Research Article

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A Case Study Addressing the Reliability of Polychlorinated Biphenyl Levels Measured at the Time of Breast Cancer Diagnosis in Representing Early-Life Exposure

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Abstract

Background: To date, breast cancer epidemiologic studies have relied on blood or tissue specimens sampled at the time of diagnosis or a few years prior to assess lifetime exposure to polychlorinated biphenyls (PCB). In this study, we evaluated whether such PCB measurements are indicative of early-life levels by reconstructing lifetime toxicokinetic profiles for women included in the CECILE case–control study, using a physiologically based pharmacokinetic (PBPK) model.

Methods: We simulated lifetime toxicokinetic profiles of PCB-153 for 2,134 French women by incorporating information on body weight history, height, pregnancies, and breast-feeding in the PBPK model. Oral dose was calculated by considering measured blood PCB-153 and the temporal trend of environmental contamination. Area under the concentration versus time curve (AUC) for each decade of life and maximum blood concentration (C_{max}) were compiled and compared with measured levels, using Pearson partial correlation analyses adjusting for age at diagnosis.

Results: When considering all individuals, simulated AUCs correlated with measured PCBs, with coefficients ranging from 0.735 to 0.981. The weakest correlations were obtained with AUCs for the first decades of life. Stratified analyses suggested that breast-feeding reduces the reliability of late-life blood levels in representing lifetime exposure.

Conclusion: Results of this study suggest that PCB levels measured at the time of diagnosis do not fully represent early-life exposures.

Impact: PBPK-derived estimates of early-life levels circumvent the limitations of current approaches in assessing PCB lifetime exposure and may be used to address hypothesized windows of breast vulnerability (e.g., puberty) in this population. *Cancer Epidemiol Biomarkers Prev; 20(2); 281–6. ©2010 AACR.*

Introduction

A large number of epidemiologic studies and reviews on polychlorinated biphenyl (PCB) exposure and breast cancer have been published over the last 20 years. Overall, available evidence does not support an association between exposure to PCBs and breast cancer incidence (1, 2). Most of these studies considered blood PCB levels monitored at the time of diagnosis or a few years prior to be proxies for cumulated lifetime exposure. Given the inter-individual variations in environmental and physiologic parameters affecting PCB toxicokinetics, it is questionable whether these levels provide accurate information on the internal exposure of women during their earlier decades of life.

Several epidemiologic studies raised concerns about organochlorine exposure assessment. When interpreting the results obtained with exposure estimation based on single biological samples, many researchers cautioned readers that although levels at the time of study might be indicative of past exposure, it remains uncertain to which extent these levels allow an appropriate evaluation of PCB carcinogenic potential during hypothesized early-life periods of vulnerability (3–6). A prospective study published by Cohn and colleagues (7) supported such contention as blood 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) measured around 26 years of age

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was associated with increased odds of developing breast cancer, although most studies based on DDE levels (DDT metabolite) at later stages reported no association (8). Thus, when taking into account the theory of breast vulnerability during certain time-windows, one might question the value of PCB levels measured in samples taken around the time of cancer diagnosis to address exposure–disease associations.

To overcome the exposure assessment issue, we have previously developed a physiologically based pharmacokinetic (PBPK) model that allows the estimation of lifetime PCB internal levels while taking into account individual information on physiology and reproductive history (9). In the present study, we aimed to assess the appropriateness of blood PCB levels measured at the time of diagnosis in representing earlier exposures by comparing these values to PBPK-derived lifetime toxicokinetic profiles of French women included in a population-based case–control study.

Methods

Population

Data from a total of 2,135 French women included in the CECILE population-based case–control study who accepted to give blood for organochlorine quantification were used. Cases were women diagnosed with invasive or *in situ* breast cancer between February 2005 and June 2007 within the administrative regions of Ille-et-Vilaine and Côte d'Or in France. Controls were matched to breast cancer cases by 5-year age groups through random-digit dialing procedure in the same residence area. One individual with no information on weight was excluded.

PCB quantification

PCB-153 was selected as a proxy for a mixture of PCB congeners because it was detected in blood samples more than twice as frequently than the other congeners, and its levels were correlated to other individual congeners and to the sum of 3 congeners (PCB-138, -153, and -180). Samples were prepared by liquid–liquid extraction followed by a solid-phase extraction. PCB-153 concentration in samples was determined by gas chromatography coupled to an ion trap mass spectrometer detector. The limit of detection (LOD) for PCB-153 was 0.50 μg/L. When levels were below the LOD ($n = 898$, 42% of study participants), values were randomly generated on the basis of the log-normal distribution function and woman's age and body mass index (BMI) change over the last 10 years, two determinants of PCB-153 levels at the time of diagnosis in this cohort (10). Whole-blood PCB-153 concentration was adjusted on a lipid basis according to the equation in Akins and colleagues (11).

PBPK modeling

PBPK modeling is a pharmacokinetic tool that describes the physiologic, biochemical, and physicochemical processes governing the absorption, distribution, metabolism, and excretion of a xenobiotic, thus enabling the simulation of its kinetics blood and tissue. Many of these processes are dependent on several physiologic parameters such as the volume and composition of organs, and the blood perfusion. The mathematical functions describing these processes are derived from population data and are designed to allow the incorporation of different profiles of body weight and height. In the case of organochlorine compounds such as PCBs, it is paramount to take women reproductive history into account, as these chemicals are extensively excreted through breast-feeding. In this study, we simulated individualized lifetime toxicokinetic profiles with a previously published PBPK model (9) modified as detailed in Verner and colleagues (12). This framework integrates women weight profile [reported for each decade of life—missing weights were imputed through multiple linear regression ($n = 212$)], height, age at deliveries, duration of each breast-feeding period, and date of birth. Consumption of breast milk in infancy was not considered, given the dearth of information on maternal levels and duration of breast-feeding. Considering the wide variation in reported PCB-153 half-life values (13), simulations were carried out with half-lives of 10 and 30 years. Simulations were done using the software acslX (Aegis Technologies Group, Inc.). Examples of simulated toxicokinetic profiles are depicted in Figure 1A and B.

Environmental exposure estimation

The daily oral dose estimation included an assessment of temporal trends in environmental levels. Because PCB production started in the 1930s, we considered environmental exposure to be null before that date. Exposure from 1930 until 1970 was characterized using production data, as no daily intake estimations were available for Europe until that time (14). Maximum exposure was assumed during the 1970s, when peak production occurred and peak daily intake estimations were made. We defined the decline in PCB daily intake after 1977, with European data taken from Baars and colleagues (15). These temporal data were transformed into fractions of peak values reached in the 1970s (F, ranging from 0 to 1). The trend in F values is depicted in Figure 1C. To integrate this temporal trend in the PBPK model, we allowed the oral dose input to change over time on the basis of the following equation:

$$\text{Dose} = F \times \text{Maximum daily dose}$$

The maximum daily dose (in the 1970s) was optimized for each woman by iterative simulations to obtain a toxicokinetic profile, with matching simulated and measured blood PCB-153 concentrations at the time of sampling.

Statistical analyses

The area under the lipid-adjusted blood concentration versus time curve (AUC) for each decade, a proxy for

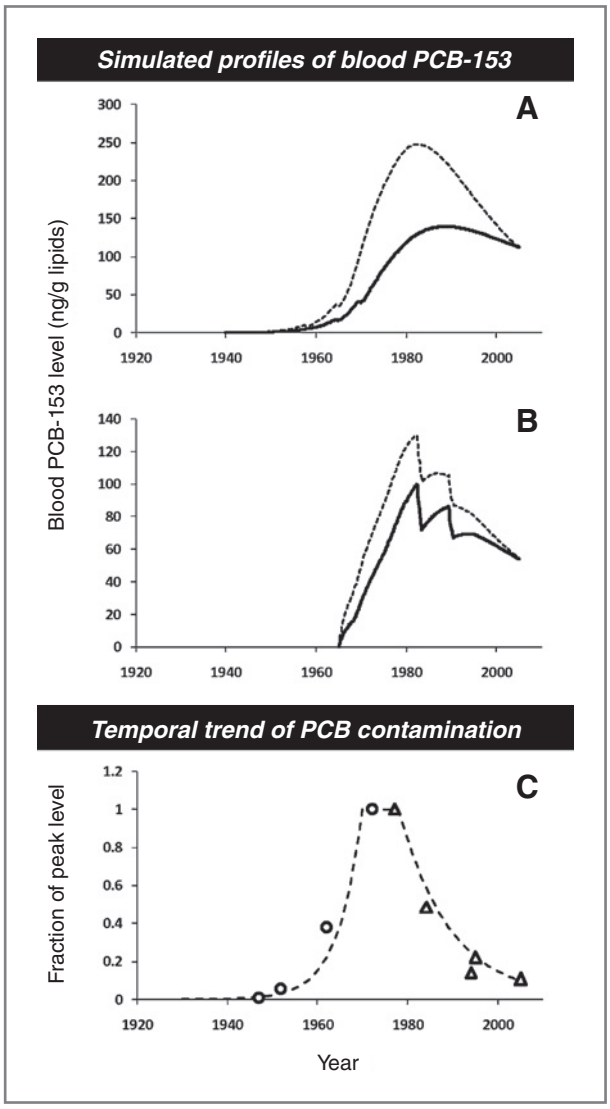

Figure 1. Examples of toxicokinetic profiles for women born in 1940 (A) and 1965 (B) with different weight and breast-feeding profiles. Lifetime blood PCB-153 profiles for half-lives of 10 (dotted line) and 30 years (continuous line) were based on levels at diagnosis, individual characteristics, and the temporal trend in actual environmental contamination levels (C) reported as fractions of peak levels [based on production data (○: ref. 14) and estimated daily intakes (Δ: ref. 15)].

total internal exposure during a time period, and the maximum blood concentration (C_{max}) were compiled for each of the 2,134 women (see Fig. 1). AUC for the first decade (0–10 years) was not used in this study, as levels during this period are strongly influenced by breast milk consumption in infancy (16), a factor that was not included in our simulations. PBPK-derived exposure estimates were compared with measured blood PCB-153 concentrations through Pearson partial correlation analyses, adjusting for age at cancer diagnosis stratified by 5-year intervals to account for case–control matching procedures in this study. We assessed these correlations in different age, breast-feeding, and BMI strata. Statistical analyses were done using SPSS for Windows 10.0 (SPSS Inc.).

Results

Women enrolled in this study were on average 55 years old (range = 25–75 years) (Table 1). Breast-feeding was relatively low in this population as 47% of the women never breast-fed, and those who breast-fed did so for an average period of 5.6 months over their lifetime. Only 6% of the women breast-fed for longer than 12 months. Mean BMI was 24.8, and 13% of individuals had a BMI of more than 30. Measured blood PCB-153 levels ranged from less than LOD to 1,218 ng/g lipids.

Blood PCB-153 levels

Median simulated PCB-153 C_{max} (5th–95th percentile) were 244 (68–703) and 126 (38–353) ng/g lipids for half-lives of 10 and 30 years, respectively. The C_{max} value was reached on average 24 and 18 years prior to blood sampling when assuming half-lives of 10 and 30 years. These values were on average 2.8 times higher than blood PCB-153 levels measured at the time of diagnosis when simulations were done with a 10-year half-life and 1.6 times higher when a half-life of 30 years was assumed.

Correlation analyses

Exposure estimates and blood levels were log-transformed prior to analyses. Because correlation coefficients between measured PCB-153 and AUCs displayed a similar pattern regardless of the half-life used in the analyses, results obtained only with a half-life of 30 years are reported. AUCs during contiguous decades were correlated with coefficients of 0.900 between $AUC_{10–20}$ and $AUC_{20–30}$, 0.666 between $AUC_{20–30}$ and $AUC_{30–40}$, 0.724 between $AUC_{30–40}$ and $AUC_{40–50}$, and 0.959 between $AUC_{40–50}$ and $AUC_{50–60}$. When all the individuals were included in the analyses, Pearson partial correlations between measured blood PCB-153 levels at diagnosis and simulated estimates decreased from 0.981 for $AUC_{50–60}$ (closest to sampling time) to 0.735 for $AUC_{10–20}$ (furthest from sampling time; Table 2). Stratification by age groups did not reveal any apparent discrepancy across correlation coefficients. However, the higher coefficients in these strata when compared with those obtained with the whole data set suggested that a residual effect of age was not accounted for by matching women by 5-year intervals. Stratification based on the duration of breast-feeding revealed that the loss in correlation strength across time is, at least in part, due to breast-feeding, with women who breast-fed for longer than 1 year showing the weakest correlations between measured levels and AUCs before the reproductive period ($r = 0.509$ for AUC = 10–20 years). Stratification on BMI did not impact the trend in correlation coefficients. Running correlation analyses solely for women with no missing weight data did not change the results. When only women

Table 1. Demographic characteristics of study participants

Categories	*n*	%	Mean	SD	Minimum	25th percentile	Median	75th percentile	Maximum
Age, y	2,134		55.0	10.85	25.5	47.3	55.3	63.4	75.0
<40	210	10							
40–50	489	23							
50–60	695	33							
>60	740	34							
Parity									
0	191	9							
1	292	14							
2	802	38							
3+	849	40							
Total breast-feeding, mo	1,005	47	5.6	7.43	0.2	1.4	3.0	7.0	64.7
0	1,129	53							
0–3	444	21							
3–12	437	20							
12+	124	6							
BMI, kg/m^2	2,131		24.8	4.8	14.3	21.3	23.8	27.2	54.7
<20	252	12							
20–25	1,045	49							
25–30	565	26							
>30	272	13							
Height, cm	2,131		161.5	6.2	134	157	161	165	180
Blood PCB-153,[a] ng/g lipids	2,134		84.9	86.7	4.2	56.2	88.3	142.5	1,218.3

[a]After imputation of PCB-153 levels below the LOD.

with blood levels above the LOD were included in the analyses, correlations were on general slightly weaker, but the impact of breast-feeding on correlation coefficients was diminished. This could be the result of the shorter duration of breast-feeding in this subgroup.

Discussion

We conducted this study to address the reliability of PCB exposure levels measured at the time of breast cancer diagnosis in representing early-life exposure. Results suggested that certain parameters could influence PCB toxicokinetics and hinder the reliability of levels sampled at the time of cancer diagnosis in representing exposure during early life. Among these parameters, the variability in the cumulative duration of breast-feeding was shown to weaken the correspondence between blood PCB measurements and estimated early-life levels even with the low prevalence of breast-feeding in this population. The traditional approach to adjust for breast-feeding in exposure–disease associations is unlikely to prevent exposure misclassification, as it does not account for the timing of breast-feeding periods and their impact on blood/tissue PCB levels during periods which could be etiologically relevant in breast cancer development. This also applies for variations in BMI. Therefore, samples collected at the time of diagnosis or a few years before may not allow the identification of associations between breast cancer and PCB levels during earlier hypothesized periods of susceptibility such as puberty.

The theory of critical windows of breast vulnerability was put forward in studies of atomic bomb survivors in Japan. These studies showed that women younger than 20 years at the time of exposure to radiations were more likely to develop breast cancer than women exposed during later stages of life (17). Cohn and colleagues (7) also suggested that early chemical insults, as indicated by DDT levels measured during the twenties, can increase breast cancer incidence. If there is a critical period of susceptibility to PCBs during early stages of life such as puberty, results reported herein suggest that studies based on blood levels at the time of diagnosis may not be able to detect exposure–disease associations. The observed discrepancy between levels measured in this study and estimated past levels during hypothesized critical windows could have biased ORs toward the null hypothesis in epidemiologic studies. Assessment of exposure during etiologically relevant periods through pharmacokinetic modeling is expected to decrease exposure misclassification as compared with the simple use of blood PCB levels measured many years after the period of breast vulnerability to carcinogens. Under the assumption of a real link between PCBs and breast cancer, PBPK modeling should thus reinforce the observed association (i.e., the OR) between PCB exposure estimate and disease. Preliminary analyses conducted in the CECILE study point to this direction (manuscript in preparation).

Table 2. Pearson partial correlation coefficients between PCB-153 levels measured at the time of cancer diagnosis and PBPK-derived exposure estimates (adjusted by age at blood sampling)

	PBPK-derived estimates of exposure					
	C_{max}	$AUC_{10–20}$	$AUC_{20–30}$	$AUC_{30–40}$	$AUC_{40–50}$	$AUC_{50–60}$
Age at diagnosis						
<40	0.903	0.891	0.902			
40–50	0.937	0.883	0.933	0.972		
50–60	0.965	0.824	0.919	0.960	0.978	
>60	0.972	0.847	0.857	0.912	0.963	0.981
Total breast-feeding, mo						
0	0.971	0.751	0.744	0.867	0.961	0.982
<3	0.976	0.752	0.751	0.884	0.969	0.981
3–12	0.932	0.743	0.751	0.892	0.967	0.984
>12	0.837	0.509	0.564	0.786	0.948	0.960
BMI, kg/m^2						
<20	0.965	0.770	0.799	0.917	0.981	0.989
20–25	0.948	0.715	0.726	0.880	0.967	0.981
25–30	0.959	0.775	0.747	0.848	0.960	0.985
>30	0.943	0.747	0.751	0.873	0.955	0.973
All	0.951	0.735	0.736	0.870	0.963	0.981

Because the reported correlation analyses were based on PBPK model simulations rather than serial blood PCB-153 measurements, some limitations of this study ought to be mentioned. First, many factors such as changes in dietary habits or place of residence were not included in the model and could have altered the extent of environmental exposure. Also, the PBPK model as a whole was not validated on repeated PCB measurements. Be that as it may, this approach incorporating breast-feeding history and weight/height profiles allows the reconstruction of lifetime PCB levels considering validated population-derived physiologic parameters relevant to PCB toxicokinetics. Given the scarcity of studies with repeated PCB measurements, the PBPK model presented herein offers a unique opportunity to evaluate the adequacy of PCB levels measured at the time of cancer diagnosis to study exposure–disease associations.

In conclusion, this study suggests that epidemiologic studies based on PCB levels measured at the time of diagnosis or a few years before might have underestimated associations that are specific to early-life periods of vulnerability. These results imply that future studies on PCBs and other lipophilic, persistent organic pollutants will need to further evaluate the temporal variability in internal levels, particularly when studying diseases with early windows of susceptibility. Although prospective studies on diseases with long latency periods are methodologically challenging, serial blood/tissue sampling would help understand the impact of different physiologic and lifestyle factors on the toxicokinetics of these compounds. On the basis of these data, statistical or pharmacokinetic models could be developed and calibrated to facilitate the back-estimation of levels during different periods of exposure.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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