SUPPLEMENTAL MATERIAL to:

Physiologically-Based Pharmacokinetic Modeling of Persistent Organic Pollutants for Lifetime Exposure Assessment: A New Tool in Breast Cancer Epidemiological Studies.

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1. Model Parameters

The parameters for the proposed generic PBPK model for POPs were obtained from the literature. The physiological parameters are estimated according to different equations listed in Table S1, they are a function of body weight, height and/or age. The functions are either taken directly from Price PS et al. (2003) or derived from the same data (Haddad et al 2006). Physiological changes relating to pregnancy are also considered by using equations from Gentry et al. (2002) (table S1). Parameters related to lactation are also estimated from equations from Neville *et al.* 1991. These show relationships between volume of milk or the milk composition and the number of days post-partum (Table S2).
Blood and tissue composition data needed to determine tissue:blood partition coefficient were also taken from the literature and are shown in Table S3. All values were assumed to be the same throughout life except for adipose tissues and muscle and skin for which data showed significant difference in lipid content between children younger than 14 years old and adults. The values in lipid composition between 14 and 18 years of age were estimated by assuming a linear increase from values at 14 to those at adulthood (i.e., 18 years old).

2. Sensitivity analysis

A sensitivity analysis was made to determine parameters most influent on blood concentrations over lifetime (AUCblood) at different ages (i.e., 5, 14, 25, 40 and 55 years old). Figure S1 displays the relative sensitivity coefficient of each parameter that is independent of BW, BH and/or age. The simulation used a PCB 153 exposure scenario with breast milk drinking in the first year of life and a single birth during adulthood with a constant environmental exposure of 20 ng/kg/day. The parameters relating to distribution in adipose tissue (Fla, Flc and Flb) were most sensitive followed by intrinsic clearance (Clint). Other parameters that were obviously influent during adulthood were those that touched excretion through breastfeeding : Duration (MDDUR) and lipid content of milk (Fmlki). The age at which the mother has her baby (Agebaby1) also had an impact only at 40 years of age. The impact of the duration of the breast milk drinking after birth was great in early life and diminishes as age increases.

3. Variability

Because all physiological parameters change as a function of age, BW and/or BH, it was not possible to include them in the sensitivity analysis performed in the previous section. Therefore, different Monte Carlo (MC) simulations were performed to determine the impact of variability of BW, BH and sensitive parameters on the blood concentration (Cblood) vs time profile.

3.1. Impact of BH, BW on toxicokinetic profile. Using data derived from distribution charts of BH vs age (Fig S2), BMI vs age (Fig S3) and BH vs BW (Fig S4) for American women (CDC 2008 and Halls.md 2008), distributions of Cblood vs time profiles were performed. In each run of the MC, the subject's baseline BH and BMI percentile was randomly selected (from 5^{th} to 95^{th}). The percentile of BMI was randomly modified by up to ±5% for each of four periods as the subject aged. For BW, an initial percentile was first determined and initial values were determined from the BH vs BW chart according to percentile chosen and BH value previously determined (fig S4). At ages above 2, the BW is then estimated by using available BMI distribution values for women from 2 to 55 years old, as follows:

$$BW = BMI * (BH/100)^2 \qquad \text{Equation S1}$$

The BMI percentile value was allowed to increase or decrease during life with change up to a maximum of 20%. The Figure S2 shows the impact of variability in BW and BH in a population on Cblood for a given exposure and life scenario. The variability in blood

concentrations introduced by BH and BW is greatest in infants after birth (i.e, variations up to 2-fold) and after lactation (i.e., variations up to 3-fold) in the population. There is a variability of about 1.3 for the rest of life.

3.2. Impact of adipose tissue:blood partioning and metabolism on toxicokinetic profile

Sensitive parameters that are difficult to obtain from epidemiological study questionnaires are Clint and those involved in the adipose tissues to blood partition coefficient (Flfa, Flfc, and Flb). To compare impact of variability on lifetime toxicokinetic profiles in the sensitive parameters Clint and those involved in the adipose tissues to blood partition coefficient (Flfa, Flfc, and Flb) with that obtained in section 3.1, MC simulations were performed by varying the parameters according to distributions published in the literature (Table S4). The results are shown in Figures S6 and S7. Using average values of these parameters can lead to errors representing 1.5 or 1.8 times the true values in blood concentrations for Clint or lipid composition in adipose tissues and blood, respectively.

3.3. Monte-Carlo simulations using distributions for BH, BW and all sensitive parameters

Using available distribution data for sensitive parameters a Monte-Carlo simulation was made to determine the expected variability in blood concentration following a given background daily dose (20 ng/kg/day). Variability in exposure to breast milk was also included and milk concentration was set at 2 µg/L. Results, shown in Figure S8, show that regardless of external exposure levels, internal concentrations can vary greatly in a population simply due to variability in physiology, breastmilk drinking in childhood, and more importantly breastfeeding during adulthood (years 20 to 40). Blood concentrations vary up to 100-fold during adulthood years when lactation occurs.

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Table S1. Physiological parameters for organ volumes and blood flows as a function of age, body weight and body height.

Parameters	***Age***	***Equations***
Volumes (L)		
Body surface cm^2 (S)[1]	0-55	$=BW^{0.5150} \times BH^{0.4220} \times 234.9$
Volume of liver (Vl) [1]	0-55	$=0.0501 \times BW^{0.780}$
Volume of richly perfused tissue (Vrp) [1]	0-3	$=(-1.919E\text{-}2 \times Age + 3.193 \times (BW^2/BH)^{0.2657} - 1.374) - Vl$
	3-18	$=(2.515E\text{-}2 \times Age + 7.619 \times (BW^2/BH)^{0.1499} - 6.098) - Vl$
	18-55	$=(2.331E\text{-}3 \times Age + 0.1253 \times BW^{0.8477} + BH^{0.3821} - 4.725) - Vl$
Volume of slowly perfused tissue (Vsp) [1]	0-55	=Vtongue + Vheart + Vskmuscles
Volume of tongue (Vtongue) [1]	0-55	$=1.190E\text{-}3 \times BW - 4.302E\text{-}4$
Volume of heart (Vheart) [1]	0-55	$=1.017E\text{-}7 \times (BH^{0.6862} \times BW^{0.3561} \times 242.7)^{1.420}$
Volume of skeletal muscles (Vskmuscles) [1]	0-3	$=9.563E\text{-}2 \times BW + 1.650E\text{-}2 \times BH + 9.102E\text{-}2 \times Age - 1.642E\text{-}1$
	3-18	$=1.629E\text{-}1 \times BW + 2.603E\text{-}2 \times BH + 4.661E\text{-}1 \times Age - 3.332$
	18-55	$=6.780 \times (S/10^4)^{1.629} - 1.492E\text{-}3 \times Age + 3.580$
Volume of skin (Vs) [1]	0-55	=Vdermis + Vepidermis
Volume of dermis (Vdermis) [1]	0-10	$=0.664 \times (S/10^4)$
	10-20	$=-9.356E\text{-}5 - 2.151E\text{-}5 \times Age - 5.058E\text{-}1 \times (S/10^4) + 1.134E\text{-}6*Age^2 + 0.117 \times Age \times (S/10^4) - 1.673E\text{-}5 \times (S/10^4)^2$
	20-55	$=1.834 \times (S/10^4)$
Volume of epidermis (Vepidermis) [1]	0-55	$=7.850E\text{-}2 \times (S/10^4)^{1.049}$
Volume of fat (Vf)	0-55	=Vf_basal + Vf_Incr + Vf_Decr
Basal volume of fat (Vf_basal) [1]	0-55	$=0.91 \times BW - (Vl + Vr + Vp + Vs)$
Fat volume increase during pregnancy (Vf_Incr) [2]		$= BWx\left(0.09x e^{\left[-12.90995862x e^{(-0.000797\,xHours\_S)}\right]}\right)$
Extra fat volume at the end of pregnancy (Vf_endP) [3]		$=BW \times 0.084026$
Fat volume decrease post-pregnancy (Vf_Decr) [4]		=Vf_endP + (-Vf_endP x (Hours _P/4380))
Volume of mammary tissue (Vmam)		=Vmam_basal + Vmam_Incr + Vmam_Decr
Basal mammary tissue volume (Vmam_basal) [2]		$=0.0062 \times BW$
Mammary tissue volume increase during pregnancy (Vmam_Incr) [2]		$= BWx\left(0.0065x e^{\left[-7.44868477x e^{(-0.000678\,xHours\_S)}\right]}\right)$
Extra mammary tissue volume at the end of pregnancy (Vmam_endP) [3]		$=BW \times 0.007088$
Mammary tissue volume decrease post-pregnancy (Vmam_Decr) [5]		=Vmam_endP - (Vmam_endP x (Hours_P/4380))
Volume of uterine tissue (Vu)	0-55	=Vu_basal + Vu_Incr + Vu_Decr
Basal volume of uterine tissue (Vu_basal) [2]		$=0.0014 \times BW$

Uterine tissue increase during pregnancy (Vu_Increase) [2]		$= BWx\left(0.02xe^{\left[-4.715669973xe^{(-0.000376xHours\_S)}\right]}\right)$
Extra volume of uterine Tissue at the end of pregnancy (Vu_endP) [3]		=BW x 0.029799
Uterine tissue volume decrease post-pregnancy (Vu_Decrease) [5]		=Vu_endP + (-Vu_endP*(Hours_P /4380))
Volume of placental tissue (VPla) [2]		$= 0.85xe^{\left[-9.434xe^{(-5.23e-4xHours\_S)}\right]}$
Fetal volume (Vfet) [2]		$= 3.50x\left(e^{\left[-16.081xe^{(-5.67e-4xHours\_S)}\right]}+e^{\left[-140.178xe^{(-7.01e-4xHours\_S)}\right]}\right)$
Blood flows (L/H)		
Blood flow to heart (Qc)	0-55	=Qc_basal+ (Qmam-Qmam_basal) + (Qu-Qu_basal) + (Qf-Qf_basal) + QPla
Basal blood flow to heart (Qc-Basal) [1]	0-55	=15.048 x $BW^{0.7609}$
Blood flow to liver (Ql) [1]	0-55	=60.00 x Vl
Blood flow to richly perfused tissue (Qr) [6]	0-55	=Qc_basal - (Qsp + Qs + Qf_basal + Ql + Qu_basal + Qmam_basal)
Blood flow to slowly perfused tissue (Qsp) [1]	0-55	=1.80 x (Vtongue + Vskeletalmuscle) + 57.60 x Vheart
Blood flow to skin (Qs) [1]	0-55	=9.0 x (Vdermis + Vepidermis)
Blood flow to fat (Qf)	0-55	=Qf_basal x Vf/Vf_basal
Basal blood flow to fat (Qf_basal) [1]	0-55	=1.80 x Vf
Blood flow to mammary tissue (Qmam)	0-55	=Qmam x Vmam/Vmam_basal
Basal blood flow to mammary tissue (Qmam_basal) [2]	0-55	=0.027 x Qc_basal
Blood flow to uterine tissue (Qu)	0-55	=Qu_basal x Vu/Vu_basal
Basal blood flow to uterine tissue (Qu_basal) [2]	0-55	=0.0062 x Qc_basal

Hours_S stands for hours after start of pregnancy
Hours_P stands for hours post-partum
[1] Equation used in Haddad *et al.* (2006)
[2] Equation used in Gentry *et al.* (2002)
[3] Calculated from Gentry *et al.* (2002)
[4] Based on Gentry *et al*. 2003
[5] Equation from present study
[6] Adapted from Haddad *et al.* (2006)

Table S2. Parameters for milk volume and contents as a function of time post-partum (adapted from Neville *et al.* 1991)

Parameters		***Equations***
Vmilk	Volume of milk (L/H)	=(1.069 – 0.001212 x Days_P)/24
Fl_milk	Lipid content of milk (%)	=3.8 + 0.0095 x Days_P
Fp_milk	Protein content of milk (%)	=0.8 + 0.0004 x Days_P
Fw_milk	Water content of milk (%)	=100 – Fl_milk – Fp_milk

Days_P stands for the number of days post-partum

Table S3. Compartment composition parameters used for partition coefficient calculation (taken from Price K *et al.* 2003).

		Compartment composition	
Compartment	***Age***	***Fl (%)***	***Fw(%)***
Blood	0-55	0.6	79.0
Brain	0-55	11.6	75.5
Muscles and skin	0-14	2.1	74.1
	14-18	*linear increase to adult values*	
	18-55	4.2	74.1
Heart	0-55	1.0	73.0
Adipose tissue	0-14	55.0	41.1
	14-18	*linear increase to adult values*	
	18-55	74.1	21.2
Richly perfused[1]	0-55	3.68	78.1

[1] Values calculated as described in manuscript. These composition values were also used to for the mammary, placenta, fetus and uterine tissues.

Table S4. Distributions of sensitive parameters.

Parameter	Type of distribution	Mean value	Standard deviation	Minimum value	Maximum value	Reference
Intrinsic clearance factor	Log normal	1	0.5	-	-	Assumed[1]
Fraction of lipids in blood	normal	0.006	0.0014	-	-	Estimated from Patterson et al 1988
Fraction of lipids in fat (adult)	uniform	-	-	0.71	0.87	Pelekis et al 2001
Fraction of lipids in fat (child)	uniform	-	-	0.53	0.65	Pelekis et al 2001[2]
Milk drinking duration	uniform	1	-	0	2	Assumed
Fraction of lipids in milk	normal	3.8	0.8	-	-	Neville et al. 1991
Duration of lactation	uniform	-	-	0	2	Assumed
Volume of milk factor	normal	1	0.05	-	-	Neville et al. 1991

[1]Variability of intrinsic clearance assumed to vary in similar proportions as in Nong and Krishnan (2007).

[2] Assumed that the child's minimum and maximum values have the same proportions to its respective mean value (from Table S3) as the adult values (i.e., maximum is 95.8 % of adult mean value and minimum is 117.4% of adult mean value).

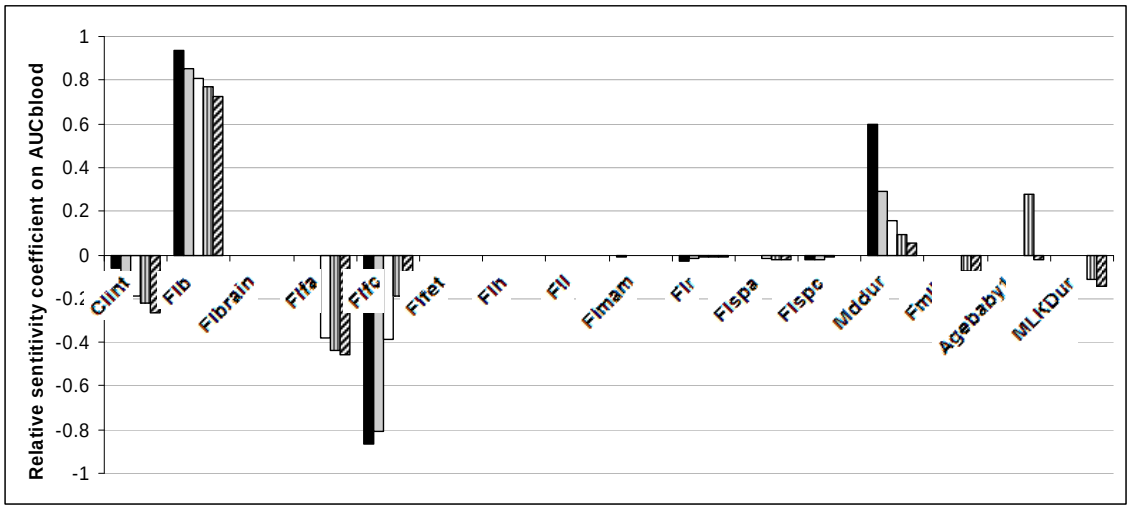

Figure S1. Analysis of parameter sensitivity for different model parameters on the AUCblood at different ages (black= 5 years, gray= 14 years, white= 25 years; vertical line= 40 years, and diagonal lines= 55 years). Clint=intrinsic clearance; Flb=lipid fraction in blood; Flbrain= lipid fraction in brain; Flfa= lipid fraction in adult fat; Flfc= lipid fraction in children fat; Flfet= lipid fraction in feotus; Flh lipid fraction in heart= ; Fll= lipid fraction in liver ; Flmam= lipid fraction inmmmay tissues; Flr= lipid fraction in richly perfused tissues ; Flspa= lipid fraction in adult slowly perfused tissues , FLspc= lipid fraction in children slowly perused tissus; Mddur= duration of breast milk drinkin g in infancy; Fmlki= lipid fraction in milk; Agebaby1= age of mother at first pregnancy; MLKDur= duration of breastfeeding after pregnancy.

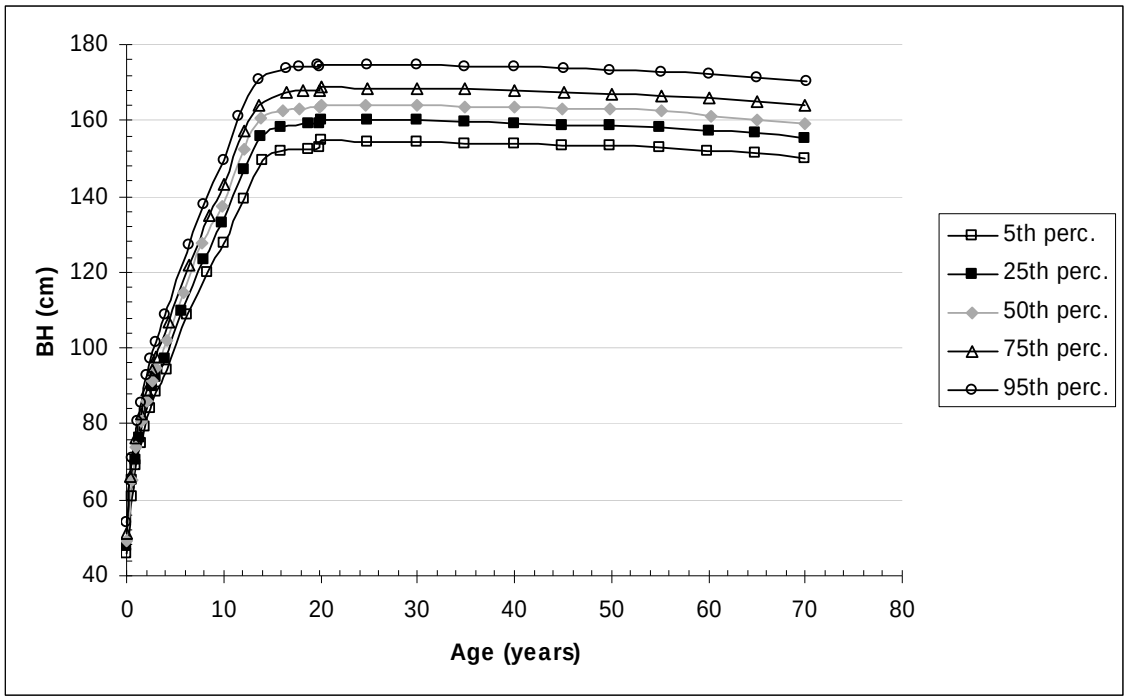

Figure S2. Female growth chart. Symbols represent extracted data (from CDC 2008 and Halls.md 2008) used for interpolation in the PBPK model.

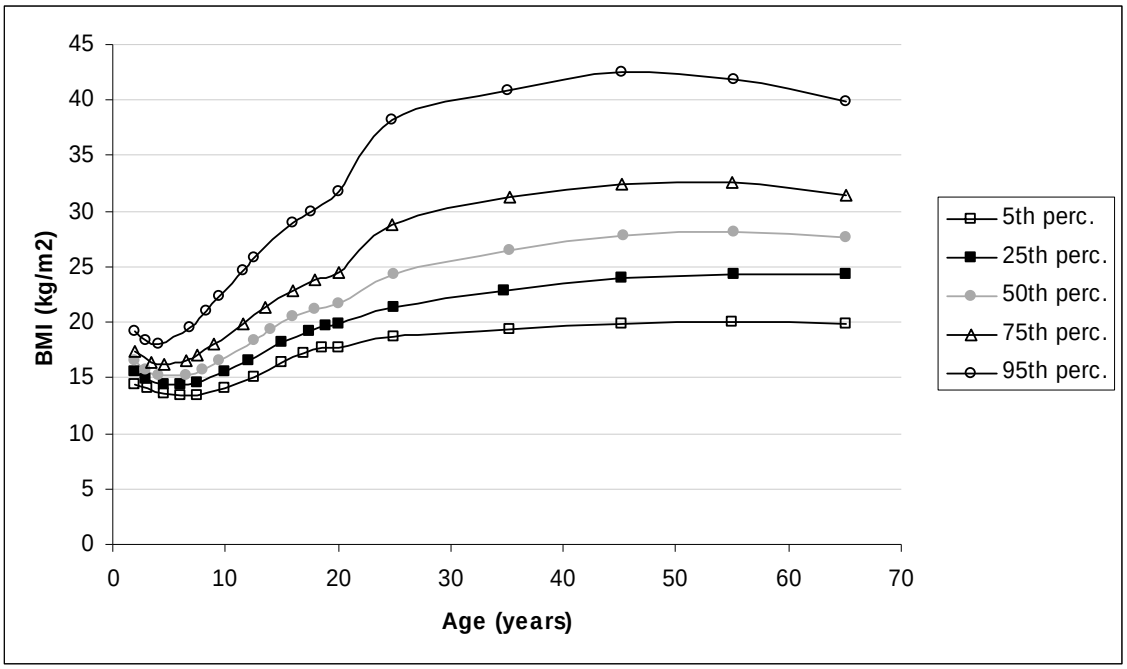

Figure S3. BMI chart in function of age for females of 2 to 65 years old. Symbols reprensent extracted data (from CDC 2008 and Halls.md 2008) used for interpolation in the PBPK model.

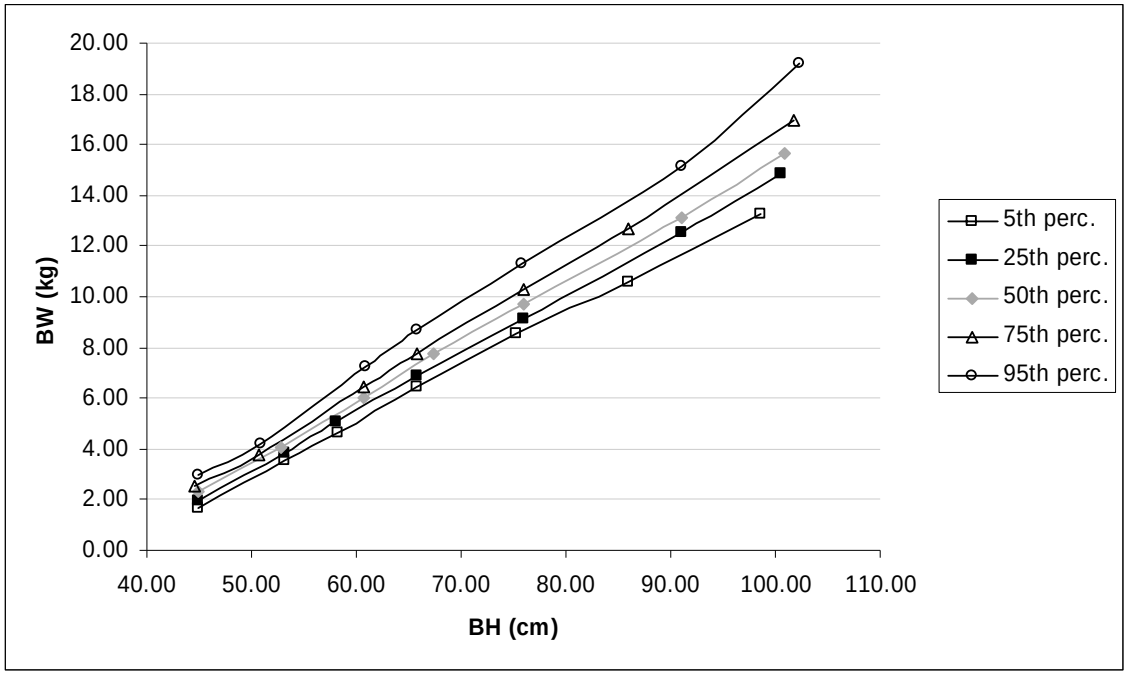

Figure S4. Body weight vs body height chart for females under 3 years of age. Symbols represent extracted data (from CDC 2008) used for interpolation in the PBPK model.

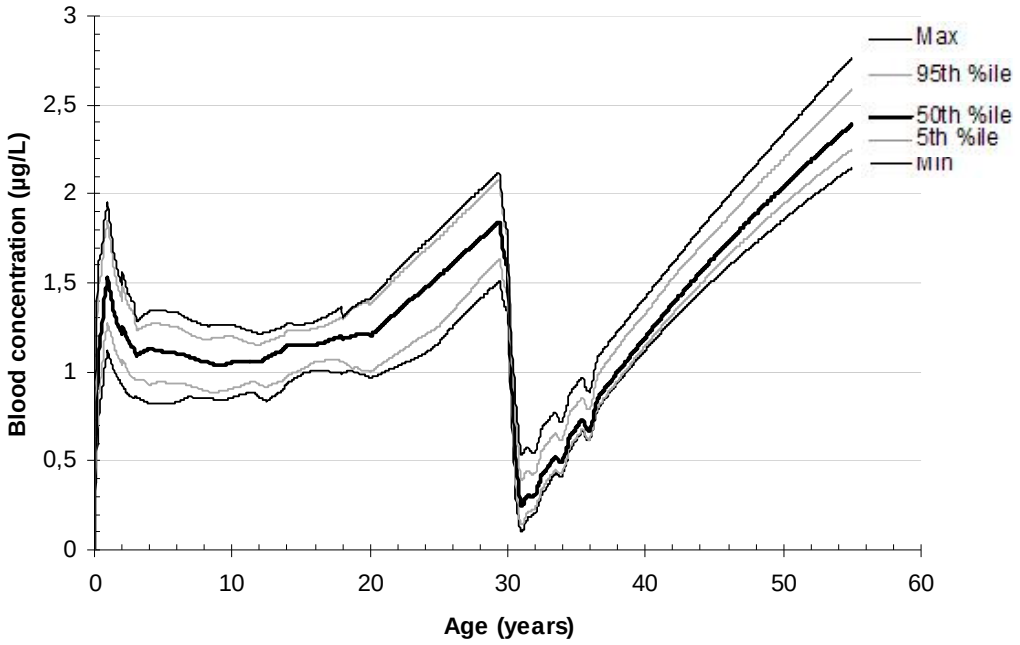

Figure S5. Monte Carlo simulations representing the impact of body height and body weight variations within a population on PCB153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations.

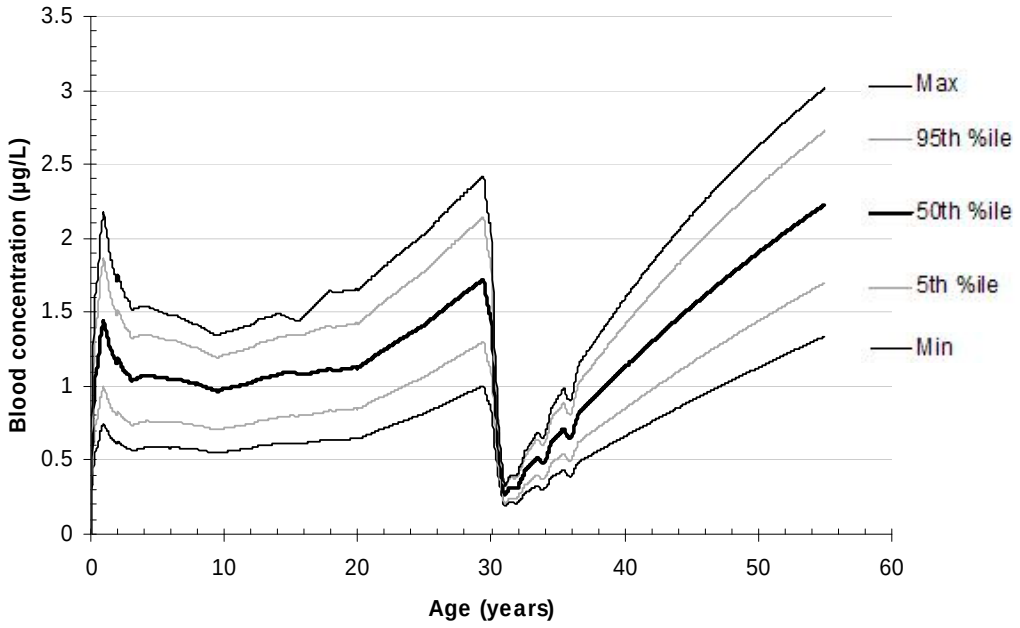

Figure S6. Monte Carlo simulations representing the impact of variations in lipid composition of adipose tissue and blood within a population (see table 4) on PCB153 lifetime toxicokinetics. . Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum

values. Other parameters were kept the same for all simulations. Body weight and height profiles were those of the 50th percentile. All other parameters were the same for all simulations.

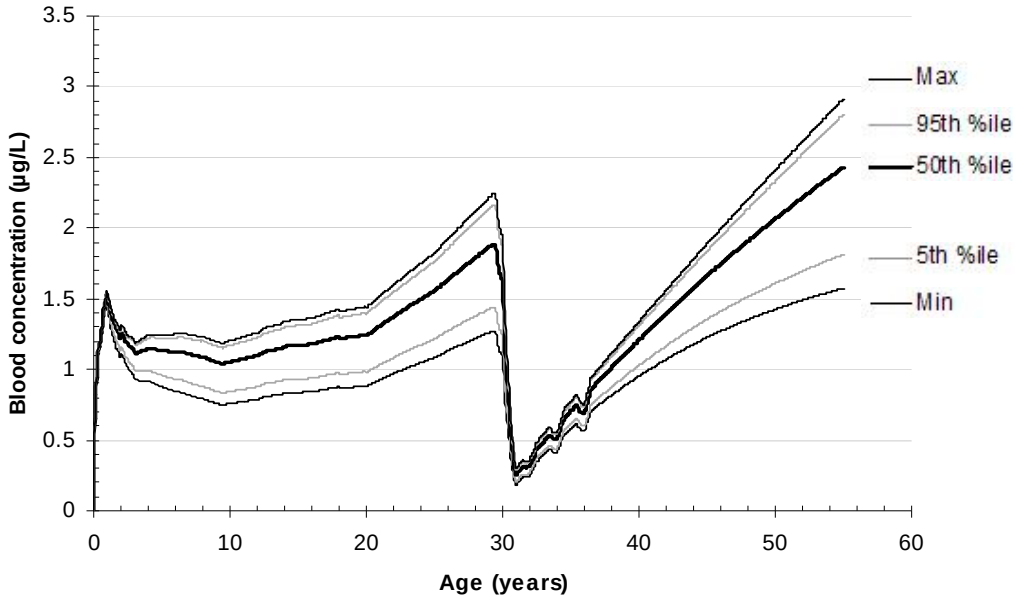

Figure S7. Monte Carlo simulations representing the impact of variations in intrinsic clearance (see table 4) within a population on PCB153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations. Body weight and height profiles were those of the 50th percentile. All other parameters were the same for all simulations.

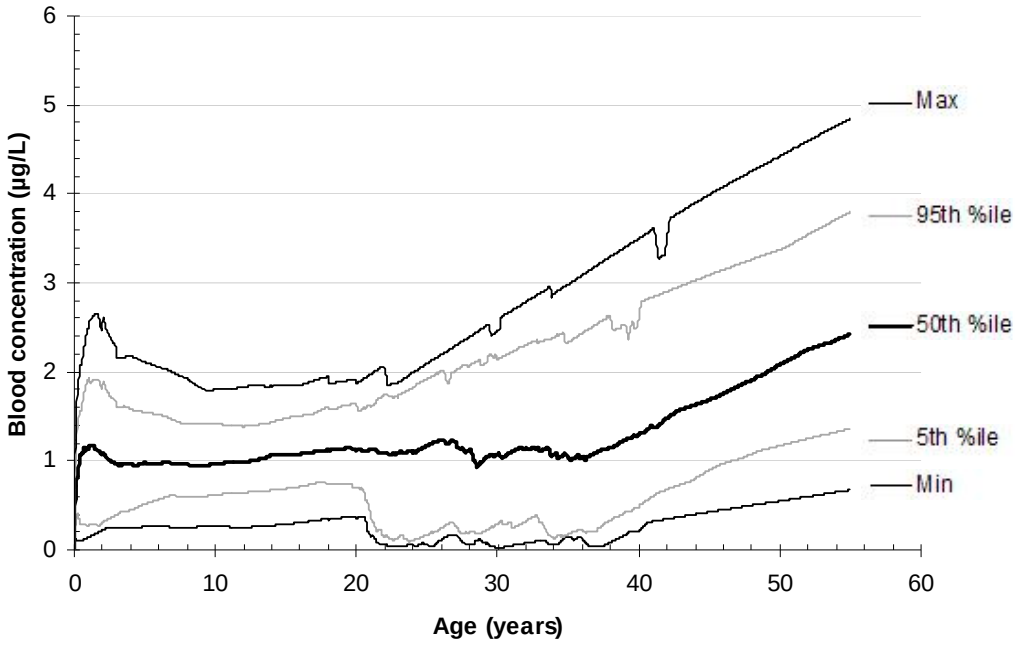

Figure S8. Monte Carlo simulations representing the impact of variations in all sensitive parameters, including body height and weight, within a population (see table 4) on PCB153 lifetime toxicokinetics. . Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations. All other parameters were the same for all simulations.