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Evaluating hexabromocyclododecane (HBCD) toxicokinetics in humans and rodents by physiologically based pharmacokinetic modeling

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ABSTRACT

Hexabromocyclododecane (HBCD) is a flame retardant largely found in textiles, electrical equipment and building materials. The potential exposure associated with adverse effects described in animals make HBCD a substance of interest. To better characterize the risk in humans, it is important to understand the dose-response relationship using available data concerning the exposure and toxicity of environmental contaminants such as HBCD. For this reason, a physiologically-based pharmacokinetic (PBPK) model was developed to describe the disposition of α-HBCD after a single oral administration. The results showed that the model can appropriately predict blood and tissue concentration in rodents. The model described that lipoproteins play a key role in the distribution of α-HBCD in the body even though its lipophilic nature would suggest preferential storage in adipose tissue. The model was also adapted to humans to predict plasma exposure to α-HBCD and showed reasonable estimates when compared against estimated diet levels and biomonitoring measures. As part of a larger study on integrating new toxicity data for human health risk assessment, the present PBPK model will serve as a supporting tool to help extrapolate and interpret *in vitro* and *in vivo* kinetics of flame retardants such as HBCD.

1. Introduction

Hexabromocyclododecane (HBCD) is a persistent organic pollutant used extensively in recent years as a flame retardant. It is found in many objects of daily life such as textiles or electrical equipment but also in many building materials (Covaci et al. 2006). While no long-term animal study has shown mutagenic or carcinogenic effects, many studies have shown some reproductive and developmental effects of HBCD in rats (Darnerud, 2003; Chengelis, 2001). For repeated exposure of HBCD, increased liver weight has been observed among rats (Van der Ven et al. 2006). The same study showed a modulation in the serum concentration of thyroid hormones T4 and TSH demonstrating that HBCD can act as an endocrine disruptor but also as a developmental neurotoxicant (Van der Ven et al. 2006; Eriksson et al. 2006; Ema et al. 2008). However, Helleday et al. (1999) showed that HBCD induces genetic recombination in in vitro assays which indicates a potential to induce cancer via a non-mutagenic mechanism. Some studies are showing mixed time trends, with both increases and decreases in HBCD concentrations in different matrices (Law et al. 2014; Esslinger et al. 2011; Hermanson et al. 2010) despite the fact that HBCD was banned in 2013 (Stockholm Convention) due to highly bio accumulative and persistent properties.

Exposure to HBCD can come from various sources: food, human milk, dust, indoor or outdoor air and soil. Food and human milk remain the major contributors of exposure with an estimated intake of HBCD of 1.12 and 2.1 ng/kg-day, respectively (Roosens et al. 2010). Food exposure is significant compared to intake through house dust (0.45 ng/kg-day) or through outdoor and indoor air (0.25×10^{-3} to 41×10^{-3} ng/kg-day) but considering the high concentrations in house dust, dust inhalation is still considered as a major contributor, especially in kids. Exposure studies have shown a rather diverse contribution of various sources, depending on the exposure scenario and the targeted population. The total daily exposure of the general population in UK or Australia is around 6–8 ng/kg-day (Driffield et al. 2008; Australian government, 2012) and the estimated upper bound adult dietary intakes in both UK and Australia is around 20 ng/kg-day which is higher but in the same order of magnitude as in Canada, where the value averages 15 ng/kg-day (Environment Canada and Health Canada, 2011). For infants and toddlers, those values are much higher because of breast milk consumption and from hand to mouth ingestion of dust

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Table 1
Physical properties of HBCD[a].

Property	Value
Formula	$C_{12}H_{18}Br_6$
Physical state	White, odourless solid
Boiling point	Decomposes at > 190 °C
Melting point	180–185 °C
Vapour pressure	6.27 x 10-5 Pa at 21 °C
Water solubility	48.8 (μg/L) (α-HBCD)
	14.7 (μg/L) (β-HBCD)
	2.08 (μg/L) (γ-HBCD)
LogKow	5.77
	5.38 (α-HBCD)
	5.47 (β-HBCD)
	5.8 (γ-HBCD)
Henry's Law Constant	1.167 x 10-4 atm m^3/mol
Dissociation constant (pKa)	Not expected to dissociate under normal environmental conditions

[a]CAS = 3194-55-6.
[a] National Center for Environmental Assessment et al., (2006)

(Australian government, 2012). Blood concentrations vary from 0.7 to 2.6 ng/g lipid. Moreover, Rawn et al. (2014) recently published the results on concentrations in sera from the Canadian population and Σ HBCD were observed at concentrations < 1 ng/g lipid which is several orders of magnitude below the provisional biomonitoring equivalent (BE) calculated by Aylward and Hays (2011).

The commercial mixture of HBCD comprises of three main stereoisomers: γ-HBCD (75–89%), α-HBCD (10–13%) and β-HBCD (1–12%) (Covaci et al. 2006), which have different physicochemical properties, particularly in their water solubility (2.1–48.8 μg/L) (Table 1). While γ-HBCD is predominant in the commercial mixture, numerous studies have shown that levels of α-HBCD were greatest in most living organisms (Janak et al. 2005; Thomsen et al. 2007). All three stereoisomers are lipophilic, with α-HBCD being the most lipophilic. Because of its higher lipophilicity, α-HBCD has a greater preponderance to bioaccumulation, while β- and γ-HBCD have limited bioaccumulation abilities and are rapidly eliminated by metabolism (Szabo et al. 2011). Some *in vitro* studies have also shown that γ-HBCD can be bio-transformed into α-HBCD by CYP450 enzymes (Zegers et al. 2005), which could account for the predominance of α-HBCD in living organisms.

Despite numerous toxicity and exposure studies, the use of toxicokinetic to link exposure and target responses to HBCD remains unknown. With the advancement and increase in the generation of non-animal data using alternative methods, there has recently been greater need for the use of computational models to help interpret biological data and integrate various types of studies that can eventually be applied in risk assessment (Thomas et al. 2013). This article describes the toxicokinetic of α-HBCD using a physiologically-based pharmacokinetic (PBPK) model that allows for the prediction of population exposure levels of HBCD. A PBPK model was developed using toxicokinetic assumptions of persistent chemicals and physico-chemical properties of α-HBCD. The model was calibrated with rodent data and human extrapolated model predictions were then compared with available biomonitoring data of HBCD from different agencies. As part of a larger study to extrapolate *in vitro* and *in vivo* data for risk assessment, the purpose of this modeling effort is to provide a tool to help understand the toxicokinetic of flame retardants such as HBCD.

2. Methods

2.1. PBPK model

2.1.1. Model representation

The basic structure of the model for α-HBCD, a highly lipophilic substance distributed mainly in fatty tissue, was adapted from the model for octamethylcyclotetrasiloxane by Dobrev et al. (2008). The current model includes seven compartments: blood, liver (the most important site of metabolism), deep liver, adipose tissue (major storage site for lipophilic substances), rapidly perfused tissues (e.g., kidneys, intestines, and lungs), slowly perfused tissues (e.g., muscles, skin and bones) and blood lipoproteins (Fig. 1). The different compartments have been described as blood flow limited tissue except adipose tissue which has been described as diffusion limited tissue. Metabolism was limited to the liver and has been shown as a saturable process described

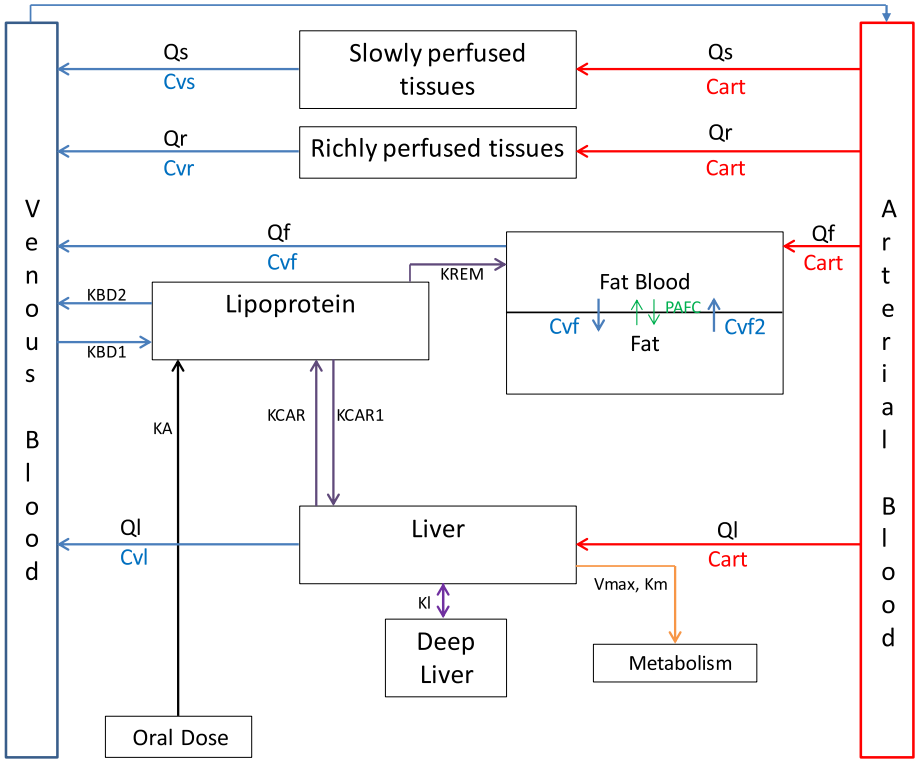

Fig. 1. Representation of the physiologically based pharmacokinetic model for a lipophilic substance with limited distribution and with links to lipoproteins in blood. Cv and Cart are for venous and arterial blood concentrations. Qt refers to cardiac output and Qs, Qr, Qf, Ql refer to blood flow to each compartment. KBD1, KBD2, KCAR, KCAR1 and Krem are transfer constants.

Table 2
Physicochemical and biochemical parameters of HBCD used for simulations for the PBPK model for mice and human.

	α-HBCD initial	α-HBCD final	Units
Partition Coefficient			
PC Adipose Tissue:blood	109.53	165	No units
PC lipoprotein:blood	200	250	No units
PC Liver:blood	5.89	4.50	No units
PC Deep Liver	25	50	No units
PC Kidneys:blood	3.56	3.56	No units
PC Muscle:blood	3.29	3.29	No units
Diffusional clearance as blood flow (PAFC)	0.2	0.04	L/h/Qf
Absorption Constant (Ka)	1	1	h^{-1}
Transfer Constant			
Blood to lipoprotein (KBD1)	10	1	h^{-1}
Lipoprotein to blood (KBD2)	0.5	0.5	h^{-1}
Liver to lipoprotein (KCAR)	0.1	0.01	h^{-1}
Lipoprotein to liver (KCAR1)	150	40	h^{-1}
Lipoprotein to adipose tissue (KREM)	0.1	3	h^{-1}
Between liver and deep liver (Kl)	0.00001	0.00001	h^{-1}
Metabolic constant			
V_{max} for liver metabolism	0.18	0.18	μmol/L/h
K_M for liver metabolism	0.47	20	μM

by the Michaelis-Menten equation. Detailed description of the model mass balance equations can be found in the supplemental materials.

2.1.2. Model parameterization

Modeling parameters are summarized in Table 2. Blood flow and tissue volume were expressed as the fraction of Qc and BW, respectively, from Brown et al. (1997).

Tissue to blood partition coefficients for α-HCBD were calculated *in silico* based on estimates using the equation by Poulin and Haddad (2012).

$$K_P = \frac{(1 + I_{wt}) \times F_{wt} + P_{nlw} \times F_{nlt}}{(1 + I_{wp}) \times F_{wp} + P_{nlw} \times F_{nlp}}$$

Where.

I_{wt} = Ionization term in tissue; F_{wt} = Fractional volume of water equivalent in tissue; P_{nlw} = Neutral lipid-water partition coefficient; F_{nlt} = Fractional volume of neutral lipid equivalent in tissue; I_{wp} = Ionization term in plasma; F_{wp} = Fractional volume of water equivalent in plasma; F_{nlp} = Fractional volume of neutral lipid equivalent in plasma.

The ionization term I_w is different for neutral, bases and acids substances: $I_w = 0$ for neutrals; $I_w = 10$pKa-pH for monoprotic bases; $I_w = 10$ pH-pKa for monoprotic acids.

Knowledge of the dissociation constant (pKa), which determines if a chemical will ionize under environmental conditions, is required to assess the importance of the other physical-chemical properties used in the hazard assessment. The pKa in water provides the amount of the dissociated and undissociated forms of an acid, base, or organic salt in water. As the percentage of ionization increases, the water solubility increases while the vapour pressure, Henry's Law constant, and octanol/water partition coefficient decrease (EPA, 2014). α-HBCD is not expected to dissociate under normal environmental conditions; thus, according to Poulin and Haddad (2012), $I_w = 0$. The partition coefficients calculated for α-HBCD are found in Table 2.

Metabolic parameters (maximum rate of metabolism V_{max} and Michaelis-Menten constant K_M) were derived from Heeb et al. (2014). These *in vitro* metabolized parameters were adjusted against *in vivo* data for better simulation. Added refinement of the metabolic parameters were done by visually fitting within the margin of errors of the dataset to increase the model's goodness-of-fit (EPA, 2006).

The absorption of the dose administered by gavage is depicted using a rate of absorption (Ka) into lipoproteins (lymphatic absorption). After oral exposure, HBCD is absorbed via the lymph and bypass hepatic first pass metabolism. Lymph absorbed through enterocytes goes directly to lymph circulation without going through the liver, which then later joins the blood circulation (O'Driscoll, 2002). Contribution of lymphatic absorption of the ingested dose is well known for highly lipophilic drugs and chemicals (Charman and Stella, 1986; Jandacek et al. 2009).

The major feature of the PBPK model is the distribution of the HBCD via different lipophilic compartments: blood lipoproteins and adipose tissues. The model assumed that by binding to blood lipoproteins, α-HBCD could circulate between this compartment and the blood in both directions. The two transfer coefficients KBD1 and KBD2 describe this process. α-HBCD is then transferred from the lipoprotein compartment to liver and adipose tissue. The adipose tissue is a limited diffusion compartment where transfer between blood and tissue is based on tissue permeability instead of blood flow. The diffusion description of distribution included separation of the blood and tissue compartments by a membrane (Lutz et al. 1980) and included a diffusional clearance as blood flow (PAFC) to describe diffusion through this membrane (Fig. 1). A similar form of distribution is described at the liver tissue where exchange between blood lipoprotein and liver blood (also known as deep liver compartment) is depicted with rate constant Kl (Andersen et al. 2001).

2.2. Model simulation and calibration

2.2.1. Model simulations

All simulations with the PBPK model were generated using acslx (The Aegis Technologies Group, Huntsville AL). The model was first calibrated against mice data and then the mice model was scaled to human physiology, including metabolic activity. Simulations of the scaled physiological human model were then compared with biomonitoring measures. A sensitivity analysis of the model parameters against the different exposure scenarios was produced to evaluate the impact of the different pharmacokinetic processes on the blood concentration estimates.

2.2.2. Calibration with experimental animal studies

Calibration of the PBPK model for α-HBCD was performed using the toxicokinetic study by Szabo et al. (2011) where female C57BL/6 mice were orally administered a single dose of 3 mg/kg of α-HBCD. The validation of the model was based directly on the dose percentages in the liver and the blood in Szabo et al. (2011). Adipose tissue dose percentages were recalculated based on blood concentrations presented in the study of Szabo et al. (2011). Model validation therefore relied upon the recalculated values based on the concentrations instead of reported percentages (See supplemental materials).

2.2.3. PBPK model extrapolation for human populations

The PBPK model developed in mice has been adapted to humans. Body weight and physiological parameters were adjusted to humans (Table 3). Metabolism values in mice were adjusted according to body surface area in humans ($BW^{3/4}$). According to Geyer et al. (2004), the average human half-life of elimination of HBCD is 64 days (range of 23–219 days). The simulation was then performed over 60000 h (6.8 years) to reach a steady state. Blood levels were simulated in humans based on different reference exposure levels found in the literature. These values were then compared to biomonitoring data from different populations (Environment Canada and Health Canada, 2011; Rawn et al. 2014).

2.2.4. Sensitivity analysis

Sensitivity analysis was conducted to examine the impact of each parameter on the maximum concentration (C_{max}), the last

Table 3
PBPK model physiological parameters used for simulations in mice and human (Brown et al. 1997).

Parameters	Mice	Human
Body Weight (BW, kg)	0.022	70
Cardiac output (Qt, L/h)	0.908	312
Blood flow (Fraction of cardiac output)		
Liver (Qlc)	0.161	0.227
Fat (Qfc)	0.07	0.052
Slowly perfused (Qmc)	0.159	0.291
Rapidly perfused (Qrc)	0.240	= Qt – Qf – Ql - Qm
Tissue volume (Fraction of body weight)		
Blood (Vvbc)	0.075	0.079
Liver (Vlc)	0.055	0.026
Fat (Vfc)	0.055	0.1605
Fat-blood (Vfbc)	0.025	0.025
Slowly perfused (Vmc)	0.384	0.60
Rapidly perfused (Vrc)	0.442	0.085
Deep Liver (Vldc) as fraction of liver volume	0.02	0.02

concentration (C_{last}) and the area under the curve (AUC) for HBCD.

The sensitivity coefficient was calculated based on the ratio: $\frac{\textit{Fractional change in model output}}{\textit{Fractional change in parameter}}$.

The concept is based on the measurement of the impact of a disturbance of model parameters on the predicted variable while setting other parameters to their typical value (Krishnan and Andersen, 2010). Each parameter was individually increased by 2% of their original value with all the other parameters held constant to determine the influence of small changes in the parameters. The output model parameters are the maximum concentration (C_{max}) in liver, blood and adipose tissue, the concentration at 320 min' post-dose (C_{last}) and the AUC.

The larger the absolute value of the sensitivity coefficient, the more important the parameter. But if the log normalized coefficient is larger than 1 in absolute value, parameter error is amplified, and model output may be highly uncertain.

3. Results

3.1. PBPK model calibration with mice data

The PBPK model parameters were adjusted to match the α-HBCD blood concentration profiles in mice exposed to a single dose of 3 mg/kg by gavage from the study of Szabo et al. (2011). Lipoprotein blood compartment helps increase the amount of substance in the blood while keeping the metabolism of the test substance high. The deep liver compartment also helps to represent the tissue-lipid accumulation in the liver. Fig. 2 shows the concentration profiles of HBCD in (A) liver, (B) adipose tissue and (C) blood with adjustment of biochemical parameters (K_M = 23.5; V_{max} = 0.18 and PAFC = 0.06). Lipoprotein-blood partition coefficient as well as transfer constants are shown in Table 2.

Overall, the simulated curves in liver, adipose tissue and blood are consistent with the concentrations measured from the mice study. The model does display some difficulties to match empirically the observed delayed absorption of α-HBCD at the early stage of exposure.

3.2. PBPK model extrapolation for human populations

Blood concentrations and dietary intake of various population were estimated by extrapolating the animal based PBPK model to human physiology. The model structure remained unchanged and all the model parameters were adjusted as described in Table 3. Fig. 3 shows the comparison between concentrations of HBCD measured in the serum of the different populations in Europe and Canada to the simulated concentrations in humans by the final model after a 24 h/day exposure over 600 days at 6, 8 and 15 ng/kg-bw/day.

The model was also used to estimate human blood levels based on two points of departure (PODs) of effects as the basis for calculating the biomonitoring equivalent (BE). These two PODs were based on 1) a no observed adverse effect level (NOAEL) from Ema et al. (2008) study based on fertility and developmental effects in rats (NOAEL = 10 mg/kg day) and 2) a lower benchmark dose (BMDL) from Van der Ven et al. (2006) based on liver weight in female rats (BMDL = 22.9 mg/kg day). In the study by Aylward and Hays (2011), the doses of 10 and 22.9 mg/kg (NOAEL and BMDL respectively) were used to calculate a provisional Biomonitoring Equivalent (BE). The authors translated these values into estimates of corresponding chronic intake (0.03 and 0.05 mg/kg day).

The results show that concentrations simulated by the model after different exposure scenarios are consistent with concentrations found in the population, except for concentrations simulated after exposure to the NOAEL and BMDL which are much higher.

3.3. Sensitivity analysis

The important model parameters identified through sensitivity analysis in liver, adipose tissue and blood are shown in Figs. 4–6. Liver volume (Vlc) and liver:blood partition coefficient (PC) were identified as the most important parameters involved in the variability of C_{max} predicted in liver with values varying from 0.82 to 0.93 (Fig. 4). Physiological parameters (liver blood flow - Qlc and cardiac output) were identified as the second most important parameters implicated in the variability of C_{max}. The third parameters involved were transfer constants KCAR1, KBD1 and KBD2. The AUC was highly negatively sensitive to V_{max} and positively sensitive to Vlc and K_M. The AUC was also positively sensitive to partition coefficient liver:blood as well as Qlc and cardiac output to a lesser extent. A 2% variation of input parameters cause variation of C_{last} for V_{max}, K_M, Vlc, PC liver:blood, Cardiac output, PC deep liver:blood and Vldc.

In adipose tissue (Fig. 5), C_{max} was positively sensitive to cardiac output, PAFC, K_M and blood flow in liver and negatively sensitive to V_{max} and KCAR1. C_{last} was highly sensitive to parameters linked to metabolic constants (V_{max} and K_M). The AUC was also sensitive to V_{max} and K_M.

In blood (Fig. 6), the transfer constant KBD1 was the most important parameter involved in the variability of C_{max} with values varying to 0.58, followed by Vvbc (0.46), KCAR1 (−0.36), KBD2 (−0.35) and PC lipoprotein: blood (0.35). A 2% variation of input parameters causes variation of C_{last} for V_{max}, K_M, cardiac output, KCAR1 and Qlc and to a lesser extent, PAFC and Qf. The AUC was highly sensitive to V_{max}, K_M, KCAR1 and cardiac output.

4. Discussion

At present, there is no PBPK model for HBCD. Therefore, the purpose of the present work was to develop a PBPK model for α-HBCD in order to better understand the pharmacokinetics of HBCD and to allow for the prediction of exposure levels in population. The model was developed for α-HBCD using data obtained from Szabo et al. (2011) and then extrapolated to humans and compared with biomonitoring data.

4.1. Animal PBPK model

Many studies have shown HBCD to be a lipophilic substance (Szabo et al. 2011; Janak et al. 2005; Zegers et al. 2005) and available toxicokinetic studies have simplified our model by combining tissues with similar physiological characteristics in the same compartment. Body adipose was lumped into a single compartment to describe the high accumulation of α-HBCD in fatty tissues. In our model, liver is considered to be the main site of metabolism and elimination of α-HBCD. Generally, most of the compartments of a model are considered flow-limited. Indeed, the simplest way to represent the distribution of a substance in a tissue is to consider that this substance is distributed

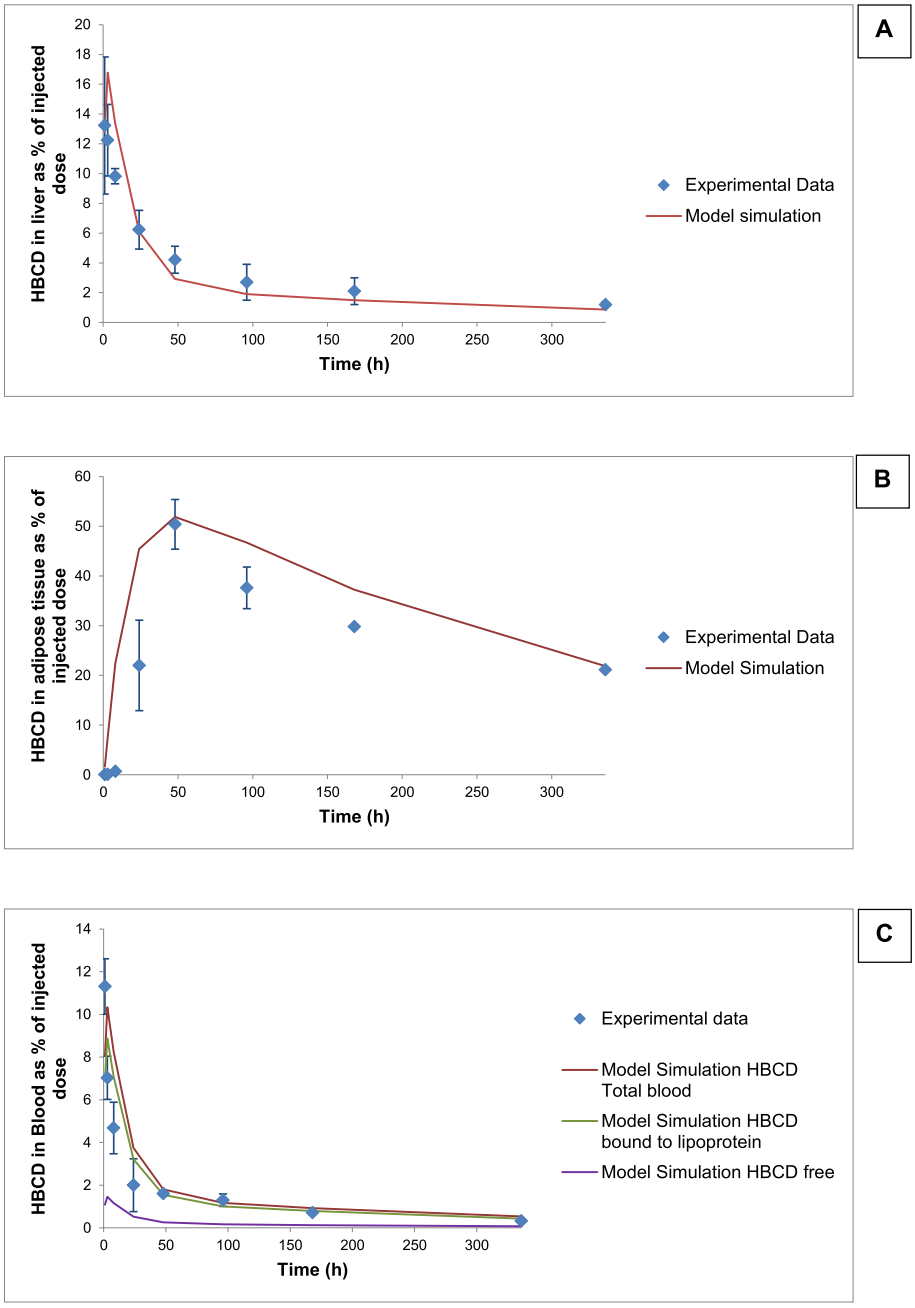

Fig. 2. Comparison of PBPK model simulations (lines) and experimental data (■) of (A) liver, (B) adipose tissue and (C) blood concentration of HBCD in mice exposed to 3 mg/kg of HBCD.

evenly without a concentration gradient, therefore, the limiting element of the distribution is blood flow (Medinsky and Klaassen, 1996). However, in this model, we did consider adipose tissue as diffusion-limited. In this case, the adipose tissue compartment is divided into fat tissue and fat blood sub-compartments with a coefficient between the two to represent the diffusional exchange (Krishnan and Andersen, 2010).

Initial model development and testing suggested that fat and lipid content of the tissues were not representative of the distribution of α-HBCD as shown in supplemental materials. The simulated concentrations in adipose tissue were consistent with the rodent data but the simulated concentrations in liver and blood were far too low, suggesting rapid elimination of the free substance in blood. A lipoprotein compartment was thus added to increase the quantities of the substance in blood and tissues other than adipose tissue. However, in adipose tissue, a delay in the absorption of the substance was observed using the model which was not considered. Overall, the PBPK model developed using descriptive lipophilic exchange between adipose tissues and lipoproteins was consistent with concentration time profiles of α-HBCD in all tissues in rodents.

Metabolic constants values (V_{max} and K_M) were taken from Heeb et al. (2014). These values were calculated from the exposure data with varying enzyme/substrate ratios. The enzyme used was LinA2, a dehydrochlorinase, which selectively binds and converts β-HBCD and the observed initial kinetics were in accordance with a Michaelis-Menten mechanism. However, there are two problems with the use of these constants. First, the choice of the enzyme used in the study, Lin A2 seems inappropriate as several studies have suggested that CYP450 enzymes are responsible for the metabolism of HBCD (Zegers et al. 2005). Second, these values were calculated for β-HBCD, which is

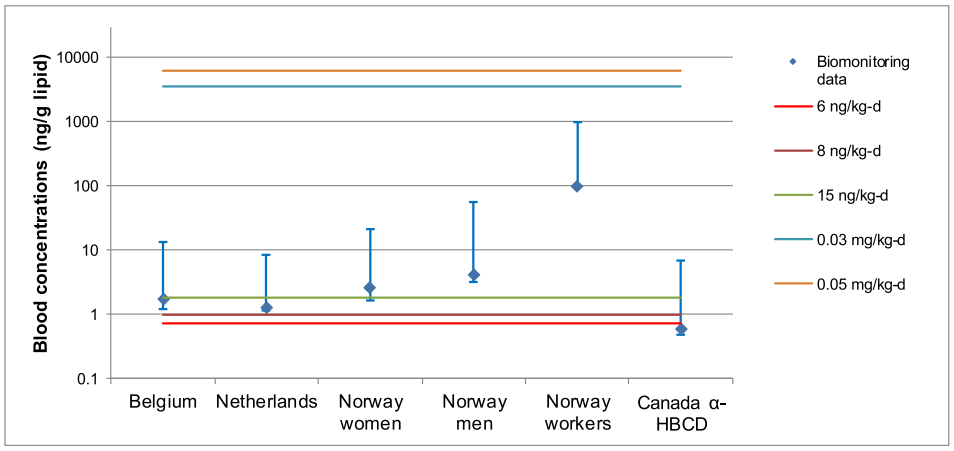

Fig. 3. Comparison of average population biomonitoring data (■) and model prediction of average blood levels (lines) based on different daily total oral doses (6, 8, 15, 0.03 and 0.05 ng/kg day representing respectively the upper bound limit of the average exposure values for adults estimated by the United Kingdom and Australia, the upper-bounding estimates of daily intake of HBCD by Canadians and the HBCD intake in humans based on the BE calculated by Aylward and Hays (2011)).

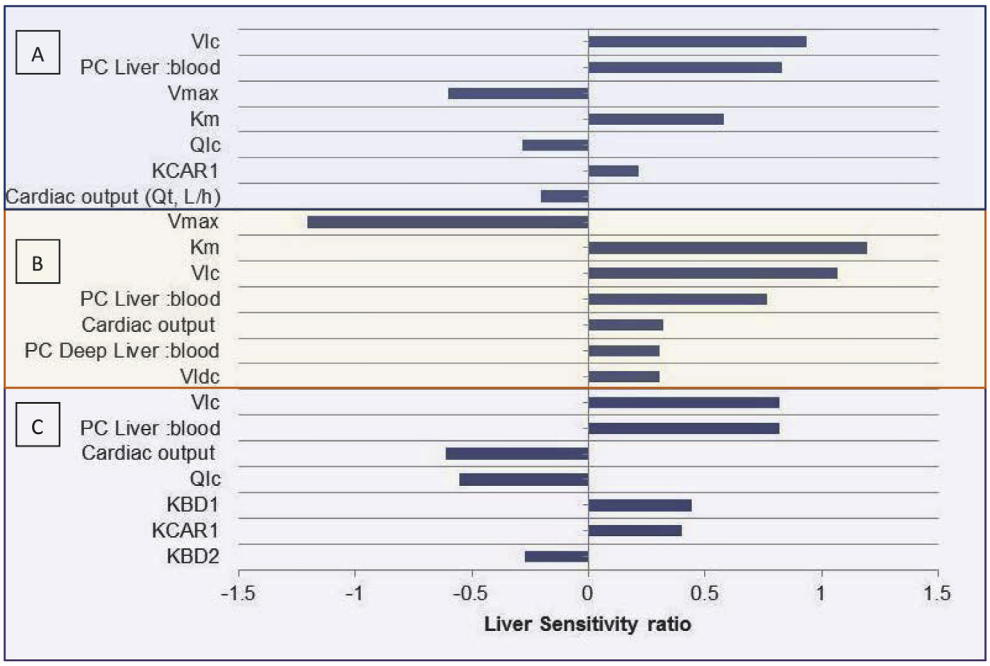

Fig. 4. Sensitivity ratios associated with selected input parameters and (A) AUC, (B) Cl_{ast} and (C) C_{max} of HBCD PBPK model in liver.

known to be metabolized faster than α-HBCD (Sanders et al. 2013). Since no other *in vitro* or *in vivo* data were available for V_{max} or K_M in the literature, K_M was optimized while V_{max} was left unchanged.

Partition coefficients (PC) are essential to determine the distribution of a substance in the body. PCs used in this study were calculated using the method described by Poulin and Haddad (2012). Their approach consists of algorithms for predicting these PCs on the basis of tissue and blood composition in terms of lipid and water content, the octanol: water (log Pow) PC of the chemical and, if necessary, binding association constants. Different simulations with different PC values showed that using higher PCs has improved simulation in adipose tissue while using smaller PCs, simulations were better in liver and blood.

The estimate of the accuracy of the input parameters is very important in modeling because slight variations can affect the model predictions. Sensitivity analysis provides an understanding of how each parameter influences the dose metric and the subsequent dose-response value, as well as which parameters have the most influence on the model predictions (Clewell et al. 1994). The sensitivity analyses showed a slight variation in model outcomes in liver, adipose tissue and blood, and identified the most important parameters involved in this variability. In each tissue, physiological parameters (metabolic constant, cardiac output, tissue volume and partition coefficient) were the most important parameters. HBCD being highly lipophilic, it was expected that the partition coefficient in the fat tissue has been a very important parameter. PAFC and the lipoprotein parameters (KBD1, KCAR1 and PC lipoprotein: blood) are less sensitive but are important nonetheless in the compartmentalization of α-HBCD in deep fat. The slight delay observed in adipose tissue seems to be linked to physiological heterogeneities of fat tissue (varying fat cell size, lipid distribution, blood flow properties), explaining the slower absorption of α-HBCD during the first hours after administration of the dose (Albanese et al., 2002). In addition, it is found that the most sensitive parameters for C_{max} are those mainly related to absorption. In contrast, for the final concentration (C_{last}), the most sensitive parameters are parameters related to metabolism (K_M and V_{max}) and thus to the elimination of the substance.

4.2. Human PBPK model

The human model for α-HBCD was simulated using 3 unique doses of exposure in humans: the upper bound limit of the average exposure values for adults estimated by the United Kingdom and Australia (6 and 8 ng/kg-day., respectively; Food Standards Agency, 2006; Australian

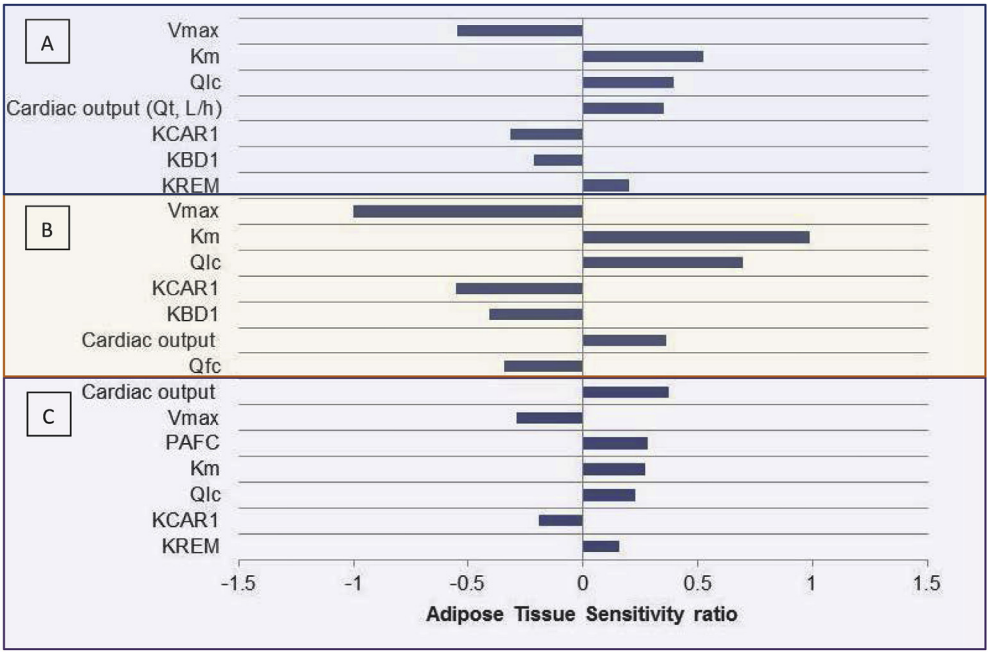

Fig. 5. Sensitivity ratios associated with selected input parameters and (A) AUC, (B) C_{last} and (C) C_{max} of HBCD PBPK model in Adipose Tissue.

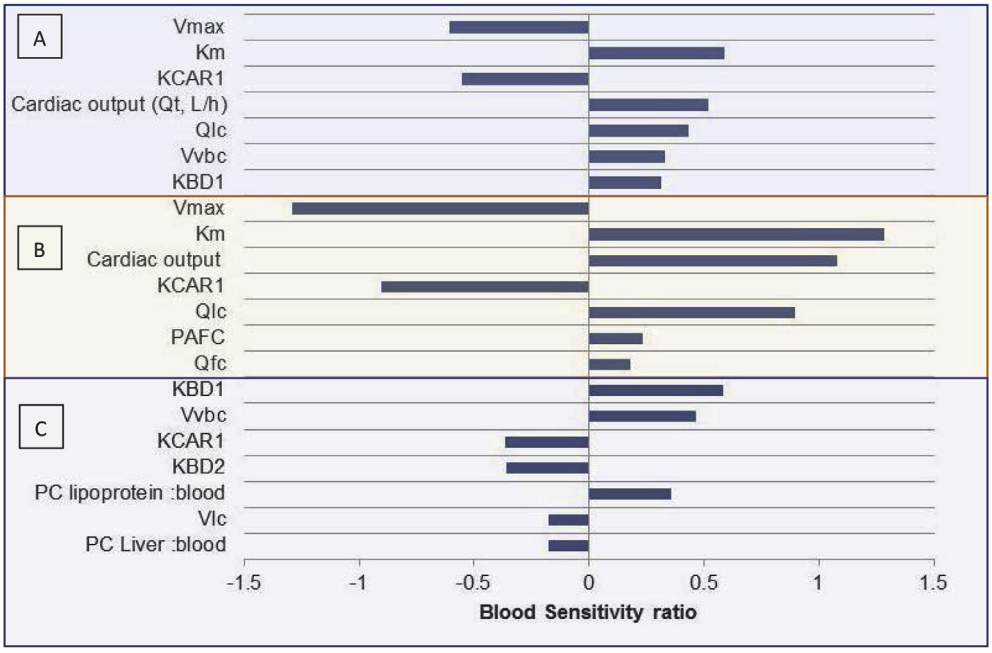

Fig. 6. Sensitivity ratios associated with selected input parameters and (A)AUC, (B) C_{last} and (C) C_{max} of HBCD PBPK model in blood.

government, 2012) and the upper-bounding estimates of daily intake of HBCD by Canadians (15 ng/kg-day; Environment Canada and Health Canada, 2011). The exposure levels in the population were estimated in numerous studies in Europe and Canada, ranging from 0.46 to 2.6 ng/g lipid (Environment Canada and Health Canada, 2011; Roosens et al., 2010). It is important to note that the exposure data in the general population give the total HBCD concentration (α, β and γ-HBCD); however, the model was built for the predominant stereoisomer, α-HBCD, which is found in greater quantities in living organisms (Janak et al. 2005; Zegers et al. 2005). Given the results of the model compared to biomonitoring data, it is reasonable to conclude that the model developed in this study is valid. However, it should be noted that the model is representative for the exposure doses based on food in Canada, Australia and England. Elsewhere in Europe, despite equivalent blood levels, the daily dietary intake calculated was much lower than those used in this model (approximately 1 ng/kg-day). These differences can be explained by the composition of selected food baskets used for the calculation of the daily intake, as well as by the incorporation or absence of dust ingestion.

In the study by Aylward and Hays (2011), the doses of 10 and 22.9 mg/kg (NOAEL and BMDL respectively) were used to calculate a provisional BE. The authors translated these values into estimates of corresponding chronic intake(0.03 and 0.05 mg/kg day) that would be required to reach the lower and upper provisional BE values. The concentrations calculated by the model after exposure to these doses were well above the biomonitoring data.

At the moment, despite the fact that the α-HBCD PBPK model appropriately predicts blood and tissue concentration, the model is not ideal for risk assessment for several reasons: First, only a single PK study was used to calibrate the model, so it would be useful to have other independent studies to validate that the calibration was appropriate. The model was developed only for mice (and humans), not rats, and the PODs mentioned in this paper was based on rat studies. The validation of the human model is only based on crude estimates of exposure (general estimates of daily intake of HBCD in a population that is not necessarily the same population as that in which blood concentrations were measured), at one time point instead of several data points over time. Second, the metabolism parameters found in the literature and used in the parameterization of the PBPK model were not appropriate and were modified to fit the experimental data. To be able to use this PBPK model to predict population exposure in a risk assessment context, exposure, physiological and biochemical parameters need to be relevant. We would recommend doing *in vitro* experiments and *in vitro* to *in vivo* extrapolation (IVIVE) to determine some key parameters for the PBPK model, e.g., metabolism parameters (Yoon et al. 2012). Another study recommended would be to measure the free concentration of HBCD to confirm our assumptions concerning lipoprotein binding. We assumed that, like other highly lipophilic compounds, HBCD is incorporated into lipoproteins during their assembly in enterocytes and absorbed sequestered in chylomicrons (CMs), impacting the free concentration *in vivo* as most of the compound is sequestered, making HBCD not readily available to metabolizing enzymes (Jandacek et al. 2009).

As part of a larger study integrating new toxicity paradigm data for human health assessment, the present provisional PBPK model for α-HCBD should be developed further based on the previous experiments recommended and could then serve as a robust scientific tool to help with the extrapolation and interpretation of *in vitro* and *in vivo* kinetics of environmental chemicals.

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Appendix A. Supplementary data

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Transparency document

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