flame retardants (BFRs), which are currently the largest market group of flame retardants produced. There are more than 75 different, commercially recognized brominated flame retardants and in a represented sample from NHANES 2003-2004, it was found that over 90% of the population had detectable levels of BFRs (Sjodin, Wong et al. 2008). Limited toxicological studies have been done on them despite their structural similarity to other persistent organic pollutants such as polychlorinated biphenyls (PCBs). BFRs are similar to PCBs in chemical structure, lipophilicity, bioaccumulation and environmental persistence (Birnbaum and Staskal 2004).

From 1973 to 1974, residents of the state of Michigan were exposed to PBBs polybrominated biphenyls (PBBs) when FireMaster FF-1 (a mixture of PBBs) was accidentally mixed with animal feed in place of a magnesium oxide feed supplement, NutriMaster (Eyster, Humphrey et al. 1983, Miceli, Nolan et al. 1985, Lambert, Schoeller et al. 1990, Small, DeCaro et al. 2009, Small, Terrell et al. 2009). This contamination resulted in loss of livestock due to their development of symptoms of acute toxicity. Residents in the surrounding communities were exposed to PBBs via the consumption of meat, milk and other tainted animal byproducts. In 1976, the Michigan Department of Community Health (MDCH), in collaboration with the US Public Health Service enrolled

individuals who were residents of quarantined farms or who consumed contaminated food products to assess potential short and long-term health effects (Terrell, Berzen et al. 2009). This cohort, officially known as the Michigan Long-Term PBB Study, has now been followed for more than 30 years, three generations, providing an unprecedented opportunity to study the long-term effects of PBBs and their congeners.

Several epidemiological studies suggest that there has been an increase in human male reproductive disorders in industrialized nations (Toppari, Kaleva et al. 2001, Bay, Asklund et al. 2006, Small, DeCaro et al. 2009). Recent studies have investigated the transgenerational endocrine effects of PBB exposure. It has been proposed that *in utero* exposure to environmental contaminants that mimic or antagonize endogenous hormonal activity are related to the increase in genitourinary disorders (Bay, Asklund et al. 2006, Birnbaum and Cohen Hubal 2006, Sharpe 2006).

Small et al. (2009a) found an association between reporting genitourinary conditions among male children and maternal enrollment PBB concentration. It has also been suggested that there is an association between *in utero*-exposure to PBBs and delayed pubertal development(Small, Terrell et al. 2009). Terrell et al. (2009) found an overall increase in the proportion of male offspring born to parents who both had high PBB exposure compared to parents with low PBB exposure. Interestingly, in models where only one of the parent's exposures was evaluated, there was a suggested increase in the odds of male birth for paternal PBB; however, this was not seen for maternal PBB exposure. Therefore, it is of interest to develop a mathematical model to estimate the relative concentration of environmental contaminants of both parents at the time of conception in relation to the manifestation of genitourinary disorders.

The United States stopped producing PBB in 1977; however, measurable serum PBB concentration levels persist in the Michigan cohort, due to its long half-life. With the availability of multiple exposure measurements collected over time, it is possible to use statistical modeling to assess the relationship between exposure outcomes and covariates. The development of a decay model will allow for the determination of the influence of covariates on PBB elimination over time and the efficient estimation of the concentration of PBB for future research (Terrell, Manatunga et al. 2008).

The majority of studies that have been conducted to evaluate the serum concentration of PBB have used simplistic techniques that only require two PBB concentration measurements, ignoring time-dependent covariates that may influence the rate of elimination of PBB from the body (Lambert, Schoeller et al. 1990, Rosen, Flanders et al. 1995, Blanck, Marcus et al. 2000, Sweeney, Symanski et al. 2001). Others that do allow multiple measurements do not account for the difference in the number of measurements between participants as well as the correlation within participants when multiple measurements were taken. Failure to address the covariance, or lack of independence, between multiple measurements for a subject may lead to an inflation of Type I error rate for time-independent variables (i.e. gender, race, treatment, etc.), may lead over estimation of the Type II error rate for time-dependent variables and underestimation of group effects(Patetta and Marovich 2002). Terrell et al. (2008) approached the limitations listed above by developing a decay model that implemented general linear mixed model for the serum PBB levels of women in the Michigan Long-Term PBB Study. This approach takes into account the number of observations between participants, as well as the correlations within participants and correctly adjusts for time-

dependent covariates. The mixed model obtains strength across all individuals to provide more reliable and precise estimates of individual decay rates when the between-subject variability is large, relative to the within-subject variability (Terrell, Manatunga et al. 2008).

The Terrell et al. (2008) study used the following inclusion criteria: female participants of the Michigan Long-Term PBB Study who were born before July 1, 1973; who were at least 16 years of age at initial measurement or who had two or more serum PBB measurements at or after the age of 16; who had two or more serum PBB measurements that were taken when the woman was not pregnant or while breastfeeding, where there were at least 6 months between the initial and successive measurements and who had initial PBB serum levels that were ≥2 ppb (n = 406). Covariates of interest included: age, BMI, smoking and pregnancy-by-breast-feeding. The decay model was validated by comparing predicted serum PBB concentrations calculated using the mixed model approach as described in this study to those predicted by the Ordinary Least Squares (OLS) two-stage modeling method used by Blanck et al. (2000) for a subset of the data that consisted of women with a minimum of three measurements. The study validated that the mixed-effects model better predicted serum PBB concentration than the model developed by Blanck et al. (mixed effects model, $r = 0.93$; OLS two-stage model, $r = 0.86$; $P<0.0001$).

The objective of this thesis is to demonstrate that the linear mixed model procedure used by Terrell et al. (2008) for women in the Michigan Long-Term PBB Study can be applied to longitudinal data of male participants of the Michigan Long-

Term PBB Study in the presence of time-independent and time-dependent covariates shown to have subject matter-importance as well as their respective interactions.

Methods

Study Population

Male participants were eligible to be included in the study if they were born before the date of contamination (July 1, 1973), had a minimum of two measurements taken at the age of 18 years or older, had measurements taken that were at least 6 months apart and where initial PBB serum levels were at least 2 parts per billion (ppb). Based on these criteria there were 904 men eligible for the study.

Serum PBB Samples

Serum PBB samples have been collected over 19 years (from 1974 – 1993) for male participants of the Michigan PBB Registry. All samples were taken from non-fasting participants and PBB concentration levels were not lipid adjusted. The level of detection (LOD) for serum PBB measurements was 1.0 ppb. Measurements below the level of detection were set to 0.5 ppb, half of the limit of detection (Hornung and Reed 1990).

The procedures by which serum concentrations have been determined have changed throughout the years. Sera collected between 1976 and 1988 were analyzed by the MDCH Bureau of Laboratories, using packed-column electron capture gas chromatography. The calibration standard used for the packed-column method was FireMaster BP-6 (standard during January – July 1976). Later, FireMaster FF-1, consisting of a mixture of BP-6 with an added 2% anti-caking agent, was used. The main congener in FireMaster FF-1 is 2,2’,4,4’,5,5’-hexabromobiphenyl, or PBB-153. The

serum samples were denatured, extracted with 1:1 petroleum ether-ethyl or 1:1 hexane-ether and chromatographed through either a Florisil or Florisil and silica gel column. Quantitation was performed by comparing the chromatography peak size with the peak size of a control sample of FireMaster FF-1.

PBB serum samples collected between 1976 and 1978 were analyzed for PBB alone; however, later samples (1978-1988) were analyzed using a modified combination method designed to identify and quantify PBB and polychlorinated biphenyl (PCB)(Needham, Burse et al. 1981). The latter method was also used to quantify and identify PBB, PCB as well as several pesticide concentrations (Price, Welch et al. 1986, Brock, Burse et al. 1996). There was high reproducibility found in samples analyzed using this method (r=0.998)(Burse, Needham et al. 1980, Needham, Burse et al. 1981, Kreiss, Roberts et al. 1982).

From 1991 to 1993, PBB serum samples were analyzed using capillary gas chromatography with electron capture detection, allowing for the separation the congeners of PBB and PCB and determine the concentration of specific pesticides (Najam, Korver et al. 1999, Humphrey, Gardiner et al. 2000). An automated integration program and a comparison of peak areas and retention times with those of a known standard PBB-153 were used to quantify PBB levels. The quality of this procedure was verified through analysis of duplicate samples and a set of spiked control samples. Quality control consisted of adding a known quantity of PBB-155 to each sample. Samples with concentration levels outside of the 95% confidence level for the spiked material were reanalyzed; 1% of the samples were reanalyzed.

The MDCH Bureau of Laboratories described the relationship among the three different standards used during the PBB study (BP-6, FF-1 and PBB-153). It was calculated that samples analyzed with BP-6 as the standard, 1ng/g BP-6 = 0.53ng/g PBB153 and for samples analyzed with FF-1 as the standard, 1ng/g FF-1 = 0.60ng/g PBB-153 (ppb=ng/g).

Data Description

Data for this study comes from the PBB records of male participants of the Michigan Long-Term PBB Study, as described by Terrell et al. (2008) and Blanck et al (2000). The exposure samples were captured from the PBB exposure database, which contained the date on which the sample was taken and serum PBB level for the respective measurement. A variable for total number of samples per individual was also created from the PBB exposure data. The enrollment questionnaire which was administered between 1976 and 1979 (with a few participants completing it in 1983-1984), was used to obtain date of birth, smoking status at enrollment, height, weight at enrollment and weight change of more than ten pounds since exposure. Date of birth was used to calculate age at exposure and age at measurement, where the exposure date was defined as July 1, 1973. A follow-up interview in the 1990's (1991 - 1993), which coincided with the latest PBB measurement dates, was used to obtain additional weight variables: weight at follow-up interview and loss of at least 10% of body weight from PBB study enrollment to follow-up. BMI (kg/m^2) at enrollment and at follow-up was calculated for each participant, if the male was age$\geq$18 at enrollment, using height at enrollment and

weight from enrollment or follow-up. All of these variables from different sources were merged to create a single dataset referred to here as the PBB dataset.

Each subject within the PBB dataset has a unique identification number. The PBB dataset contains multiple serum PBB measurements per male, distinguishable by date of measurement. All participants had to have a minimum of two measurements. Men had varying differences in the number of measurements and the time between measurements. Men with high initial PBB levels may have had more measurements in the registry as a result of being included in multiple studies over the years.

The outcome variable, PBB level, was continuous and measured in parts per billion (ppb). Evaluation of the distribution of PBB levels of male participants were checked graphically. Previous studies have shown that PBB serum measurements are positively skewed, indicating that log transformation is appropriate. Histograms for initial serum PBB levels and all serum PBB measurements regardless of time were created with and without the log transformation; this data coincided with previous observation of the distribution of serum PBB levels.(Terrell 2004).

A time variable was also created to represent the time from PBB exposure (date of July 1, 1973) to PBB measurement date. The other variables considered in the statistical models are time independent covariates. Covariates Age at exposure and BMI were evaluated as both continuous and ordinal variables. As continuous variables, Age at exposure and BMI were measured as differences from mean age at exposure and mean BMI, respectively. As an ordinal variable, Age at exposure was divided into tertiles: <25 years of age, 35-44 years of age (reference) and >44 years of age. BMI categories were: underweight/normal (reference, BMI <25), over-weight ($25 \leq$ BMI <30) and obese

(BMI≥30), interpreted according to Centers for Disease Control (CDC) classification (CDC 2011). Smoking status at enrollment was categorized as non-smoker (reference), past cigarette/cigar/pipe smoker or current cigarette/cigar/pipe smoker.

A separate sub analysis utilized a time-dependent BMI variable, categorized to describe whether or not a participant had no change in BMI (from enrollment to follow-up), an increase of ≥5% of their BMI (reference, n = 182) or a decrease of ≥5% of their BMI (n = 38) from enrollment to follow-up. A second sub analysis used a variable describing whether or not the participant had lost 10% of their body weight from enrollment to follow-up.

Statistical Analysis

A linear mixed model was used to address the repeated nature of the data, the variability in the number of measurements as well as the time between measurements. The mixed model, using the notation of Verbeke and Molenberghs (2000), is as follows:

$$Y_{ij} = \beta_0 + b_{0i} + \beta_1 + b_{1i} * t_{ij} + \varepsilon_{ij} \qquad (1.0)$$

This model takes into account the variation that exists in the intercepts and slopes among each individual. In Model 1.0, the exposure of interest is denoted by Y_{ij}, β_0 and $\beta_1$represent the fixed effects, and random effects b_{ij} and ε_{ij} are assumed to be independent, where $b_i = b_{il} \dots b_{in_i} \sim N(0, D)$ and $\varepsilon_i = \varepsilon_{il} \dots \varepsilon_{in_i} \sim N(0, \sum_i)$, are normal with mean zero and variance components D and $\sum_i$, respectively. Dependent on the random effect b_i, $Y_i = (Y_{il} \dots Y_{in})$ is normally distributed with mean vector $(X_i\beta +$

$Z_i b_i$) and covariance matrix $\sum_i$, where X_i and Z_i are the appropriate fixed and random effects design matrices. Therefore, based on (1.0) the predicted exposure level for a person at time *t* becomes:

$$Y_{ij} = (\beta_0 + b_{0i}) + \ \beta_1 + b_{1i} \ * t \qquad (1.1)$$

Model 1.1 can be generalized to include other fixed covariates that may or may not interact with time. Parameter estimates for model (1.1) and its generalizations are based on maximum likelihood estimation (MLE) or restricted maximum likelihood estimation (REML). Predicted estimates using the mixed models are based on a combination of fixed and random effects, resulting in estimates that are the best linear unbiased predictors (BLUP)(Geert and Geert 2000, Terrell 2004).

Building the Decay Model

The development of the main decay model included 904 men. Exploratory graphs were first created to observe trends in PBB levels over time among participants by age, smoking status at enrollment, BMI at enrollment and having had a weight change more than 10lbs since 1973. Similarly, trends of PBB levels over time for covariate subgroups were observed; missing values were excluded from these graphs. A saturated linear mixed-effects model was then fit with the created time variable intercepts for , all covariates (age, smoking status at enrollment, BMI at enrollment), and interaction terms (slope parameters) of each covariate and time (age*time, smoking status at enrollment*time, BMI at enrollment*time, time and a time-squared term (time^2) as

potential predictors. The saturated model was used to test for random-effects covariance structures. In addition to random intercepts, it was found to be appropriate to include a random slope in the decay model. Akaike information criteria(Akaike 1974) were used to compare models fit with various residual covariance structures. The spatial exponential structure was selected as the residual covariance structure because of its ability to improve the fit of the model and adjust for the unequal spacing among measurements appropriately(Littel, Miliken et al. 1996). A backward elimination approach was used to obtain a reduced model. All covariates significant at α=10% level were retained in the model.

Validating the Model

The mixed effect decay model was validated by comparing predicted serum PBB concentration levels to actual serum PBB measurement levels from a subset of the study population. Men with at least three serum PBB concentration measurements, one of which was collected between 1991 and 1993 (n=316 men), were included in the subset population. Serum PBB concentration measurements collected between 1991 and 1993 were first excluded from the dataset. The model was then re-estimated using 2163 serum PBB concentration measurements taken between 1974 and 1987 for individuals included in the subset population. Model re-estimation was conducted using the mixed-effects modeling procedure previously described in the Methods section. The re-estimated model was used to predict serum PBB concentration at time t_i*, time (years) from exposure to the date at which the 1991-1993 serum PBB sample was collected for the *i*th subject. Spearman's correlation coefficient was calculated for the observed and predicted

serum PBB concentration. A scatter plot of the observed and predicted serum PBB concentrations was also created.

Prediction of Offspring's Paternal in utero PBB exposure

Estimated serum PBB concentration levels at the time of the sons' conception were predicted for fathers enrolled in the Michigan Long-Term PBB Study using the mixed effect decay model described above (n=134 offspring). Offspring were initially reported by the mother in reproductive health questionnaires or identified by matching demographic information of mothers within the cohort that were born before July 1973 to maternal information in the Michigan electronic birth files. Paternal relationships were then matched using cohort registry records and checked with copies of the birth certificate. This data was obtained from studies that have undergone human subjects review and approval by IRBs at the Michigan Department of Community Health and Emory University. Informed consent was obtained from all participants. The conception date was calculated from the gestational age reported for each offspring in the mother's reproductive health questionnaires or from the offspring's birth records. All analyses were conducted in SAS 9.3, and models were fit using the mixed procedure (SAS Institute, 2012).

Results

Descriptive characteristics and exposure details for the 904 participants included in the study are shown in Table 1. The average age at exposure was 35 years; 34% of the men were between ages 25 and 44years at exposure. At the time of enrollment, the majority of participants were classifiable as either underweight/normal (43%) or overweight (40%), and were non-smokers (47%). Most men had three or fewer PBB measurements (79%) over the course of the study. The majority of men had a total of two (46.2%) or three (32.9%) PBB measurements collected. Initial serum PBB measurements were obtained between 1974 and 1987, with a median exposure PBB level of 6.0 ppb. The majority of initial measurements were above the LOD (83.3%). Only 3.18% of subsequent PBB measurements were below the limit of detection (<1 ppb, n=53 of 1665 samples). Approximately forty percent of men (n = 361) had serum PBB measurements collected between 1991 and 1993, with the majority of PBB concentration PBB measurements in this time bracket still being above the level of detection.

Figure 2 contains the individual profiles of the log-transformed serum PBB concentration levels for men in the study population (n = 904). Overall, there does appear to be a slight decline in serum PBB concentrations over time, with greater variability between subjects than within subjects. Initial serum PBB concentration levels for men in the study population ranged between 2.0 and 1900.0 ppb, justifying the need for a random intercept to be included in the decay model.

The final mixed-effects model is displayed in Table 2. Non-significant intercepts were removed from the model, as were non-significant interactions between covariates.

An overall intercept and overall slope as well as random intercepts and random slopes for individual were retained in the model. The final model covariate estimates are representative of their effects on the initial log (PBB) level (intercepts) or the rate of serum PBB decay (slopes). There is a significant decline in log (PBB) values over time. A time-squared ($Time^2$) variable was also found to be significantly associated with the decay rate of log(PBB) (Table 2). A male participant with an initial BMI of 25 kg/m^2, who did not smoke at the time of enrollment into the Michigan Long-Term PBB Study and, who had not had a weight change of greater than 10 lbs since 1973 had, on average, a decay rate of -0.1188 log(PBB) ppb per year (Table 2). For every kg/m^2 increase above the mean BMI, there was a 0.0017 increase in the rate of decay of log(PBB). There was a decrease in the initial PBB level by -0.2230 log(PBB) ppb for having had a weight change of greater than 10lbs from 1973 to the enrollment date.

` As an alternative to the final mixed-effects model, the continuous BMI variable was replaced with a categorical BMI variable. BMI was categorized as 'Underweight/Normal' (BMI<25), 'Overweight' (25≤BMI<30) or 'Obese' (BMI≥30. This categorical BMI variable remained significant, suggesting that those who were classified as overweight at enrollment had an increase in their log(PBB) decay rate by 0.0089ppb and those classified as obese at enrollment had an increase in their log(PBB) decay rate by 0.0124ppb.

Two sub-analyses were conducted, through the development of secondary decay models that used data from 319 male participants. Participants of the sub-analyses had an enrollment and a follow-up BMI measurement (taken between 1991 and 1993). These models consisted of all covariates retained after the backwards elimination process of the

development of the main decay model, in addition to either an interaction term between the time and the time-dependent BMI variable [Time*BMI (change of ±5%)] or an interaction term between time and a categorical variable for having lost at least 10% of body weight from the time of enrollment to follow-up [Time*lost 10% of body weight].

Participant height and weight was not collected at regular intervals; therefore, BMI was not able to be treated as a true time-dependent covariate. In the first sub-analysis, the effect of BMI over time was calculated using a categorical variable that describes whether or not a participant had an increase of ≥5% of their BMI (reference, n = 182) or had a decrease of ≥5% of their BMI (n = 38) from enrollment to follow-up (1991 – 1993). Individuals who had less than a 5% change in BMI in either direction or for whom information was missing were categorized together. This model indicates a slight increase in the rate of decay of log(PBB) by 0.0062ppb per year for persons who lost at least 5% of their initial BMI, compared to those whose BMI increased by at least 5%, at the time of their 1991-1993 measurement (Table 3). Alternatively, the second sub-analysis incorporated an interaction variable between time and whether or not an individual had lost 10% of their body weight from enrollment to follow-up. This model suggested a decrease of -0.0013 ppb per year in the rate of decay of log(PBB) for those who lost at least 10% of their body weight from enrollment to follow-up. The latter interaction variable (of time* lost 10% of body weight from enrollment to follow-up) was not found to significantly contribute to the mixed effects decay model (P = 0.8479, Table 4).

A validation study was conducted on the final mixed-effects model described above. The subset of the study population used for this second model included

individuals with at least three measurements, one of which was taken between 1991 and 1993 (n = 319). Serum PBB measurements taken between 1991 and 1993 were not included in the development of the second model. Table 5 shows the parameter estimates obtained using serum PBB measurements taken between 1974 and 1987 for a subset of the study population. Comparison of tables 2 and 5 show that there was not a dramatic difference between the parameter estimate values and no change in the direction of the estimates. The highest serum concentration measurement for this subset of the population was 954.6ppb. The highest predicted serum PBB concentration level was 783.4ppb (Table 6). Figure 3 depicts how the predicted log(PBB) values compare to the observed log(PBB) levels. The points cluster closely around the line, suggesting a fairly strong relationship between the predicted and observed values. Due to the non-parametric nature of the data, a Spearman Correlation Coefficient was calculated to quantify the relationship between the predicted and observed log(PBB) levels for participants in the subset. There was a strong association between the predicted and observed log(PBB) levels ($\rho_s = 0.80$).

Estimated serum PBB concentration levels at the time of the sons' conception were predicted for 134 offspring. Sons were conceived between 1974 and 1993 and born between 1975 and 1994. The median estimated *in utero* PBB concentration for the 134 was predicted to be 6.7ppb, ranging between 1.0 and 1535.5ppb.

Discussion

The linear mixed-modeling method used for this longitudinal study takes into account the number of observations between participants, as well as the correlations within participants and correctly adjusts for time-dependent covariates. Furthermore, its ability to provide subject-specific parameter estimates allows for the development of a more powerful predictive model. These model characteristics allowed for inclusion of time-dependent variables, such as change in weight and BMI with respect to time in the study.

A strength of this study is that the results can be generalized to the majority of male cohort members. This is because there was a wide range of PBB values for the males included in our study (range: 2.0 – 1900 ppb). However, the mixed-effects decay model does have some limitations. Nearly 80% of male participants had three or fewer PBB measurements, possibly leading to inaccurate trends of the effects of time-dependent covariates on the log(PBB) levels. BMI was not able to be treated as a true time-dependent variable for all males in the study, due to participant height and weight not being regularly recorded. In the absence of an actual time-dependent BMI variable, the effect of BMI over time was calculated using information of male participants who had a second weight obtained from a follow-up survey completed around the time of their 1991-1993 serum PBB measurement. This allowed us to create a categorical variable describing whether or not a participant had an increase of ≥5% of their BMI (reference, n = 182) or a decrease of ≥5% of their BMI (n = 38) since enrollment. We were also limited in that those who we categorized as no change in BMI included the group of

males who had a <5% BMI change (n=168) and those were missing a subsequent BMI, due to weight not reported at the follow-up survey (n=350). This resulted in misclassification for those who were missing weight at follow-up. In this sub-analysis model of percent change in BMI, the rate of change contributed by the initial BMI*time remained significant (P=0.0443, Table 3). The second sub-analysis model included a variable for change in weight of greater than 10% from enrollment to follow-up. Though not significant (P = 0.8858, Table 4), the interaction term between time and loss of at least 10% of body weight from enrollment to follow-up (time* lost 10% of body weight from enrollment to follow-up) suggested that there was a very small decrease in the rate of decay of -0.0010 ppb per year. Interestingly, the time-dependent BMI variable (initial BMI*time) did suggest that there was a trivial increase in the rate of decay of 0.0062 ppb per year for those who lost greater that 5% of their initial BMI, compared to those who gained greater than 5% of their initial BMI (Table 3). Terrell et al. (2008) observed a slower rate of decay among women who either gained or had not change in weight since their initial PBB measurements; however, the effect of including a time-varying BMI variable among their study population was small. A possible explanation of this phenomenon is that PBB, which is stored in body fat, may be released into the bloodstream during phases of weight loss, leading to fluctuations in serum PBB levels and thus altering decay rate estimates. Further investigation on the effect of weight change over time on halogenated organic compounds is necessary to better understand this association.

PBB conversion factors were used to calculate approximate serum congener FF-1 levels due to changes in laboratory analytic methods and standards for the quantification

of serum PBB measurements over the years. However, Terrell et al. (2008) altered the conversion factors to ±5% and refit the mixed-effects model, finding no change in the direction of the estimates. In addition, because time of analysis is accounted for in our final model, it is not likely affect our conclusions.

Serum PBB concentrations that were used were not lipid adjusted, which could have potentially led to within-subject variability due to the fact that measurements were taken on non-fasting subjects, which could affect serum lipid concentration variations due to what was eaten at the last meal when the last meal was eaten, in addition to the genetic factors of the subjects (Blanck et al., 2000; Terrell et al., 2008). Furthermore, it has been suggested that lipid adjustment may introduce bias (Schisterman et al., 2005).

The validation study indicates a high correlation between the predicted and observed PBB levels ($\rho_s = 0.80$); however, the predictive performance of our final mixed-effects model at levels near the level of detection or at extremely high levels may be less accurate. The Spearman correlation coefficient is not able to reflect decreased accuracy at lower or extremely high levels, allowing potential outliers to influence this value.

Meaningful conclusions are still able to be drawn from these analyses. The mixed effects model suggests a higher initial BMI was associated with a decrease in the rate of decay of log(PBB), which is consistent with previous studies (Flesch-Janys et al., 1996; Michalek et al. 1996; Blanck et al., 2000; Terrell et al., 2008). Also, when a categorical BMI variable is used, there was a decrease in the rate of decay of PBB with respect to BMI for those men classified as obese. Changes in weight greater than 10lbs from 1973 (exposure) to enrollment, which is associated with BMI measurements, was also found to have a significant effect on the initial log(pbb) level. Changes in body fat may be

influential in the distribution of halogenated compounds, resulting in longer half-lives. Previous PBB studies have suggested equilibrium is established between serum and adipose tissue after approximately 2 after exposure years (Wolf et al., 1979). Furthermore, it is possible that despite public health interventions, there was a continued exposure to PBB following the PBB contamination period which lasted about 1 year.

The median estimated in *utero* PBB concentration levels for offspring was 6.7ppb and ranged from 1.0 ppb to 1535.5 ppb. Previous studies have reported associations between i*n utero* exposure to PBB and genitourinary disorders and/or pubertal development among the offspring. It may be of interest for future analyses to use this model to investigate potential associations between estimated *in utero* PBB exposure to health outcomes in the offspring with respect to the father's serum PBB concentration predicted to the time of the offspring's conception.

Conclusion

The mixed-effects decay model developed in this study is able to describe how covariates of interest influence the rate of PBB decay. Initial BMI and weight change of greater than 10% from enrollment to follow-up were found to be significant contributors to the rate of decay among male participants. The strength of the model was demonstrated through a validation study comparing predicted to observed PBB levels for a subset of the study population, resulting in a high Spearman Correlation Coefficient ($\rho_s = 0.80$).

This model provides the advantage of being able to estimate an exposure level of PBB from a single PBB serum level. This allows for the estimation of paternal contribution to *in utero* exposure of offspring to PBB exposure. Due to the expected similarity in toxicokinetics, the decay model developed in this study is also relevant to studies of other halogenated compounds such as PCB and TCDD and their congeners.

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Tables

Table 1. Demographic characteristics of men from the Michigan Long-Term PBB Study included in the mixed-effects decay model (n=904).

Variable	Sample Number	n	% (of Individuals)	Initial Median FF1 p.p.b. (min-max)
Age at Exposure (years)[a]				
<25	831	303	33.5	6.0 (2.0 - 744.0)
25 - 44	901	305	33.7	5.3 (2.0 - 1900.0)
>44	837	296	32.7	5.0 (2.0 - 1700.0)
BMI at Enrollment (kg/m^2)				
<25	1117	391	43.3	6.0 (2.0 - 1900.0)
25-30	1049	364	40.3	5.0 (2.0 - 1744.0)
>30	240	85	9.4	4.0 (2.0 - 661.0)
Missing	163	64	7.1	-
BMI at Follow-up (kg/m^2)				
<25	469	153	16.9	6.0 (2 - 1900.0)
25-30	945	298	33.0	5.0 (2.0 - 1744.0)
>30	698	258	28.5	5.0 (2.0 - 661.0)
Missing	457	195	21.6	-
Smoking Status at Enrollment				
Non-Smoker	1248	424	46.9	6.0 (2.0 - 1900.0)
Past Cigarette/Cigar/Pipe Smoker	516	185	20.5	5.0 (2.0 - 744.0)
Current Cigarette/Cigar/Pipe Smoker	710	257	28.4	6.0 (2.0 - 1744.0)
Missing	95	38	4.2	-
Number of PBB Measurements				
≤3	1727	715	79.1	5.0 (1.0 - 1700.0)
>3	842	189	20.9	13.2 (2.0 - 1900.0)
*Decade of Measurement**				
1970's	1491	868	58.0	6.0 (0.5 - 2560.0)
1980's	717	552	28.0	7.0 (0.5 - 1317.0)
1990's	361	361	14.1	5.5 (0.5 - 954.6)
Had a weight change of more than 10lbs since 1973 (at enrollment)				
No	2137	748	82.7	6.0 (2.0 - 1900.0)
Yes	400	146	16.2	5.0 (2.0 - 1194.0)
Missing	32	10	1.1	-
Loss of 10% of Body Weight from Enrollment to Follow-up				
No	1600	528	58.4	6.0 (2.0 - 1900.0)
Yes	361	116	12.8	5.24 (2.0 - 1744.0)
Missing	608	260	28.8	-

[a]Exposure defined as July 1, 1973.; *Measurements include all individuals.

Table 2. Results of the mixed effects decay model for males in the Michigan Long-Term PBB Study using measurements between 1974 and 1993 (n = 904).

Variable	Parameter[a]	Estimate	SE	P-value
Intercept	β_0	2.4129	0.0536	<0.0001
PBB decay rate (p.p.b./year)[b]				
Time (years)	β_1	-0.1234	0.0084	<0.0001
BMI (kg/m^2)	β_2	-0.0238	0.0126	0.0608
Weight change of greater than 10 lbs since 1973 (at enrollment)				
No	-	-	-	-
Yes	β_3	-0.2230	0.1134	0.0503
Time (years)*BMI (kg/m^2)	β_4	0.0017	0.0006	0.0087
$Time^2$ [b]	β_5	0.0046	0.0004	<0.0001

[a] $Log(PBB)_{ij} = \beta_0 + (\beta_1 + BMI\ \beta_2 + \textit{Weight change of greater than 10lbs since 1973}\ \beta_3 + (Time*BMI)\ \beta_4) + Time^2\ \beta_5$

[b] Average decay rate for a man with BMI of 25 kg/m^2, who did not smoke at the time of enrollment into the Michigan Long-Term PBB Study and, who had not had a weight change of greater than 10 lbs since 1973 = $\beta_{1+}\ \beta_5$.

Table 3. Results of the mixed effects decay model that includes a time-dependent BMI variable for males in the Michigan Long-Term PBB Study using measurements between 1974 and 1993 (n = 904).

Variable	Parameter[a]	Estimate	SE	P-value
Intercept	β_0	2.5990	0.0948	<0.0001
PBB decay rate (p.p.b./year)				
Time (years)	β_1	-0.1326	0.0165	<0.0001
BMI (kg/m^2)	β_2	-0.0085	0.0221	0.7024
Weight change of greater than 10 lbs since 1973 (at enrollment)				
No	-	-	-	-
Yes	β_3	-0.3306	0.2061	0.1103
Time (years)*BMI (kg/m2)	β_4	0.0016	0.0008	0.0443
Time (years)* ±5% Change in BMI (at follow-up)				
≥ 5%	-	-	-	-
≤ -5%	β_5	0.0062	0.0060	0.3077
No Change[b]	β_6	0.0071	0.0039	0.0687
$Time^2$	β_7	0.0049	0.0007	<0.0001

[a] $Log(PBB)_{ij} = \beta_0 + (\beta 1 + BMI\ \beta_2 +$ *Weight change of greater than 10lbs since 1973* β_3
$+ (Time*BMI)\ \beta_4 + (Time*(\leq\text{-}5\%\ Change\ in\ BMI))\ \beta_5$
$+ (Time*(No\ Change\ in\ BMI))\ \beta_6) + Time^2\ \beta_7$

[b] No Change category includes missing values

Table 4. Results of the mixed effects decay model that includes an interaction variable between time and having lost at least 10% of body weight from enrollment to follow-up. Model estimates were calculated using males in the Michigan Long-Term PBB Study using measurements between 1974 and 1993 (n = 904).

Variable	Parameter	Estimate	SE	P-value
Intercept	β_0	2.5714	0.0927	<0.0001
PBB decay rate (p.p.b./year)				
Time (years)	β_1	-0.1164	0.0135	<0.0001
BMI (kg/m^2)	β_2	-0.0087	0.02206	0.6838
Weight change of greater than 10 lbs since 1973 (exposure) to enrollment				
No	-	-	-	-
Yes	β_3	-0.3271	0.2061	0.1141
Time (years)*BMI (kg/m2)	β_4	-0.0019	0.0007	0.0135
Time (years)* Loss of at least 10% of body weight from enrollment to follow-up				
No	-	-	-	-
Yes	β_5	-0.0010	0.0067	0.8858
$Time^2$	β_6	0.0042	0.0006	<0.0001

[a] $Log(PBB)_{ij} = \beta_0 + (\beta_1 + BMI\ \beta_2 +$ *Weight change of greater than 10lbs since 1973* β_3
$+ (Time*BMI)\ \beta_4$
$+ (Time*$Loss of at least 10% of body weight from enrollment to follow-up$)\ \beta_5$
$+ (Time*(No\ Change\ in\ BMI))\ \beta_6) + Time^2\ \beta_7$

Table 5. Results of the validation of the mixed effects decay model for males in the Michigan Long-Term PBB Study using measurements between 1974 and 1987 (n = 319).

Variable	Parameter[a]	Estimate	SE	P-value
Intercept	β_0	2.5300	0.0585	<0.0001
PBB decay rate (p.p.b./year)				
Time (years)	β_1	-0.1617	0.0117	<0.0001
BMI (kg/m^2)	β_2	-0.0285	0.0130	0.0306
Weight change of greater than 10lbs since 1973 (at enrollment)				
No	-	-	-	-
Yes	β_3	-0.2267	0.1147	0.0503
Time (years)*BMI (kg/m^2)	β_4	0.0024	0.0009	0.0069
$Time^2$	β_5	0.0077	0.0007	<.0001

[a] $Log(PBB)_{ij} = \beta_0 + (\beta 1 + BMI\ \beta_2 +$ *Weight change of greater than 10lbs since 1973* $\beta_3 + (Time*BMI)\ \beta_4) + Time^2\ \beta_5$

Table 6. Validation study: comparison of observed and predicted serum PBB levels between 1991 and 1993 for men in the Michigan Long-Term PBB Study (n = 319).

Model	Median	Mean	SD	Range	
				min	max
Observed PBB Values	5.5	6.6	4.6	ND	954.6
Predicted PBB Values[a, b]	8.5	10.3	3.0	ND	783.4

[a] Mixed Effect Decay Measurements 1974 – 1993: $Log(PBB)_{ij} = 2.53 + (-0.1617 + BMI\ (-0.285) +$ *Weight change of greater than 10lbs since 1973* $(-0.2267) + (Time*BMI)\ 0.0024) + Time^2\ 0.0077$

[b] Predicted serum PBB concentration levels below the level of detection are set to 0.5 ppb.

Figures

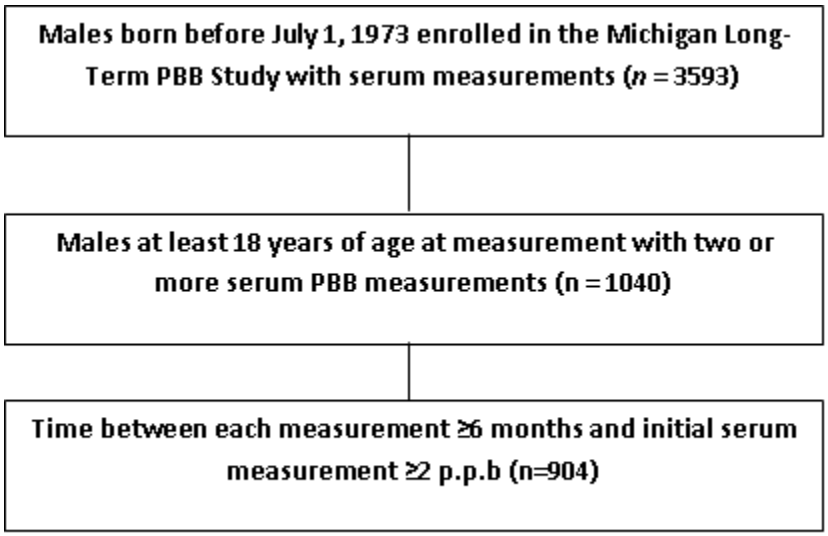

Figure 1. Flow chart of inclusion criteria for male participants in the Michigan Long-Term PBB Study that were included in the mixed-effects decay model.

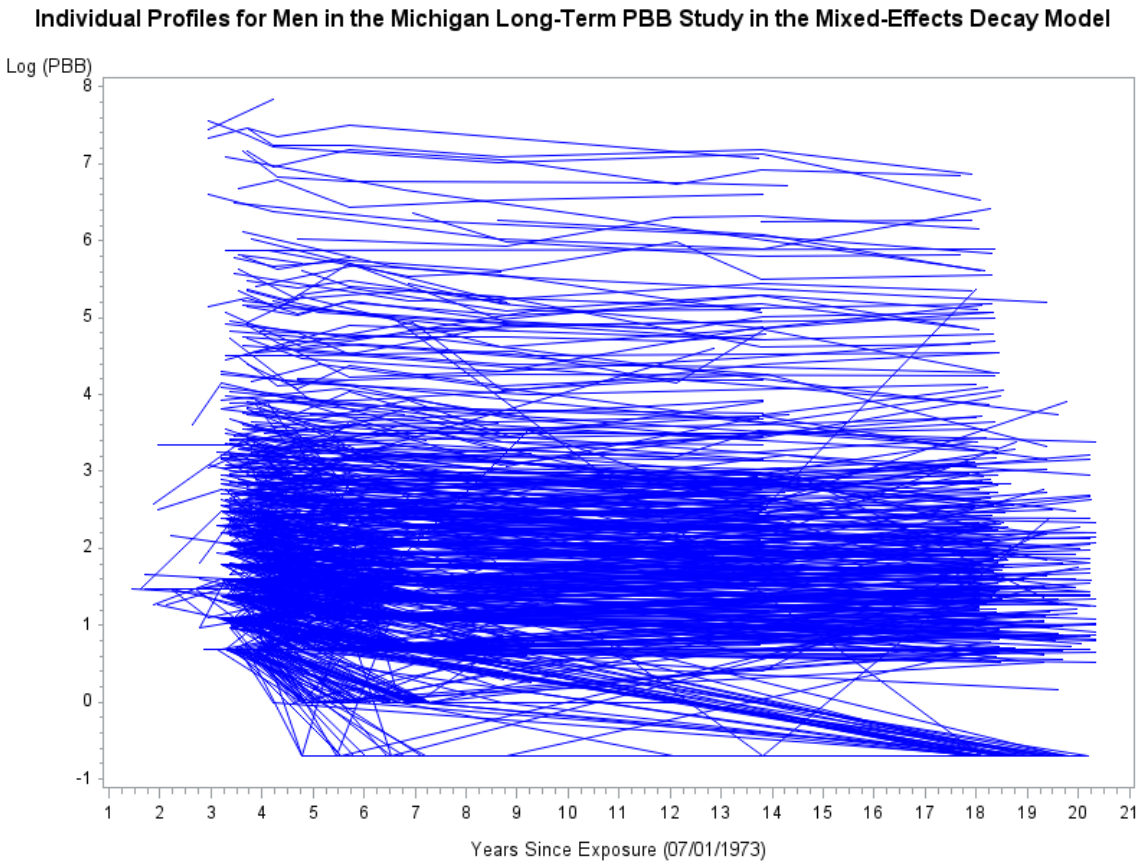

Figure 2. Individual profile for male participants from the Michigan Long-Term PBB study that were included in the mixed-effects decay model (n = 904).

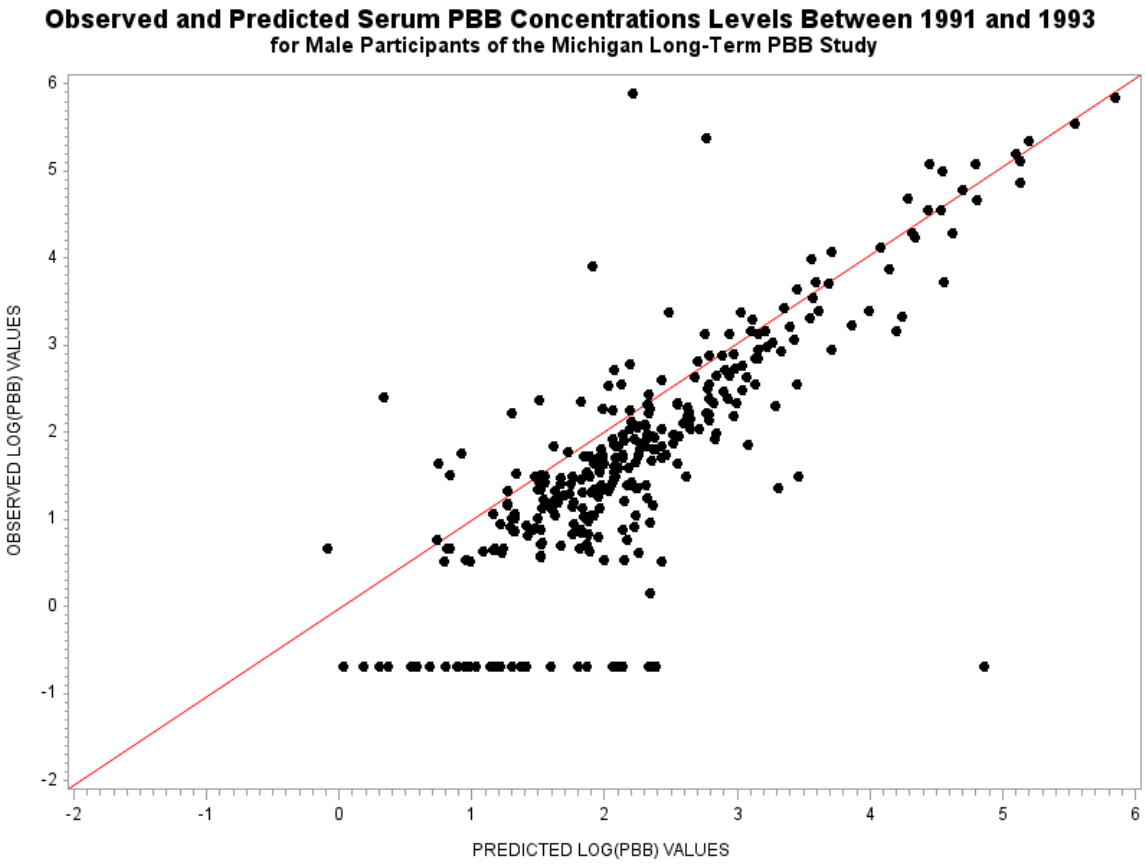

Figure 3. Observed and predicted serum PBB concentrations levels between 1991 and 1993 for male participants of the Michigan Long-Term PBB Study. Predicted values were calculated using the mixed-effects decay model described in Table 5 and Table 6. Spearman Correlation Coefficient $\rho_s = 0.80$.