A LIFETIME PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR CB 153 IN HARBOUR PORPOISES: *IN SILICO* TOOL FOR PREDICTING CONCENTRATIONS OF FUTURE LIPOPHILIC POLLUTANTS?

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Abstract
Physiologically based pharmacokinetic (PBPK) models are mathematical models which are largely based upon the physiological characteristics of the species and upon the biochemical properties of the selected chemical. They give important information about the kinetics and bioaccumulation of pollutants inside the body. As such, they can have a great predictive value and they can be of major importance for risk assessment of chemicals in marine mammals. In the present study, a preliminary PBPK model was developed for the most persistent and prevalent polychlorinated biphenyl (CB 153) in order to find out more about its bioaccumulation, distribution and kinetics in several tissues of harbour porpoises (*Phocoena phocoena*). The model consists of 4 compartments, namely liver (metabolism), blubber or adipose tissue (storage), kidney, and brain (neurotoxicity) and was developed using Berkeley Madonna software. All physiological/biochemical parameters were found in the literature. This PBPK model is capable of simulating the bioaccumulation of CB 153 during the entire life span of approximately 20 years of the harbour porpoises. The intake of CB 153 was from milk from birth to 6 months and after weaning, principally from fish as a food source. The model was evaluated using existing datasets from the literature and data from own analyses performed with GC-MS. Preliminary computer simulation results were consistent with the available data. It is believed that a well constructed PBPK model is a good reflection of reality and that the model can be used as a non-invasive and non-destructive tool for predicting pollution in harbour porpoises or perhaps in marine mammals in general.

Introduction
Physiologically based pharmacokinetic (PBPK) models are a mathematical and computational approach of reality. Based upon the physiology of the organism and the biochemical properties of the selected compound, these models provide insights into the kinetics of the compound in the body which includes processes such as the absorption, distribution, metabolism and excretion (ADME)[1,2]. As such, PBPK models can be used as a tool to describe biological reality and to predict future and unstudied situations with similar pollutants, both are needed for risk assessment[3-5] and for the conservation of this species.
PCBs (polychlorinated biphenyls) are banned since the 1970s, but are still a threat to wildlife, including marine mammals, because of their persistence in the environment. PBPK models for PCBs have been developed for rats, mice and humans[6-8], but are rare for marine mammals. Hickie et al. (1999 and 2005)[9,10] developed models for selected chemicals (including some PCBs) in ringed seals (*Phoca hispida*) and beluga whales (*Delphinapterus leucas*). However, no PBPK models are available for any chemical in harbour porpoises (*Phocoena phocoena*) so far.

Harbour porpoises are common cetaceans in European waters. They are known to accumulate high concentrations of chemicals because of their long life spans and their top position in the food chains. These animals are suggested to have low metabolic capacities for PCBs, and possibly other chemicals as well, compared to other top predators, such as the harbour seals[11]. This makes them particularly vulnerable and sensitive to pollution. In the last decade, observations showed that harbour porpoises were moving from more northern waters (Norway) to the North Sea (Belgium, The Netherlands, UK, Germany). A a results of their limited metabolic capacities, surviving in a contaminated region like the North Sea might be a challenge for the porpoises. Assessing the health situation of harbour porpoises in the North Sea (and other parts of the World) now and in the future requires a more profound knowledge about the kinetics of chemicals inside their body. In

the present study, a preliminary PBPK model for CB 153, as one of the best known pollutants in marine mammals, in harbour porpoises was developed and evaluated. It is our hope that, as more information becomes available, continuing improvements will be made to this PBPK model.

Materials & Methods

Although there are 209 different PCB congeners, the PBPK model presented here will only focus on exposure to CB 153. This congener is, without a doubt, the most dominant and best known PCB in marine mammals and hence provides enough data for model parameterization and validation. The development of this new PBPK model for lifetime exposure to CB 153 in harbour porpoises can be separated into two parts: model conceptualization and parameterization and model verification.

Model conceptualization and parameterization. In the present study, harbour porpoises are described as consisting of 4 tissue compartments perfused by blood (Fig 1). All compartments are selected because of their relevance for exposure to and bioaccumulation of CB 153 and because of the data availability in the literature. Due to the high lipophilic nature of CB 153, blubber was chosen as a compartment for storage. Kidneys represent the tissue where excretion of the parent compound occurs via urine. The intake of CB 153, enterohepatic clearance (elimination through faeces) and possible biotransformation is set in the liver. Brain was included as well due to the possible neurotoxic effects of CB 153. All tissues in the model are considered to be flow-limited, consistent with a PBPK model for lactational transfer of CB 153 in human[7].

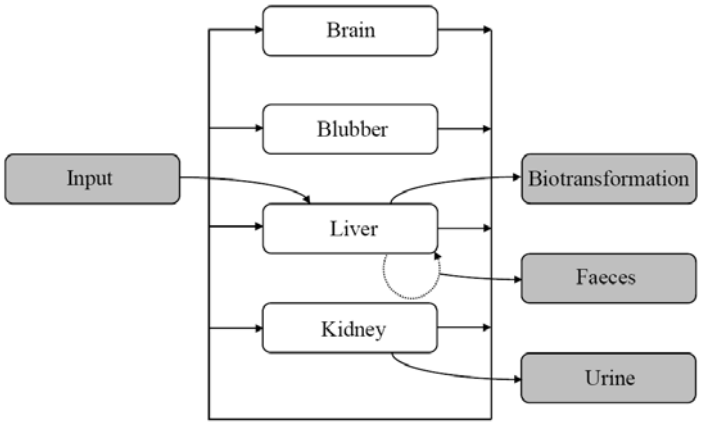

Fig 1. Conceptual diagram of the PBPK model for CB 153 in harbour porpoises.

The absorption of CB 153 occurs in marine mammals mainly through food intake[9]. From birth to the age of 6 months, the porpoises obtain their nutrients (and thus CB 153) exclusively from the milk from their respective mothers. After weaning, the major exposure route is through feeding on fish for the rest of their lives. CB 153 intake was assumed to be directly into the liver, due to the lack of information about the intestinal uptake of chemicals in marine mammals. For both fish and milk, an assimilation efficiency (AE) of 90 % of the total amount of CB 153 ingested was used for the actual uptake of CB 153[10]. The average daily input (ADI) for the fishdiet, expressed in ng CB153/day, was calculated by:

$$ADI = DC \times TOTDIET \times AE$$

With $DC = 0.123 \times BW^{0.80}$ (kg/day) as the daily consumption for porpoises as a function of the body weight[12,13] and TOTDIET as the amount of CB 153 in prey of harbour porpoises[14,15]. The model was coded so that milk would be the only source of CB 153 for porpoises for the first 6 months of their lives. DC for the milk diet was kept constant during this period at 540 g milk/day[16] with an estimated CB 153 concentration of 127.6 ng/g wet weight[17] (Weijs et al., in preparation). Milk or fish was given twice a day in the model.

All physiological parameters used for model development are given in Table 1. The growth of the animals during their entire lifetimes was modeled with a Von Bertalanffy age-dependent growth equation fitted to data

from Gaskin et al. (1983)[18], Duinker et al. (1989)[19], Szefer et al. (2002)[20], Strand et al. (2005)[21] and Law et al. (2006)[22]. Variables in this equation compare favorably with variables found by Lockyer et al. (2001)[23] for harbour porpoises from West Greenland or other areas mentioned in the same study. Changes in tissue or organ volumes with age in harbour porpoises were described by equations, dependent on body weight, taken from McLellan et al. (2002)[24].

The distribution of CB 153 was determined by the blood flow to the four compartments and the partition coefficients from the blood to each tissue. An equation for cardiac output as a function of the body weight was derived from Altman and Dittmer (1971)[25]. Blood flow rates to various compartments were achieved by multiplying the cardiac output with a constant factor for fractional blood flow to each tissue based on human data[26]. The fat/blood partition coefficient was calculated by transforming the equations in Parham et al. (1997)[27] for rat – human extrapolation to rat – bottlenose dolphin (*Tursiops truncatus*) extrapolation since the composition of blood of dolphins (or other marine mammals) is highly comparable with the blood of humans. Other tissue/blood partition coefficients were found by taking the lipid percentage of the tissues[28] into account according to Parham et al. (1997)[27] and Redding et al. (2008)[7]. The distribution process was coded using the following mass balanced differential equations:

$$\frac{dA_t}{dt} = Q_t \times \left(C_{Blood} - \frac{C_t}{P_t} \right)$$

With A_t the amount of CB 153 in tissue t, Q_t the blood flow to tissue t, C_{Blood} the concentration of CB 153 in venous blood, C_t the concentration of CB 153 in tissue t and P_t the partition coefficient between tissue t and blood.

Table 1. Physiological parameters of harbour porpoises used for PBPK model development

Parameter	Value/Equation	Reference
Body Weight (BW; g)	$0.5455 \times BS^{2.275}$	Fitted to data from Strand et al. (2005)[21]; Szefer et al. (2002)[20]; Strandberg et al. (1998)[29]; Ciesielski et al. (2004)[30]; Duinker et al. (1989)[19]
Body Size (BS; cm)	$143.9 \times (1-0.4029e^{(-0.00006378 \times age)})$	Von Bertalanffy equation, data from Gaskin et al. (1983)[18]; Duinker et al. (1989)[19]; Szefer et al. (2002)[20]; Strand et al. (2005)[21]; Law et al. (2006)[22]
Cardiac output (QC; L/min)	$0.1017 \times BW^{0.9988}$	Altman and Dittmer (1971)[25] –mammals
Mass of fat (V_F; g)	$18.41 \times BW^{0.607}$	McLellan et al. (2002)[24]
Mass of brain (V_B; g)	$49.20 \times BW^{0.211}$	McLellan et al. (2002)[24]
Mass of liver (V_L; g)	$0.060 \times BW^{0.932}$	McLellan et al. (2002)[24]
Mass of kidney (V_K; g)	$0.002 \times BW^{1.137}$	McLellan et al. (2002)[24]
Mass of blood (V_{Blood}; g)	$0.143 \times BW$	Reed et al. (2000)[31]
Density of fat ($DENS_F$; g/L)	920	Maruyama et al. (2002)[32] – human
Density of brain ($DENS_B$; g/L)	1050	Maruyama et al. (2002)[32] – human
Density of liver ($DENS_L$; g/L)	1040	Maruyama et al. (2002)[32] – human
Density of kidney ($DENS_K$; g/L)	1050	Maruyama et al. (2002)[32] – human
Density of blood ($DENS_{Blood}$; g/L)	1068	Niels van Elk (Dolfinarium Harderwijk) pers.comm. – bottlenose dolphins
Fractional blood flow to fat (Q_FC; %)	8.2	Recalculated from Brown et al. (1997)[26] – human: 5 %
Fractional blood flow to brain (Q_BC; %)	19.7	Recalculated from Brown et al. (1997)[26] – human: 12 %
Fractional blood flow to liver (Q_LC; %)	41.0	Recalculated from Brown et al. (1997)[26] – human: 25 %
Fractional blood flow to kidney (Q_KC; %)	31.1	Recalculated from Brown et al. (1997)[26] – human: 19 %

Since CB 153 is the most dominant PCB congener in marine mammals, representing about 20 % of the total PCB body burden[11], metabolism or biotransformation of CB 153 was assumed to be only a minor pathway. However, to account for possible biotransformation, the metabolism was limited to the liver and described by the hepatic extraction ratio, the liver blood flow and the blood concentration. All equations were taken from Verner et al. (2008)[8] and rely partly upon the half-life value of 27.5 years for CB 153[8,33]. Excretion of CB 153 through faeces or urine is poorly described in literature. CB 153 is a highly lipophilic compound and is therefore assumed to be only a minor component in urine. Excretion of CB 153 was therefore estimated between 0 and 1 % of the concentration present in the kidney.
Elimination of CB 153 through faeces was thought to be more important compared to excretion through urine. The production of faeces is a part of the enterohepatic circulation. This latter process represents a route of circulation where nutrients are secreted into the bile from where they can be transported to the intestines for reabsorption or eliminated from the body via faeces. Elimination of CB 153 as part of the enterohepatic circulation was estimated to be between 0 and 9 % of the concentration present in the liver, similar to BDE 47[34] which is the most dominant PBDE congener in marine mammals.

Model verification. The model was verified using a dataset of CB 153 concentrations in liver, blubber, brain and kidney of 20 male harbour porpoises from the Black Sea, all stranded or bycaught in 1998. Methods for sample analyses and all results can be found in Weijs et al. (in preparation). An additional dataset from the literature, with results of harbour porpoises from UK waters, was also used to make comparisons[35].

Results & Discussion
PBPK model simulations of CB 153 in blubber, liver, kidney, brain and blood were within the bounds of the data from analyses performed on animals from the Black Sea. However, initial results revealed that minor changes (partition coefficients and elimination factors) in the code were needed for a better fit of the data as shown in Fig 2 for blubber. Given the scattering of the literature data, it is hard to determine the correct values of these parameters. The continuing biomonitoring of the harbour porpoises would be necessary to provide additional data for the improvement of this PBPK model.

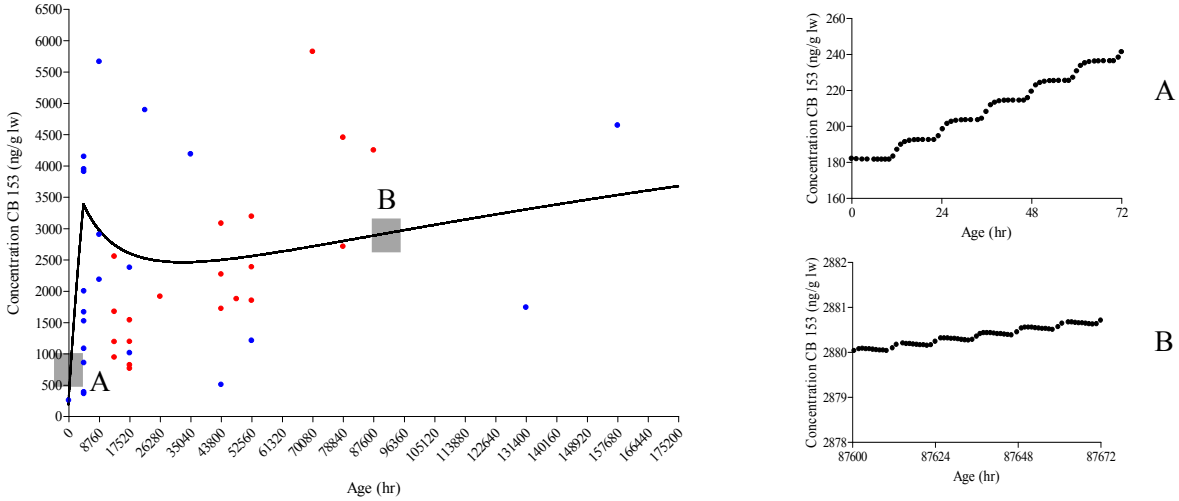

Fig 2. Bioacccumulation of CB 153 (expressed in ng/g lw) in harbour porpoises. The X-axis represents the entire lifetime of the harbour porpoises, expressed in hours. Age 0 is the animal's birth, maximum age is 20 years or 175200 hours. ● = the preliminary model, ● = individual data of harbour porpoises from the Black Sea, ● = individual data of harbour porpoises from UK waters. A and B are close-ups of the first 72 hours of their lives and the first 72 hours at age 10 (87600 hours).

Partition coefficients. The partition coefficients of liver, brain and kidney, calculated and estimated by using the equations from Parham et al. (1997)[27], lipid data from Tilbury et al. (1997)[28] and blood parameters from bottlenose dolphins[36], were found to provide simulation results inconsistent with the literature data and the data from analyses. These partition coefficients might need to be adjusted during further computer simulation exercises.

Excretion through urine and elimination through faeces. CB 153 is the most persistent PCB in marine mammals and ranges for excretion and elimination (between 0-1% and 0-9%, respectively) were therefore narrow and low. Model simulations improved when both processes approached 0, indicating that elimination through urine and faeces plays only a minor role in the whole bioaccumulation process or basically: 'What they eat, is what they get'.

General discussion. Results generated by this preliminary model were compared with data from harbour porpoises from UK waters[35] and they were, coincidently, within ranges of these data (for blubber: Fig 2). This suggests that the uptake of CB 153 in harbour porpoises from both regions was comparable. Concentrations in blubber were highest, followed by liver, kidney, blood and brain. Concentrations in blubber (Fig 2) increase rapidly during the first 6 months of their lives (Fig 2A) influenced by the 'background concentration' at birth and the concentrations in the lipid-rich milk. After weaning, the animals were modeled to switch completely to a 100 % fish diet. Because of the lower concentrations in fish and a fast growth (growth-dilution), concentrations decrease until the age of 3-4 years. After that, growth occurs very slowly, elimination (through faeces or urine) is very low, resulting in increasing bioaccumulation of CB 153 with age (Fig 2B). This pattern was seen in all tissues. CB 153 concentrations in harbour porpoises do not reach a steady-state during the entire life of the animals.

Non-invasive and non-destructive tool. Models like this are highly dependent on the concentration in the diet (fish or milk), which is, especially for fish, relatively easy to investigate *in vivo* and/or *in vitro*. The physiology of the porpoises and physico-chemical features of the chemical (CB 153 in this case) can be found in literature or extrapolated from data of other species (human, rat). This makes such models useful for investigating bioaccumulation of this chemical in harbour porpoises in their entire distribution range.
Future endeavors may involve the testing of this preliminary model for other (lipophilic) chemicals and to investigate its predictive power, not only for predictions in time, but also for the fate of new chemicals in harbour porpoises or marine mammals in general.

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