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Pharmacokinetic bias analysis of the epidemiological associations between serum polybrominated diphenyl ether (BDE-47) and timing of menarche

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ABSTRACT

Background: Associations between serum levels of polybrominated diphenyl ether (PBDE) and timing of pubertal development in adolescent girls (*e.g.*, menarche) have been reported in both a cross-sectional and in a longitudinal study. The associations may be biased by growth dilution and pharmacokinetic changes during pubertal development.

Objectives: To use a physiologically-based pharmacokinetic (PBPK) model to assess how much of the epidemiologic association between PBDE and altered timing of menarche might be attributable to growth dilution and pubertal maturation.

Methods: We developed a PBPK model of BDE-47, a major congener of PBDE, to perform Monte Carlo (MC) simulation of plasma BDE-47 levels in a hypothetical target population aged 2 to 22 years old. The model used realistic distributions of physiological parameters including timing of growth spurts and menarche. The simulated data were analyzed as if they had come from an epidemiologic study. We compared the results based on the simulated population to those reported.

Results: The population characteristics, including age and body mass index (BMI) were similar between the simulated and reported groups. In the cross-sectional study design, the association between proportion of subjects with menarche before age 12 years and BDE-47 serum concentration was inverse in our simulated population, whereas the reported association was positive. In the longitudinal study design, simulated data were not suggestive of an association, whereas a delay in pubertal onset with higher concentrations of BDE-47 was observed in the epidemiologic study.

Conclusion: Results of our simulation suggest that in the previous cross-sectional study there was a small negative bias due to pharmacokinetics in the reported relationship between BDE-47 and age at menarche. However, in the longitudinal study there was little evidence of bias. Our study showed how PBPK modeling can be used to quantify the potential bias in epidemiological studies and also suggested that further studies on the optimal approach to modeling exposure are warranted to better understand and quantify the potential bias in the epidemiological associations with BDE-47 due to pharmacokinetics.

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1. Introduction

Quantitative methods for evaluating potential biases in epidemiologic studies, called bias analysis, has been used to estimate the effects of unmeasured confounders, misclassification, and selection bias (Rothman et al., 2008). We have developed a new type of bias analysis using Monte Carlo pharmacokinetic modeling, which extends the previously described approaches with a probabilistic, physiologically relevant method that can evaluate confounding and reverse causality related to physiology and pharmacokinetics. For example, we have conducted pharmacokinetic bias analyses of associations between serum concentration of polychlorinated biphenyls and birthweight (Verner et al., 2013) and perfluoroalkyl substances (PFAS) and birthweight (Verner

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et al., 2015). Recently, we used our approach to evaluate bias in an epidemiological report linking serum PFAS with delayed menarche (Wu et al., 2015). In that recent report, we found that one third of the observed association between serum PFAS concentrations and age at menarche was explained on the basis of pharmacokinetics. Thus, we speculated that the associations between serum polybrominated diphenyl ethers (PBDEs) and age at menarche observed in epidemiological studies might be similarly biased.

PBDEs are a class of chemicals used as flame retardants in a wide variety of consumer products. Though production has been increasingly regulated and reduced, exposure is ongoing (Law et al., 2014). 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) is the most abundant congener detected in human samples. Toxic effects of BDE-47 have been found in animals, although at much higher concentrations than those observed in humans (Darnerud et al., 2007). In humans, PBDEs are suspected of endocrine activity at relatively low concentrations and have been associated with altered thyroid hormone levels and decreased fecundability (Costa et al., 2009; Harley et al., 2010). Two epidemiologic studies of PBDE and timing of puberty have been published, one from a cross-sectional study of adolescent females (Chen et al., 2011) and another from a longitudinal study of girls (Windham et al., 2015). We hypothesized that the association observed in these types of studies would depend on study design and that results could be biased by growth-spurt induced "dilution" of PBDE body burden in a cross-sectional study like Chen et al. (2011). As shown in previous studies, pharmacokinetic modeling is particularly well-suited to quantify potential bias related to physiologic and pharmacokinetic processes in epidemiologic studies.

To quantify potential bias in these two epidemiologic studies of BDE-47 and timing of menarche, we developed a physiologically-based pharmacokinetic (PBPK) model of BDE-47 for humans. The PBPK model describes BDE-47 pharmacokinetics during growth and development and includes a submodel that describes BDE-47 exposure. A Monte Carlo process was implemented to simulate plasma BDE-47 concentrations and age at menarche for subjects in epidemiologic studies. The simulated data were analyzed statistically as if they had come from an epidemiologic study. Any association observed between BDE-47 exposure metrics, such as plasma concentration, and the timing of menarche in the statistical analysis of the simulated sample reflects a potential pharmacokinetic bias in the epidemiologic results. Because no pharmacodynamic models are involved, associations apparent in the statistical analysis of simulated data must arise from the correlations that exist among variables in the PBPK model rather than toxic activity of the contaminant. Using this approach, we simulated the two epidemiological studies noted above (Chen et al., 2011; Windham et al., 2015).

2. Materials and methods

2.1. PBPK model for BDE-47

The PBPK model for BDE-47 presented here is, to our knowledge, the first human PBPK model for PBDEs. The only other PBPK

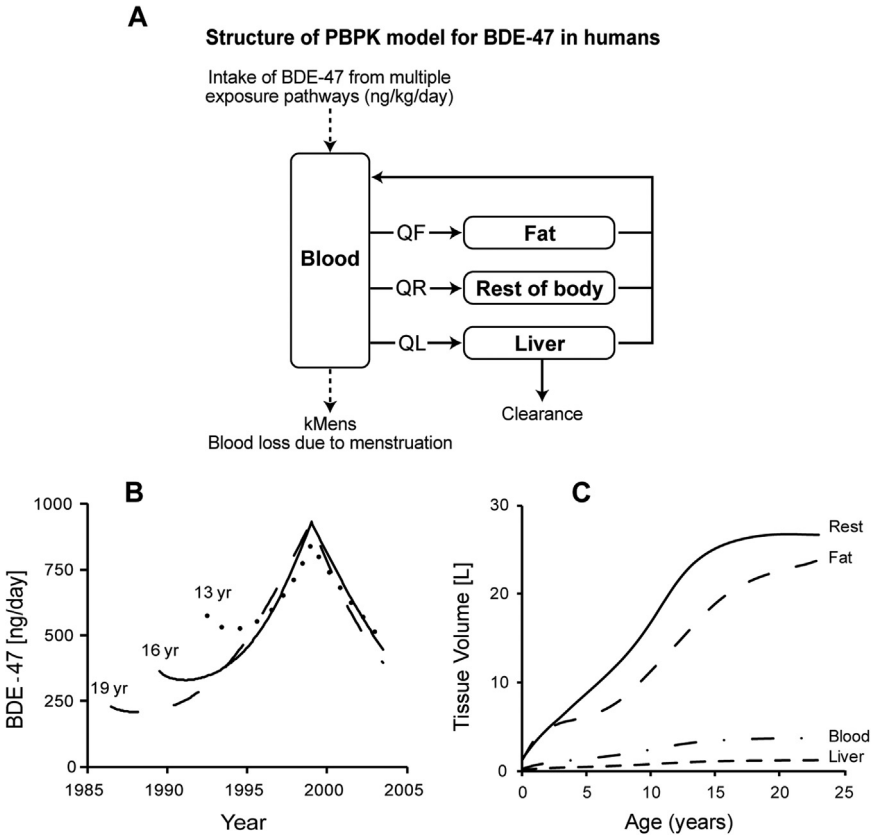

Fig. 1. A) Structure of PBPK model compartments B) Dynamic exposure is calculated based on Age and Calendar date (trajectories represent median exposures for individuals of age 13, 16, and 19 years in mid-2003). Note that for the simulated subject who in 2003 was 19 years old, their year of birth, 1984, was at a time when exposure was much lower. When this subject was 2 years old, it was after the age of crawling, and dust exposure would have been decreasing. Their second peak in exposure corresponds to the point in calendar time when exposure was at its height. In comparison, for the simulated subject who in 2003 was 13 years old, when they were 2 years old and after the age of maximal exposure to dust, this was when BDE exposure levels were much higher. When this subject was aged 9, it was again at the point in calendar time when exposure was at its height. The small difference in exposure in 1999 among subjects of different ages is due to differences in mean body weights for the different age groups, and C) Growth of compartment volumes (median values are shown).

model of BDE-47 was developed for rats by Emond et al. (2010). The model consists of four compartments including blood, liver, adipose tissue, and the rest of the body (Fig. 1A). These compartments were selected based on their relevance to the pharmacokinetics of BDE-47 in humans (Geyer et al., 2004). For this highly lipophilic compound, we used tissue neutral lipid content as the basis to describe BDE-47 partitioning between blood and tissue as suggested by Haddad et al. (2000), and adipose tissue was included as a major storage compartment BDE-47 (ASTDR, 2004). Hepatic metabolism was assumed to account for BDE-47 clearance (Erratico et al., 2013). A perfusion-limited description was considered adequate for all the tissue compartments given the long half-life of BDE-47 in humans in this long-term exposure simulation study. Chemical specific and physiologic parameters used for the human PBPK model for BDE-47 are described in Supplementary Tables S1 and S2. Intrinsic hepatic clearance (Clint) was estimated to yield a 1.4 years half-life for BDE-47 in adults. There is considerable disagreement among published estimates of the BDE-47 half-life, as discussed by Wong et al. (2013), and 1.4 years is a moderate value that aligns with the median estimate of Trudel et al. (2011).

Daily exposure to BDE-47, estimated from the exposure submodel (Fig. 1B) and described below, was modeled as a continuous input to the blood compartment to take into account combined daily intake of BDE-47 from multiple pathways (Trudel et al., 2011; Lorber, 2008). To describe physiological changes throughout adolescence, the life stage model of changes in body growth (e.g., body weight, height, and BMI) reported in Wu et al. (2015) was used (Fig. 1C, and Supplementary Fig. S1), and is also described in more detail below. The model was run for females aged 2–22 years of age.

Data for BDE-47 concentrations at age 2 in females in the US are not available. Serum concentration of BDE-47 in newborn (cord blood) reported by Toms et al. (2009) was used to estimate plasma BDE-47 concentration at age 2 (12 ng/g lipid in serum). The modeled concentrations of BDE-47 later in childhood were not sensitive to the initial concentration (data not shown). The initial concentrations in each tissue compartment were calculated using the tissue to plasma partition coefficients. The complete model code and script, implemented in acslX (3.0.2.1) are included in Supplementary materials.

2.2. Exposure model

Exposure data for simulated subjects from age 2–22 years was generated by Eq. (1).

$$DX_i(age, date)\left[\frac{ng}{day}\right] = D(age)\left[\frac{ng}{kg - day}\right] * BW_m(age)[kg] * F_T(date) * V_i * M \quad (1)$$

$DX_i(age, date)$, the individual daily exposure, was calculated as function of *age* and *date*. $D(age)$ is a function that describes the age-dependence of exposure that is believed to be a consequence of behavioral changes throughout life. $D(age)$ was based on exposure estimates published by Lorber (2008). $BW_m(age)$ represents the mean body weight of the population at a given *age* (See Growth Model, below). The temporal exposure factor, $F_T(date)$, depends only on the calendar *date*. It can be thought of as representing the general trend of environmental contamination levels. The exponential increase in exposure, with a doubling time of 4–6 years, ending around 1999, has been well characterized (Hites, 2004). Evidence for a decline thereafter is less well documented, but has some support (Ryan and Rawn, 2014; EFSA 2011). Individual exposure variability is represented by the factor, V_i. Finally, M is an empirical multiplier, and it was manually adjusted to fit observed BDE-47 plasma concentrations. Even though most exposure to BDE-47 is from dust, few data are available about the concentration of BDE-47 in dust. Lorber's exposure estimates, on which we relied, were based on just 10 dust samples. In addition, the estimated daily exposure is highly sensitive to the BDE-47 half life in the body, the most uncertain parameter in the exposure estimation process, as stated in Lorber (2008). The half-life we selected was shorter than the one Lorber used to estimate exposure, so it is not surprising that a correction factor, M, was needed. Therefore, substantial uncertainty in exposure levels exists, and thus the need for a 3-fold correction factor may not be very surprising. (Note that our results were not sensitive to F_T or M, as discussed below.) Detailed descriptions of the exposure model factors can be found in the Supplementary materials.

2.3. Growth model

Models of growth described by others were tuned to match age-specific distributions of height, weight, and BMI reported in NHANES (Wu et al., 2015). In these models, growth in height was primarily a function of age and a parameter controlling the age of the adolescent growth spurt; related parameters were varied across subjects to create realistic variation in individual trajectories. Similarly, growth in weight was primarily a function of age, height, and a parameter controlling the age of the adolescent growth spurt; related parameters varied across subjects to create realistic variation in individual trajectories. Age at menarche was a function of the growth spurt parameter noted above, the average length of time between peak height velocity and age at menarche, and related parameters that varied across subjects. In the growth model, the age-specific BMI calculated from height and weight had a realistic correlation with age at menarche (Wu et al., 2015). The age-specific body height and weight were used to calculate physiological parameters in the PBPK model, such as tissue volume and tissue blood flow. The growth model (Wu et al., 2015) included blood loss from menstruation. Relevant details of the growth model are summarized in the Supplementary materials, Supplementary Table S3.

2.4. Monte Carlo population variability

Variability in simulated populations was introduced through independently sampling values for a number of model parameters that determine characteristics of simulated individuals. This method of inducing variability was applied to physiological and chemical specific parameters in the PBPK model, and it was also applied in the exposure model. The distributions of parameters (*i.e.*, mean, SD, range) were drawn independently from distributions that were either normal or $\log_e$-normal, and the type of distribution, mean, variance, and truncation limits, if any, are described in Supplementary Tables S2 and S3. The parameters were selected to produce simulated populations comparable to NHANES 2003–2004 participants in terms of distributions of body height, body weight, body mass index (BMI), age at menarche, and plasma concentration of BDE-47.

2.5. Simulation of study populations

We simulated data to replicate the population from the Chen et al. (2011) study (Chen study hereafter), a cross-sectional study of 271 adolescent females from the National Health and Nutrition Examination (NHANES) Survey 2003–2004 database. The Chen study population was simulated by generating lifetime data for 40,000 individuals, which was sufficient for convergence of the epidemiologic regression results. BMI, body height, body weight, and plasma concentration of BDE-47 were recorded once for each

simulated individual, at the blood sampling time. Each individual's age at blood sampling time was randomly selected from a discrete uniform distribution of 0.1 year increments in the range of 12.0–19.9 years old. The calendar date at measurement (July 1, 2003) was constant for all subjects. The calendar date is relevant only to the exposure sub-model.

We also simulated data to replicate the population from the Breast Cancer and the Environment Research Program (BCERP) study described in Windham et al. (2015) (BCERP study hereafter). This study was an investigation of determinants of puberty within the BCERP study, a longitudinal study in which 1170 girls between the ages of 6 and 8 years in 2004–2007 were enrolled and monitored for seven years with annual follow-up for pubertal development. This study was simulated by generating data for 40,000 individuals. In the simulation, BMI, body height, body weight, and plasma concentration of BDE-47 were recorded at enrollment. Calendar date at enrollment (January 1, 2006) was constant for all subjects. Age and BMI at enrollment for each individual was selected from a distribution defined by the distribution of age at enrollment for the subjects of the BCERP study.

2.6. Statistical analysis

We evaluated the relationship between simulated BDE-47 levels and age at menarche using the statistical approaches selected by Chen et al. (2011) and Windham et al. (2015). The regression analysis of the simulated population data for comparison to the results from Chen study was done by fitting a log-binomial model using the 'glm' function in the R software package (v. 3.1.2). Relative risks were determined, for the quartiles and for the natural logarithm of BDE-47 concentration, with age as the only covariate.

The BCERP simulation study was analyzed by fitting an accelerated failure time (AFT) model using the SAS package (Version 9.3). Time ratios were calculated to compare the median age at menarche among girls in each of the higher 3 quartiles of BDE-47 in comparison to the girls in the reference category (the first quartile of BDE-47). The age at enrollment and BMI at enrollment were included as covariates in the AFT model.

2.7. Sensitivity analyses

2.7.1. One-at-a-time and global sensitivity analyses

The sensitivity of plasma concentration for simulated subjects at three ages: 7.5 years, 15 years, and 19 years were evaluated. First, one-at-a-time (OAT) sensitivity coefficients were obtained by varying each of the input parameters by 1% and noting the resulting changes in the output. The OAT sensitivity coefficients were expressed as the ratio of the percentage change in the BDE-47 serum concentration to the percentage change in a model input parameter (Clewell et al., 1999). Pearson correlations of plasma concentrations of BDE-47 with the values of input parameters in the dataset from Monte Carlo simulations were also analyzed (Clewell et al., 1999). The parameters with higher correlation coefficients were considered more influential. Lastly, a sensitivity analysis of the BDE-47 plasma concentration was conducted using the Morris global sensitivity method implemented in acslX v3.0.2.1. The list of parameters varied and their ranges are given in Supplementary Table S4.

2.7.2. Regression sensitivity analyses

The sensitivity of the coefficients from the regression models (e.g., for risk ratios or time ratio) to our assumptions were studied by altering the simulation in significant ways, repeating the entire population generation process under the new assumptions, and analyzing the risk ratios or time ratios of the new population as described above. Specifically, we tested our assumption about description of age-dependent exposure: a) with an alternative step function so that exposure was directly based on Lorber's (2008) estimates, (i.e., with M = 1), and b) with individual body weight scaling rather than mean body weight for the population at a given age. We also evaluated the effect of removing time trends in exposure levels. In addition, different half-lives of BDE-47 in humans (0.7 years and 2.8 years) were tested in comparison to the 1.4 years value used in the baseline simulation scenario. We also evaluated the changes in risk ratios or time ratios made by altered average menstrual blood loss (i.e., no menstrual blood loss). The preceding set of specific assumptions was chosen for examination because the sensitivity analyses described in Section 2.7.1 indicated that these parameters affected the PBPK model output and because these were the parameters about which we had the least certainty. Finally, for the age at puberty (BCERP) simulation, we also examined the effect of using onset of puberty (breast development) as the outcome, calculated as age at menarche – 1.96 y (Martí-Henneberg and Vizmanos, 1997; Largo and Prader, 1983).

3. Results

3.1. Characteristics of the simulated and reported study populations

The simulated and reported characteristics of the Chen study population were similar with respect to age, age at menarche, BMI, and geometric mean serum BDE-47 concentration (Table 1). BDE-47 serum concentrations by age in the simulated population were in good agreement with the reported data (Fig. 2). For two of the three key characteristics of the Windham et al. (2015) study population that were available (age and BMI), the simulated and reported values were also similar; the geometric mean BDE-47 in the simulated population, however, was about 20% higher (Table 1).

Table 1
Comparison of sample characteristics for simulated and reported epidemiologic studies.

Variable	Chen et al.		Windham et al.	
	Simulated	Reported[†]	Simulated	Reported
Age (y)				
6.0–6.9			28.0%	30.4%
7.0–7.9			68.4%	66.7%
≥ 8.0			3.6%	3.0%
12–15	49.9%	53.1%		
16–19	50.1%	46.9%		
Age at menarche				
Mean (y)	12.3	12.1		
% < 12	24.8%	24.0%		
BMI				
< 85th %ile			71.6%	70.9%
≥ 85%ile			28.4%	29.2%
2nd Quartile [kg/m^2][a]	19.2	19.6		
3rd Quartile	22.5	21.8		
4th Quartile	26.7	24.9		
Serum BDE-47 concentration (ng/g)				
Median	24.5	26.2		
Geometric mean	24.6	24.1	51.7	42.2

[a] Means within category.

[†] Secondary analysis of the Chen study was completed in R using the 'survey' package, Lumley (2004, 2014). NHANES 2003–2004 data were obtained from CDC. The same filtering criteria described in Chen et al. (2011) were applied to arrive at the same sub-sample of 271 subjects. In this way, it was possible to obtain statistics of the Chen study sample that were not previously reported, such as some of those in Table 1.

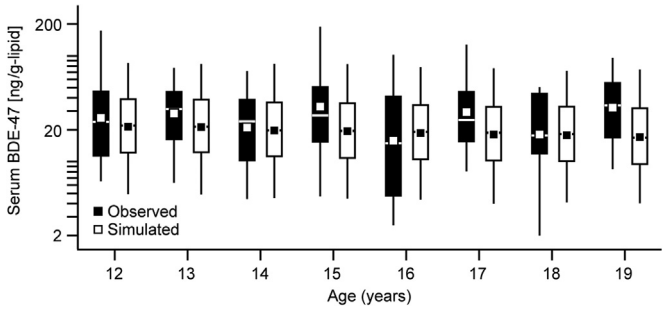

Fig. 2. Comparison of simulated and observed BDE-47 blood levels in a cross-sectional study by age group. Small rectangle, mean; horizontal line bisecting large rectangle, median; large rectangle, lower and upper quartiles; whiskers, 5th and 95th percentiles. Observed data from NHANES 2003–2004. At age 18, for the observed value, the estimate of the 95th percentile is not stable because of the small number of 18 year old females (n=35) and the use of the weights, clustering, and stratification factors in the analysis.

3.2. Epidemiologic analysis of the simulated data

In the simulation of the Chen study the association between proportion of subjects with menarche before age 12 years and BDE-47 serum concentration was inverse, with relative risks just slightly below one (Table 2). In contrast, the relative risks reported by Chen et al. (2011) were greater than one. In the simulated BCERP study, the association between age at menarche and BDE-47 serum concentration was null, whereas in the results reported by Windham et al. (2015) pubertal transition was later across increasing quartiles of BDE-47 (Table 2).

3.3. Sensitivity analysis

The sensitivity of serum BDE-47 concentration for simulated subjects at 7.5 years, 15 years, and 19 years were evaluated (Table 3). The parameters identified as most influential on serum BDE-47 concentrations by the OAT sensitivity analysis were: maximum height in cm (H1), intrinsic hepatic clearance (ClintC), empirical correction factor (Dosemultiply, M), fraction of neutral lipid in blood (NLPB), a timing parameter controlling the age of the growth spurt (THETA), maximum weight in the later section of the growth curve (MWADULT), maximum weight for the early hyperbolic section of the growth curve (MWCHILD), exposure doubling time constant for BDE-47 between 1982 and 2004 (XPDLTIMEC), prepubertal linear growth (S0), and time difference (years) between the beginning of the exponential decline of BDE-47 exposure and the epidemiological study (CUT1). The same parameters were also identified as significant by the correlation analysis, with the exception of CUT1. The sensitivity of the model results to these parameters was similar at different ages. The results for the Morris global sensitivity analysis were also similar and a ranking of the top 10 parameters for each of the three ages from the Morris analysis is described in Supplementary Table S5.

3.4. Regression sensitivity analyses

In the regression sensitivity analyses, for the Chen study, the results were essentially unaffected by a different method of modeling age-specific exposure, by setting menstrual clearance to zero, by removing the time trend in exposure, or by using substantially different half-lives (Table 4). Whether exposure was calculated on the basis of individual body weight or mean body weight for age, however, did affect the association, and changed its direction from negative to positive in the simulation of Chen study. For the BCERP simulation, the regression results were sensitive to the same factor as for the Chen study, though the effect was not as large as for Chen study. Of particular note is that the regression results were not sensitive to the half-life, the absolute exposure level (the correction factor M was set to 1 when the alternative age-dependent exposure was used), or having the age-dependent exposure held constant across calendar time (with $F_T=0$). In addition, when we considered pubertal onset instead of age at menarche in our simulation of the BCERP study, the Adjusted Time Ratio was not sensitive to the altered pubertal timing (onset vs. menarche).

4. Discussion

Our analysis indicated that, under the values we chose for the baseline simulation parameters, the epidemiologic results obtained in the cross-sectional study by Chen et al. (2011) would have a slight negative bias because of growth dilution. In other words, in the absence of the pharmacokinetic bias we characterized, the associations observed by Chen et al. would have been slightly larger (10%, using relative risk – 1 as the effect size) than what they reported. On the other hand, using the prospective and longitudinal design in the BCERP program, our simulation indicates little bias would be expected. Because of the different statistical approaches used by Chen et al. (2011) and Windham et al. (2015), it may not be obvious that Chen et al.'s results mean higher BDE-47 exposure was associated with earlier age at menarche (RR > 1), while Windham et al.'s results mean higher exposure was associated with later menarche (TR > 1). A complete discussion of potential reasons for the discrepancy is beyond the scope of the present exercise. However, the study designs were different and, in general, the results of a prospective, longitudinal study would be expected to have higher validity.

In the Chen cross-sectional study based on NHANES it was assumed that serum PBDE concentrations measured after

Table 2
Comparison of association between serum BDE-47 concentrations and age at menarche in simulated and reported epidemiologic studies.

	Chen study (Chen et al. 2011) Adjusted Relative Risks (95% CI) for menarche < 12 years[a]		BCERP study (Windham et al. 2015) Adjusted Time Ratio (95% CI) for puberty[†]	
	Simulated	Reported	Simulated	Reported
BDE-47				
1st Quartile	1.00 (Reference)	1.00	1.0000	1.00
2nd Quartile	0.98 (0.93–1.04)	2.73 (1.07–6.92)	1.0001 (0.9979–1.0023)	1.02 (0.99–1.05)
3rd Quartile	0.91 (0.86–0.97)	1.94 (0.51–7.42)	0.9987 (0.9965–1.0009)	1.02 (0.99–1.05)
4th Quartile	0.93 (0.88–0.99)	4.17 (1.52–11.44)	0.9983 (0.9962–1.0005)	1.04 (1.01–1.07)
ln([BDE-47])	0.95 (0.92–0.98)	1.54 (1.07–2.23)		

[a] The simulated and adjusted results for the Chen study are both adjusted for age.
[†] In the BCERP study (Windham et al., 2015), the onset of puberty was based on age at Tanner Stage 2+ breast development. In the simulation, the onset was based on age at menarche. See text for discussion. The simulated and reported results are both adjusted for age and body mass index at the beginning of follow-up.

Table 3
Calculated one-at-a time sensitivity coefficients (SCs) and correlation coefficients for model parameters for BDE-47 with respect to serum concentrations of BDE-47 (ng/g) at 7.5, 15, and 19 years old.

Parameter	7.5 years old		15 years old		19 years old	
	SC	Correlation coefficient	SC	Correlation coefficient	SC	Correlation coefficient
NLPB	−0.99	−0.20	−0.99	−0.18	−0.99	−0.19
ClintC	−1.13	−0.18	−1.95	−0.17	−1.11	−0.17
MWCHILD	0.37	0.06	0.16	0.13	0.12	0.03
MWADULT	0.39	0.04	0.93	0.04	0.52	0.11
S0	−0.31	−0.04	−0.15	−0.03	−0.11	−0.02
Dosemultiply	1.00	0.03	1.00	0.03	1.00	0.04
THETA	0.89	0.03	0.66	0.01	0.23	0.00
XPDBLTIMEC	0.25	−0.03	0.26	−0.03	0.33	−0.03
PR	0.01	0.01	0.06	0.01	0.11	0.01
H1	−1.42	−0.01	−1.47	−0.01	−1.45	−0.01
CUT1	0.08	0.00	0.11	0.00	0.18	0.00

NLPB: fraction of neutral lipid in blood, ClintC: intrinsic hepatic clearance, MWCHILD: maximum weight for early hyperbolic section curve (kg), MWADULT: maximum weight for late hyperbolic section curve (kg), S0: prepubertal growth, Dosemultiply: empirical correction factor exposure, THETA: a timing parameter controlling the location of the adolescent growth spurt along the time axis, XPDBLTIMEC: exposure doubling time constant for BDE-47 from 1982 to 2004, PR: BDE-47 Rest of body: plasma partition coefficient, H1: maximum height in cm, CUT1: time difference between the year beginning the exponential decline of BDE-47 and the epidemiological study.

Table 4
Regression result sensitivity analysis.

Modification	Chen study simulation	BCERP study simulation
	Adjusted Risk Ratio ln([bde47])	Adjusted Time Ratio ([bde47 Q4 vs. Q1])
None (baseline simulation)	0.95 (0.92–0.98)	0.9983 (0.9962–1.0005)
Alternative age-dependent exposure[a]	0.95 (0.92–0.98)	0.9997 (0.9953–0.9996)
BW_i[†]	1.04 (1.01–1.07)	0.9969 (0.9947–0.9991)
Age-dependent exposure held constant		
No time trend[§]	0.94 (0.92–0.97)	0.9980 (0.9959–1.0002)
Alternative elimination $T_{1/2}$		
0.7 years	0.95 (0.92–0.98)	0.9979 (0.9958–1.0000)
2.8 years	0.94 (0.91–0.97)	0.9986 (0.9965–1.0008)
Menstrual clearance=0	0.95 (0.92–0.98)	0.9983 (0.9961–1.0005)
Age at puberty onset		0.9983 (0.9958–1.0008)

[a] Step function based directly on Lorber estimates (Lorber, 2008). Estimated BDE-47 exposure for those of age 0–6 years is 12.8 (ng/kg/day), for those of age 6–11 years is 3.7 (ng/kg/day), for those of age 11–20 years is 2.4 (ng/kg/day), and for those of age 20+ years is 2.0 (ng/kg/day).

[†] Exposure calculated based on individual body weight rather than mean for age.

[§] With F_T=0, exposure is constant across calendar time.

menarche would approximate the perimenarcheal concentrations (Chen et al., 2011). However, the physiological changes accompanied by the pubertal onset may have a significant impact on the serum concentrations of BDE-47 in girls. The adolescent growth spurt, a period of rapid growth in height and weight during puberty, begins at 10 years of age for girls on average (Bogin, 2010) and is related to the timing of menarche (Power et al., 1997). The growth spurt can lead to growth dilution where a rapid increase in body volume causes a decrease in BDE-47 concentrations. Other than growth, the physical manifestations of puberty begin with the development of pubic hair and of the breasts and conclude with menarche (Martí-Henneberg and Vizmanos, 1997; Largo and Prader, 1983). Thus, in a cross-sectional study of postmenarcheal girls, those experiencing earlier pubertal development would have lower BDE-47 levels, whereas girls with later onset of growth spurt and menarche would have higher BDE-47 levels. The regression sensitivity analysis showed that in the Chen study, the bias was due to growth dilution, with no contribution from menstrual onset. This result is qualitatively similar to our related analysis in perfluoroalkyl substances and menarche, where the bias was also due to growth dilution (Wu et al., 2015).

Accurate Monte Carlo simulation requires correct specification of the correlations among variables (Ferson, 1995). Many of the correlations among variables in our simulation stem from the growth model. Even though the inputs for the growth model are selected independently and at random, together they determine a simulated person's rate of development, growth trajectory, relative size, and body composition. Because body size determines organ size and organ blood flow, many correlations among variables derive from functional relationships. Dosing and elimination rate were also assigned independently and at random. Dosing rate was not dependent on individual body size, but elimination rate was, as the final elimination rate was a function of individual liver size, which in turn was dependent on body size. Other than the functional relationships mentioned, correlations were not induced between exposure, elimination, or variables affecting body size.

The regression results were sensitive to how we modeled exposure. We scaled daily exposure to BDE-47 by average body weight in the population at a given age. With exposure scaled by individual body weight, the direction of the association changed for the simulation of the Chen study. Because dust ingestion is the major route of BDE-47 exposure, Lorber (2008) estimated the PBDE intake dose per average body weight per age group. However, individual body weight at a given age could have some residual ability to improve the dose estimate because weight is related to inhalation rate (USEPA, 1997), and it could also improve prediction of the minor portion of exposure that comes from food. More data on the best way to express PBDE exposure in relation to body weight could improve the accuracy of our PBPK model and simulation. Nonetheless, with dose expressed per average body weight or per individual body weight, the amount of bias indicated by the simulation was relatively small in either case. In addition, when the dose was expressed by average body weight for age, the regression results were not sensitive to the accuracy of the BDE-47 half-life or to the levels of daily exposure at each age in the PBPK model. This implies that more precise simulation of the geometric mean serum concentration in the BCERP study would not have been critical for evaluating the BDE-47 and age at menarche association. Although it did not affect the conclusions from the simulation, the fact that an empirical multiplier, M, was needed in the exposure submodel to reproduce the serum BDE-47 concentrations in the epidemiological studies indicates uncertainty about half-life, exposure, or both, which may be important to address in future applications of the PBPK model.

We have developed a PBPK model for BDE-47 in humans and, using this PBPK model, demonstrated that growth dilution can influence the association between PBDE and age at menarche. The growth model linked to our PBPK model was developed so that the age-specific distributions of BW, BH, and BMI for females matched that of the general U.S. population (Wu et al., 2015). Selection probabilities for simulated subjects can be applied as needed to make the characteristics of a given population match a given target population. In addition, the correlation between BMI and age at menarche was incorporated into the growth model. Although the onset of breast and pubic hair development used in the BCERP study has been shown to be highly correlated with the age at menarche (Largo and Prader, 1983; Martí-Henneberg and Vizmanos, 1997), these reflect slightly different aspects of the maturational process. However, we demonstrated that the regression results were not sensitive to timing of the specific pubertal outcome used. Our results demonstrated that quantitative assessment of bias using Monte Carlo-pharmacokinetic models allows incorporation of current knowledge of physiology and pharmacokinetic variability into simulations of diverse populations.

5. Conclusion

Given our assumptions, we found clear evidence of bias due to kinetics in the cross-sectional epidemiologic results on BDE-47 and age at menarche, although the amount of bias was modest. The tools we have developed here can be applicable to other chemicals and health end points to evaluate potential bias in epidemiological associations due to pharmacokinetics. The most important data gap identified was how best to model exposure as a function of weight: individual body weight or of mean weight for a given age? Additional studies on this data gap would improve the assessment of the bias introduced by the physiologic contributions to the epidemiological association.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.07.004.

References

ATSDR, Agency for Toxic Substances and Disease Registry., 2004. Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available online at ⟨http://www.atsdr.cdc.gov/toxpro2.html⟩.

Bogin, B., 2010. Evolution of Human Growth. Human Evolutionary Biology, pp. 379–395.

Chen, A., Chung, E., DeFranco, E.A., Pinney, S.M., Dietrich, K.N., 2011. Serum PBDEs and age at menarche in adolescent girls: analysis of the National Health and Nutrition Examination Survey 2003–2004. Environ. Res. 111 (6), 831–837.

Clewell, H.J., Gearhart, J.M., Gentry, P.R., Covington, T.R., VanLandingham, C.B., Crump, K.S., et al., 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. Risk analysis 19 (4), 547–558.

Costa, L.G., Giordano, G., Tagliaferri, S., Caglieri, A., 2009. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potentialadverse health effects. Acta BioMed. Atenei Parm. 79 (3), 172–183.

Darnerud, P.O., Aune, M., Larsson, L., Hallgren, S., 2007. Plasma PBDE and thyroxine levels in rats exposed to Bromkal or BDE-47. Chemosphere 67 (9), S386–S392.

EFSA, Panel on Contaminants in the Food Chain (CONTAM), 2011. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA Journal, 9(5), 2156. [274 pp.] doi:10.2903/j.efsa.2011.2156. Available online: ⟨www.efsa.europa.eu/efsajournal⟩.

Emond, C., Raymer, J.H., Studabaker, W.B., Garner, C.E., Birnbaum, L.S., 2010. A physiologically based pharmacokinetic model for developmental exposure to BDE-47 in rats. Toxicol. Appl. Pharm. 242 (3), 290–298.

Erratico, C.A., Szeitz, A., Bandiera, S.M., 2013. Biotransformation of 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) by human liver microsomes: identification of cytochrome P450 2B6 as the major enzyme involved. Chem. Res. Toxicol. 26 (5), 721–731.

Ferson, S., 1995. Quality assurance for Monte Carlo risk assessment. In: Proceedings of the Third International Symposium on Uncertainty Modeling and Analysis and Annual Conference of the North American Fuzzy Information Processing Society, IEEE Computer Society Press, Los Alamitos, CA.

Geyer, H.J., Schramm, K., Darnerud, P.O., Aune, M., Feicht, E.A., Fried, K.W., et al., 2004. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Organomet. Compd. 66, 3867–3872.

Haddad, S., Poulin, P., Krishnan, K., 2000. Relative lipid content as the sole mechanistic determinant of the adipose tissue: blood partition coefficients of highly lipophilic organic chemicals. Chemosphere 40 (8), 839–843.

Harley, K.G., Marks, A.R., Chevrier, J., Bradman, A., Sjodin, A., Eskenazi, B., 2010. PBDE concentrations in women's serum and fecundability. Environ. Health Perspect. 118 (5), 699–704.

Hites, R.A., 2004. Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. Environ. Sci. Technol. 38 (4), 945–956.

Largo, R.H., Prader, A., 1983. Pubertal development in Swiss girls. Helv. Paediatr. Acta 38 (3), 229–243.

Law, R.J., Covaci, A., Harrad, S., Herzke, D., Abdallah, M.A., Fernie, K., et al., 2014. Levels and trends of PBDEs and HBCDs in the global environment: status at the end of 2012. Environ. Int. 65, 147–158.

Lorber, M., 2008. Exposure of Americans to polybrominated diphenyl ethers. J. Expo. Sci. Environ. Epidemiol. 18 (1), 2–19.

Lumley, T., Survey: analysis of complex survey samples, R package (version 3.30), 2014.

Lumley, T., 2004. Analysis of complex survey samples. J. Stat. Softw. 9 (1), 1–19.

Martí-Henneberg, C., Vizmanos, B., 1997. The duration of puberty in girls is related to the timing of its onset. J. Pediatr. 131 (4), 618–621.

Power, C., Lake, J.K., Cole, T.J., 1997. Body mass index and height from childhood to adulthood in the 1958 British born cohort. Am. J. Clin. Nutr. 66 (5), 1094–1101.

Rothman, K.J., Greenland, S., Lash, T.L., 2008. Modern Epidemiology. Lippincott Williams & Wilkins, Philadelphia, PA.

Ryan, J.J., Rawn, D.F., 2014. The brominated flame retardants, PBDEs and HBCD, in Canadian human milk samples collected from 1992 to 2005; concentrations and trends. Environ. Int. 70, 1–8.

Toms, L.L., Sjodin, A., Harden, F., Hobson, P., Jones, R., Edenfield, E., et al., 2009. Serum polybrominated diphenyl ethers PBDE in pooled serum are higher in children (2–5 years of age) than in infants and adults. Environ. Health Perspect. 117 (9), 1461–1465.

Trudel, D., Scheringer, M., von Goetz, N., Hungerbühler, K., 2011. Total consumer exposure to polybrominated diphenyl ethers in North America and Europe. Environ. Sci. Technol. 45 (6), 2391-–22397.

USEPA Exposure Factor Handbook, 1997. National Center for Environmental Assessment, Office of Research and Development. U.S. Environmental Protection Agency, Washington, D.C.

Verner, M., McDougall, R., Glynn, A., Andersen, M.E., Clewell, H.J., Longnecker, M.P., 2013. Is the relationship between prenatal exposure to PCB-153 and decreased birth weight attributable to pharmacokinetics? Environ. Health Perspect. 121 (10), 1219–1224.

Verner, M.A., Loccisano, A.E., Morken, N.H., Yoon, M., Wu, H., McDougall, R., et al., 2015. Associations of perfluoroalkyl substances PFASs with lower birth weight: an evaluation of potential confounding by glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK). Environ. Health Perspect. 123 (12), 1317–1324.

Windham, G.C., Pinney, S.M., Voss, R.W., Sjodin, A., Biro, F.M., Greenspan, L.C., et al., 2015. Brominated flame retardants and other persistent organohalogenated

compounds in relation to timing of puberty in a longitudinal study of girls. Environ. Health Perspect. 123 (10), 1046–1052.
Wong, F., Cousins, I.T., MacLeod, M., 2013. Bounding uncertainties in intrinsic human elimination half-lives and intake of polybrominated diphenyl ethers in the North American population. Environ. Int. 59, 168–174.
Wu, H., Yoon, M., Verner, M., Xue, J., Luo, M., Andersen, M.E., et al., 2015. Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environ. Int. 82, 61–68.