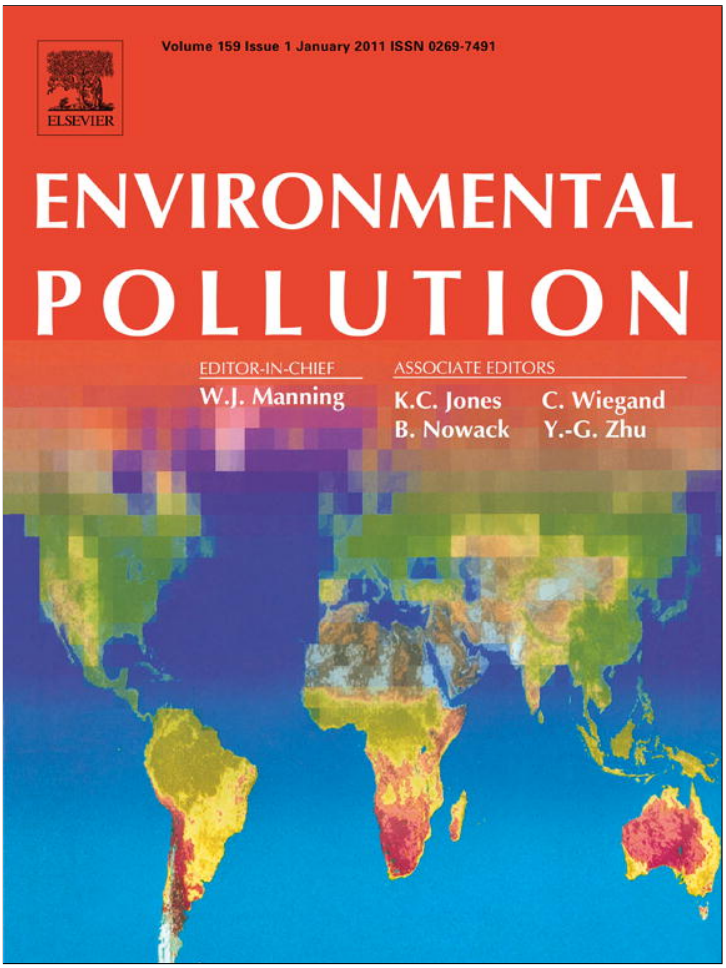

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Computational toxicology: Physiologically based pharmacokinetic models (PBPK) for lifetime exposure and bioaccumulation of polybrominated diphenyl ethers (PBDEs) in marine mammals

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ABSTRACT

Due to migration of harbour porpoises towards more polluted areas like the North Sea and their sensitivity towards pollution, there is a need for proper conservation measures for this species. As a consequence, knowledge about the pollutant's kinetics is required. The present study is the first to investigate the kinetics of PBDEs in marine mammals using PBPK modeling as a non-destructive tool for describing the chemical's kinetics in a protected animal species. The models were developed and parameterized using data from the literature and Black Sea harbour porpoises through computer optimization. The predictability of these models in time was assessed by reverse dosimetry modeling using data from North Sea porpoises (1990–2008). From these predictions, PBDE 99 levels were found to decrease the fastest, followed by PBDE 153, 47 and 100. Results show that the PBPK models can be applied for harbour porpoises from different regions and also simulate time trends.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in various products and equipment ranging from textiles to electronics. They have been banned in many countries (ATSDR, 2004); however, they are still ubiquitous in the environment due to their stability and persistence (Birnbaum and Staskal, 2004). Some PBDEs have the potential to biomagnify in terrestrial and aquatic food webs (Kelly et al., 2007; Voorspoels et al., 2007). Biomagnification is of particular concern for organisms at the top of the food chains, such as marine mammals, as they receive high concentrations of PBDEs from their prey. In addition, lactational transfer of chemicals through the lipid rich milk (up to 40% fat) represents also a major source of contaminants for these animals (Debier et al., 2003; Hickie et al., 2007; Weijs et al., 2009a). Several PBDEs were shown to be toxic on the reproductive, immune and endocrine systems of marine mammals (Sonne et al., 2009; Beineke et al., 2010; Frouin et al., 2010).

Physiologically based pharmacokinetic (PBPK) models represent a mass-balanced system of compartments where the pollutant's distribution is regulated according to the physiological properties of the compartments or tissues and the biochemistry of the pollutants (Reddy et al., 2005; Chiu et al., 2007). Recently, PBPK modeling has been applied for showing the kinetics of specific environmental pollutants in marine mammals (Hickie et al., 1999, 2005; Weijs et al., 2010a). PBPK models for marine mammals are very helpful since these animals are endangered and protected, so that *in vivo* exposure experiments cannot be undertaken. *In vitro* exposure tests can be performed in marine mammal derived cell and tissue cultures of blood and liver (McKinney et al., 2006; Das et al., 2008; Frouin et al., 2010). However, the sampling of liver biopsies or blood from the same animal of a wild marine mammal species is too invasive. Therefore, blubber was the most investigated tissue and is still the best known source of biomonitoring results of lipophilic compounds to date.

PBPK models have the potential to integrate *in vitro* experimental data in liver or blood with biomonitoring information from blubber, so that a non-destructive, *in silico* approach can be developed. With the help of PBPK models, blubber concentrations can be used to predict levels in liver, blood and other tissues thus giving more realistic exposure scenarios than working with hepatic or blood cell

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lines alone. Since blubber samples can be taken in a non-destructive manner from dead and living marine mammals, the PBPK models would, in addition to the *in vitro* tests done with liver cells, allow the assessment of toxicity in a more rapid and instantaneous way. Furthermore, PBPK models are important to describe the dynamics of the exposure-accumulation process. This can simulate the impact of releases of pollutants and remedial measures on their long-term fate and accumulation in marine mammals.

Harbour porpoises have relatively long life spans and feed at the top of the aquatic food chains, resulting in high body burdens of pollutants (Weijs et al., 2009a, 2010a). Previous studies have suggested that harbour porpoises are less able to metabolize several pollutants compared to harbour seals. They were found to have higher proportions of PBDEs in their blubber compared to harbour seals even though both species had a comparable diet (Weijs et al., 2009a,b). In addition, hydroxylated PBDE metabolites (HO-PBDEs) could not be detected in serum of harbour porpoises (Weijs et al., 2009c). During the last decade, movements of harbour porpoises from northern areas to the more polluted Southern North Sea have raised concerns about the viability of the population. These movements and their sensitivity to pollution justify the need for proper conservation measures for these animals.

Similarly to models developed for polychlorinated biphenyls (PCBs; Weijs et al., 2011), the goals of the current study were 1) to develop a non-destructive computational toxicology approach to study protected marine mammals such as harbour porpoises by formulating PBPK models for PBDEs, and 2) to assess temporal trends of lifetime exposure to PBDEs in North Sea harbour porpoises using data from 1990 to 2008.

2. Materials and methods

The models developed for PBDEs are based on the PBPK model for the bioaccumulation of PCB 153 in male harbour porpoises (Weijs et al., 2010a). In general, PBPK models for females include processes (e.g. lactation, gestation) which are responsible for the transfer of contaminants to the offspring thereby reducing the overall concentrations of the chemicals in the females. PBPK models for males lack these processes, so the models developed in the present study are suitable for males only. Similar as in Weijs et al. (2010a), all models consisted of 5 compartments, liver, blubber, kidneys, brain and rest of the body. All tissues were considered to be flow-limited similar as in Weijs et al. (2010a). Dietary uptake, either through the consumption of fish or milk, was set directly to the liver compartment as the liver was the only tissue of the gastrointestinal tract used in the models. Models were developed and parameterized using Berkeley Madonna software version 8.3.14 (Berkeley Madonna Inc) and model codes are available on request to the corresponding author.

2.1. Parameters

All models were developed using parameters taken from the literature or fitted to the data if clearly indicated. The same physiological parameters and equations of the male harbour porpoises (such as the relationships between body size, age and daily intake) as in Weijs et al. (2010a) were used. Biochemical parameters were adjusted according to the specific PBDE congener (Table 1). The parameters that were fitted (elimination half-lives, assimilation efficiency for the milk or AE2 and brain/blood partition coefficients or PB) were chosen upon visual inspection of the position and shape of the curves compared to the real-life data from animals from the Black Sea. Of these three parameters, elimination half-lives were fitted first as this parameter affects the slope of the curve in each compartment. AE2 was fitted after that as it determined only the concentrations in all compartments for animals < 1 year. PBs only had an impact on the curve of the brain compartment and were therefore fitted last.

Exposure was assumed to be through fish and milk consumption only which were both set in the liver as this compartment was the only tissue of the gastrointestinal tract represented in the models. Lipophilic compounds like PBDEs do not dissolve easily in sea water and dermal exposure was earlier found to play only a negligible role for the bioaccumulation of PCBs in beluga whales (Hickie et al., 1999). Tanabe et al. (1997) reported on the concentrations of several PCBs, but not of PBDEs, in the fish prey of the harbour porpoises from the Black Sea. The lack of PBDE data in marine mammals and their prey appears to be the norm, especially for Black Sea porpoises. Thus, we relied on PBPK modeling using the best available data to determine the validity of the model. An assumption was made that PBDEs share similar physical and chemical properties (e.g. two phenyl rings with halogenated atoms, comparable log K_{ow} values for matched congeners) with PCB congeners. This assumption was applied to estimate the dietary fish input of PBDEs using the concentrations of PBDEs in milk samples ($n = 7$) of Black Sea porpoises. In the PCB 153 model, there was a 116 times difference between the concentration in the milk (127.6 ng/g ww) and the concentration in the fish (1.1 ng/g ww; Tanabe et al., 1997; Weijs et al., 2010a). This factor, together with the results of the milk samples (Supporting Information Table S1), was used to calculate the concentrations in the fish prey of the Black Sea porpoises leading to a concentration in fish of 0.054, 0.008, 0.009 and 0.002 ng/g ww for PBDE 47, 99, 100 and 153, respectively.

Physiological parameters were kept as general as possible by using preferentially physiological information of harbour porpoises in general and not only from Black Sea or North Sea harbour porpoises. This was done to make the models species-specific rather than population-specific. As such, the models should allow comparisons between harbour porpoises from different areas (e.g. Black Sea and North Sea).

Table 1
Compound specific parameters for several PBDEs. The original values of the parameters are given between brackets for parameters that were fitted to the data.

	PBDE 47	PBDE 99	PBDE 100	PBDE 153
Log (K_{fp})[a]	2.35750	2.41682	2.41682	2.35750
PF[b]	331.6	380.2	380.2	331.6
PL[b]	7.9	9.0	9.0	7.9
PK[b]	4.6	5.3	5.3	4.6
PB[b]	1.6 (13.3)	2.2 (15.2)	8.2 (15.2)	6.3 (13.3)
PR[b]	8.1	9.2	9.2	8.1
AE1 (%)[c]	95	98	99	97
AE2 (%)[d]	40	31	25	25
CFoetusF[e]	14.1	1.8	2.0	0.3
CFoetusL[e]	5.5	0.8	1.1	0.7
CFoetusK[e]	6.3	5.6	0.5	ND
CFoetusB[e]	1.0	ND	0.2	ND
Half-life (yr)	4.24 (3.1[f])	5.17 (2.9[g])	4.63 (1.6[g])	9.43 (6.5[g])

K_{fp} – adipose tissue/plasma partition coefficient, PF – adipose tissue/blood partition coefficient, PL – liver/blood partition coefficient, PK – kidney/blood partition coefficient, PB – brain/blood partition coefficient, PR – muscle/blood partition coefficient, AE – assimilation efficiency.

[a] Adipose tissue to plasma partition coefficients (K_{fp}) from Parham et al. (1997) are actually for PCBs, but are used here for PBDEs as well; e.g. the log (K_{fp}) for PBDE 47 is actually for PCB 47. Parham et al. (1997) does not report on PCB 100, so for PBDE 100 the same value as for PBDE 99 (or PCB 99) was used.

[b] Equations from Parham et al. (1997) were transformed to equations for bottlenose dolphins (blood composition from Bossart et al., 2001). For partition coefficients of tissues (liver, kidneys, brain) as given in Table 1, the average lipid content was used (Weijs et al., 2010b). For the 'rest of the body'-compartment, the average lipid content of muscle was used (Weijs et al., 2010b).

[c] Assimilation efficiency for the fish diet or the percentage of PBDE absorbed by the juveniles and adults after ingestion of the fish prey. Values taken from Thomas et al. (2005).

[d] Assimilation efficiency for the milk diet or the percentage of PBDE absorbed by the calves after milk ingestion. Values were fitted to the Black Sea data.

[e] Results from own analyses (Weijs, unpublished data) and expressed in ng/g lipid weight (lw). For modeling reasons, values of 0.01 ng/g lw were used for concentrations below limit of detection (ND). Muscle tissue of the fetus was not available, so a value of 0.01 ng/g lw was used here for the 'rest of the body' compartment as well.

[f] Staskal et al. (2005) found a terminal half-life of PBDE 47 in mice of 23 days after a single exposure, which is comparable with the half-life values of TCDD in mice (Miniero et al., 2001). Therefore, the elimination half-life for PBDE 47 in harbour porpoises was derived from the half-life of TCDD which is body weight-dependent (Miniero et al., 2001). The body weight of the harbour porpoises from the Black Sea in the present study varies from 4.1 to 48.5 kg, resulting in half-life values for TCDD (and thus PBDE 47) ranging from 330 days to 5.4 years with an average of 3.1 years.

[g] Geyer et al. (2004) expressed in years.

2.2. Datasets

The datasets used include results of PBDEs in tissues of harbour porpoises from the Black Sea (milk, liver, blubber, kidney, brain and muscle) for goal 1 and blubber results from the North Sea for goal 2. For goal 1, results of one neonate were also used (Table 1).

2.2.1. Black Sea dataset

This dataset was used to parameterize the PBDE models (goal 1), similar to the PCB models in male harbour porpoises (Weijs et al., 2010a, 2011). The dataset contains results for 8 PBDEs in 20 male harbour porpoises (9 juveniles, 11 adults). All animals were by-caught or found stranded in 1998 in the Black Sea and results of PBDEs in blubber, liver, kidney, brain and muscle are discussed thoroughly in Weijs et al. (2010b).

2.2.2. North Sea dataset

The blubber data were used to investigate the usefulness of the PBPK models to predict PBDE accumulation in time (goal 2), as done for PCBs (Weijs et al., 2011). A part of the animals included in this dataset were found stranded or were by-caught along the Belgian coast of the North Sea. These animals were from 1999–2004, the PBDE results in blubber can be found in Weijs et al. (2009a,b). The other part of the animals from this dataset were found alive on the coasts of Belgium and The Netherlands, but died during rehabilitation in SOS Dolfijn, Harderwijk, The Netherlands. These animals were sampled between 1990 and 2008 and data of PBDEs in blubber can be found in Weijs et al. (2010c).

2.3. Sensitivity analysis

To assess the impact of some physiological parameters independent of the body weight on the model outcome, sensitivity analyses were performed as was done previously for PCB 153 (Weijs et al., 2010a). For each parameter, 3 runs (a batch run) were set simultaneously using the original value of the parameter and a coefficient of variation of 5%, resulting in a run with the original parameter, a run with the original parameter increased with 5% and a run with the original parameter decreased with 5%. The impact of the parameter changes on the concentration of PBDE 47, PBDE 99, PBDE 100 and PBDE 153, respectively, in blood was determined by calculating sensitivity coefficients (%) according to the following equation (modified from Mörk and Johanson, 2006):

$$S_c = \left(\frac{AUC_5}{AUC_{Orig}} - 1\right)100$$

With AUC_{Orig} the area under the blood concentration curve with the original parameter value and AUC_5 the areas under the blood concentration curves with the original parameter value increased and decreased with 5%. Blood is the circulation medium between all tissue compartments (liver, brain, blubber, kidney and rest of the body), so changes in one or more of these compartments are reflected in the blood. Therefore, the blood concentration curves were used for the sensitivity analyses.

3. Results

3.1. Goal 1: computational toxicology approach: development of PBPK models for PBDEs

Elimination half-lives of PBDEs, or the time at which 50% of the chemical is eliminated from the body by metabolic biotransformation, fecal or urinary excretion, are scarce in the literature. Geyer et al. (2004) estimated elimination half-lives for several PBDEs in humans (Table 1) which are higher than PBDE half-lives in rodents (Staskal et al., 2005). Nevertheless, considering the continuous exposure and the higher lipid deposits of marine mammals compared to rodents, the human values were here preferred. However, when the human PBDE elimination half-lives were incorporated into the PBPK models, the resulting simulation results were not consistent with the tissue concentration dataset from Black Sea harbour porpoises. Therefore, optimization for suitable elimination half-lives for each PBDE congener was obtained by using the Black Sea dataset (Table 1).

3.1.1. PBDE 47

When the optimized half-life for PBDE 47 of 4.24 years was incorporated into the PBPK model, the computer simulation results seemed more appropriate, not only for all compartments, but also for all ages (Fig. 1A–E). In all compartments, the model reached a peak in concentrations at the end of lactation followed by a steady decline until the age of about 3 years. After that, levels of PBDE 47 increased slightly in the males until the end of their lives. Despite this increase, the calves at the end of the nursing period were predicted to have the highest concentrations of all ages, as the concentrations of PBDE 47 never reach the same level again according to the model predictions (Fig. 1A–E). Typically, concentrations of PBDE 47 were highest in the blubber, followed by the muscles (rest of the body), liver, kidney and brain (Fig. 1A–E).

3.1.2. PBDE 99

According to the model simulations, the half-lives from Geyer et al. (2004) needed to be almost doubled to provide a better fit compared to the Black Sea data (Table 1). The resulting curves reached a peak after the first 4 months due to lactational transfer, followed by a decline until the age of 3 years. The increase after the age of about 3 years was however steeper compared to the increase seen in the PBDE 47 model. Consequently, the predicted concentrations of PBDE 99 from the age of 9 years onward were higher than those in calves at the end of the nursing period (Supporting Information Fig. S1A–E). This pattern was observed in all compartments with the highest concentrations in blubber, followed by the muscle (rest of the body), liver, kidney and brain (Supporting Information Fig. S1A–E).

3.1.3. PBDE 100

Although the individual data from the Black Sea porpoises were more scattered for PBDE 100 (for example in the liver, Supporting Information Fig. S2B), there was an increase in concentrations of PBDE 100 in all compartments. The final value for the elimination half-life of 4.63 years, fitted by the model, was almost three times higher than the original value of 1.6 years for humans (Geyer et al., 2004). The model predicted a peak concentration at the end of lactation, a decrease in concentrations from the end of lactation until the age of about 3 years and a steep increase after that, resulting in higher concentrations of PBDE 100 in adults than in calves (Supporting Information Fig. S2A–E). Again, higher levels of PBDE 100 were found in blubber, muscle (rest of the body), liver, kidney and brain, respectively (Supporting Information Fig. S2A–E).

3.1.4. PBDE 153

The Black Sea animals lacked a general trend for PBDE 153 in the liver compartment compared to all other tissues (Supporting Information Fig. S3B), which could not be solved by other values for the blood/liver partition coefficient because of the high degree of scattering. In accordance with PBDE 99 and 100, the concentrations in the adult animals after the age of 3 years were higher than the levels in the calves. The final value for the elimination half-life of 9.43 years, fitted by the model, was 50% greater than the literature value of 6.5 years (Geyer et al., 2004). As for the other PBDE congeners, the general profile was conserved also for concentrations of PBDE 153 in all compartments (Supporting Information Fig. S3A–E).

3.1.5. Sensitivity analysis

Sensitivity coefficients are available in Tables S2–S5 in Supporting Information for the model of PBDE 47, 99, 100 and 153, respectively. In all models, the most sensitive parameters are the parameters related to the blood (e.g. density of blood, fat percentage of blood), followed by the parameters that are responsible for the intake of fish (e.g. concentration in the fish and assimilation efficiency for the fish diet), the elimination half-life values and some tissue-related parameters (e.g. blood/blubber partition coefficient, density of the liver and density of the blubber. Parameters that are responsible for the intake of milk have an impact on the bioaccumulation of PBDE 47 (Table S2), but not on PBDE 99, 100 and 153 (Tables S3–S5).

3.2. Goal 2: assessing temporal trends for PBDEs

Using the PBDE results in blubber of North Sea harbour porpoises from 1990 until 2008 ($n = 46$) (Weijs et al., 2009a, 2010c), changes over time were investigated. The models used for the time trends were those described in goal 1, except for the specific input values for PBDE concentrations in the fish and in the milk. Although the input parameters changed with reverse dosimetry modeling (PBDE 99: 0.42 to 0.10 ng/g; PBDE 100: 0.38 to 0.24 ng/g; PBDE 47: 1.08 to 0.54 ng/g; PBDE 153: 0.43 to 0.17 ng/g) meaning that they were

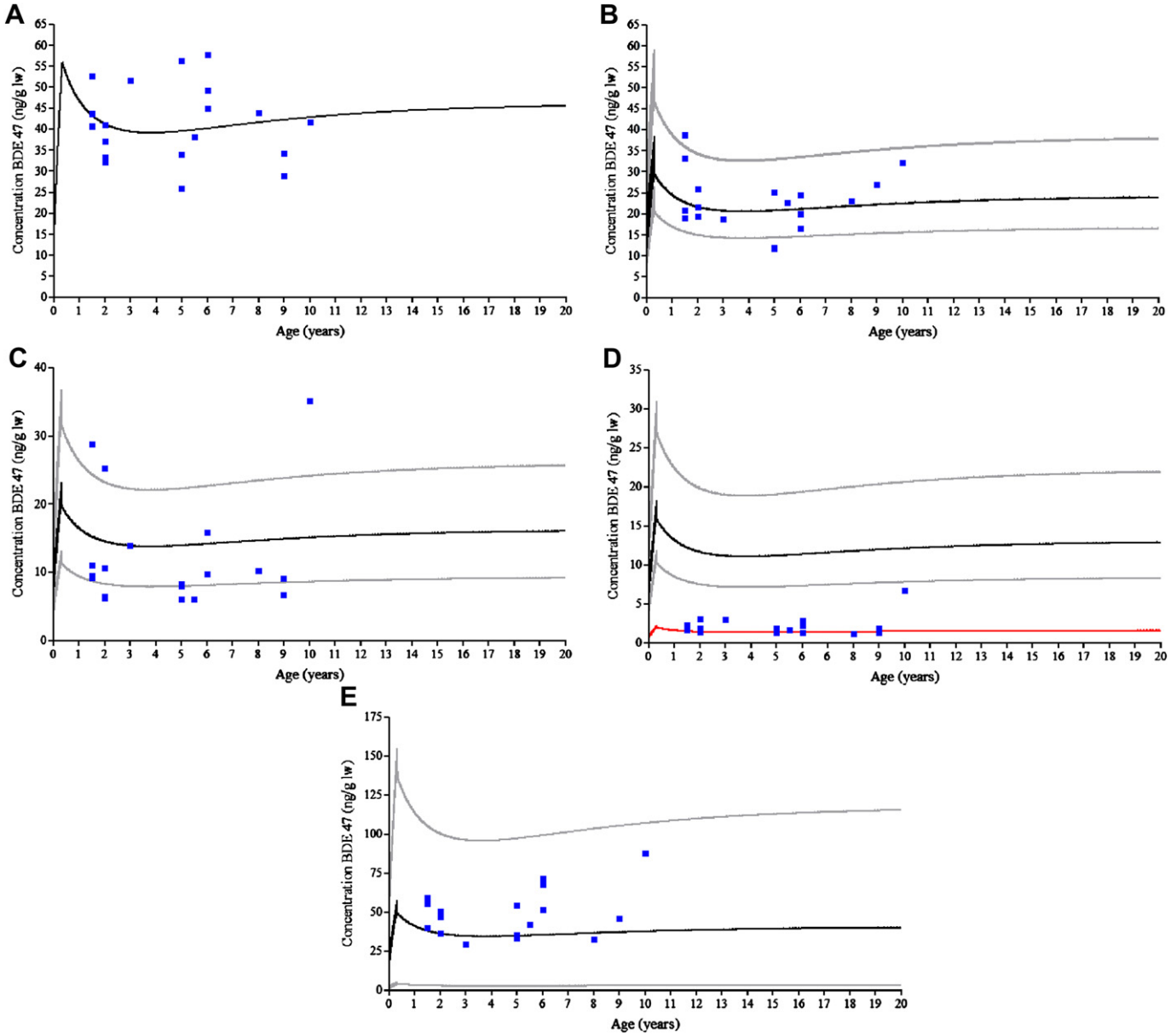

Fig. 1. Age-dependent bioaccumulation of PBDE 47 (expressed in ng/g lw) in (A) blubber, (B) liver, (C) kidney, (D) brain and (E) muscle (rest of the body) of male harbour porpoises from the Black Sea. ■ = Individual data from male harbour porpoises from the Black Sea from 1998 (Weijs et al., 2010b), ▬ = model prediction with tissue/blood partition coefficient calculated with the average lipid content of the respective tissue, ▬ = model predictions with tissue/blood partition coefficients calculated with the minimum and maximum lipid percentage of the respective tissue, ▬ = model prediction with fitted tissue/blood partition coefficient.

adjusted in order to find curves that would fit to the North Sea data, the proportion between the concentrations in milk and fish remained unchanged (116 times difference) throughout this modeling exercise. As in Weijs et al. (2011), the North Sea data were initially added to the models per year, but since the input parameters were very similar for data from 1990–2001 and from 2002–2008, all data were pooled in these two groups (Fig. 2A–D). Between the two groups, there were differences with the highest concentrations of PBDEs in the older data (1990–2001). Results reveal that, in a period of about 18 years, levels of PBDE 99 decreased the fastest with 4.2 times lower levels in 2002–2008 than in 1990–2001 (Fig. 2B). Levels of PBDE 100 decreased the slowest with only a 1.6 times difference between concentrations in 1990–2001 compared to 2002–2008 (Fig. 2C). For PBDE 47 and 153, there was a difference of 2.0 and a 2.5 times, respectively, between the levels in 1990–2001 and 2002–2008 (Fig. 2A, D).

4. Discussion

Although PBDEs have been banned in the EU in 2004 and 2008, their presence and high levels in wildlife can still be toxic. For decades, risk assessment of pollutants in marine mammals has mainly been performed through biomonitoring on blubber from dead animals. Marine mammals are protected species, so these blubber data are generally the only experimental data available. Recently, *in vitro* exposure studies in hepatic or blood cells of marine mammals have gained more attention (McKinney et al., 2006; Das et al., 2008; Frouin et al., 2010). Unfortunately, the connection between reported levels of pollutants in the blubber (from dead or live animals) and the effects found in liver (from dead animals) or blood (from live animals) is often blurred or even non-existent. This can be solved through PBPK modeling as a non-destructive approach in marine mammal toxicology. It combines computational

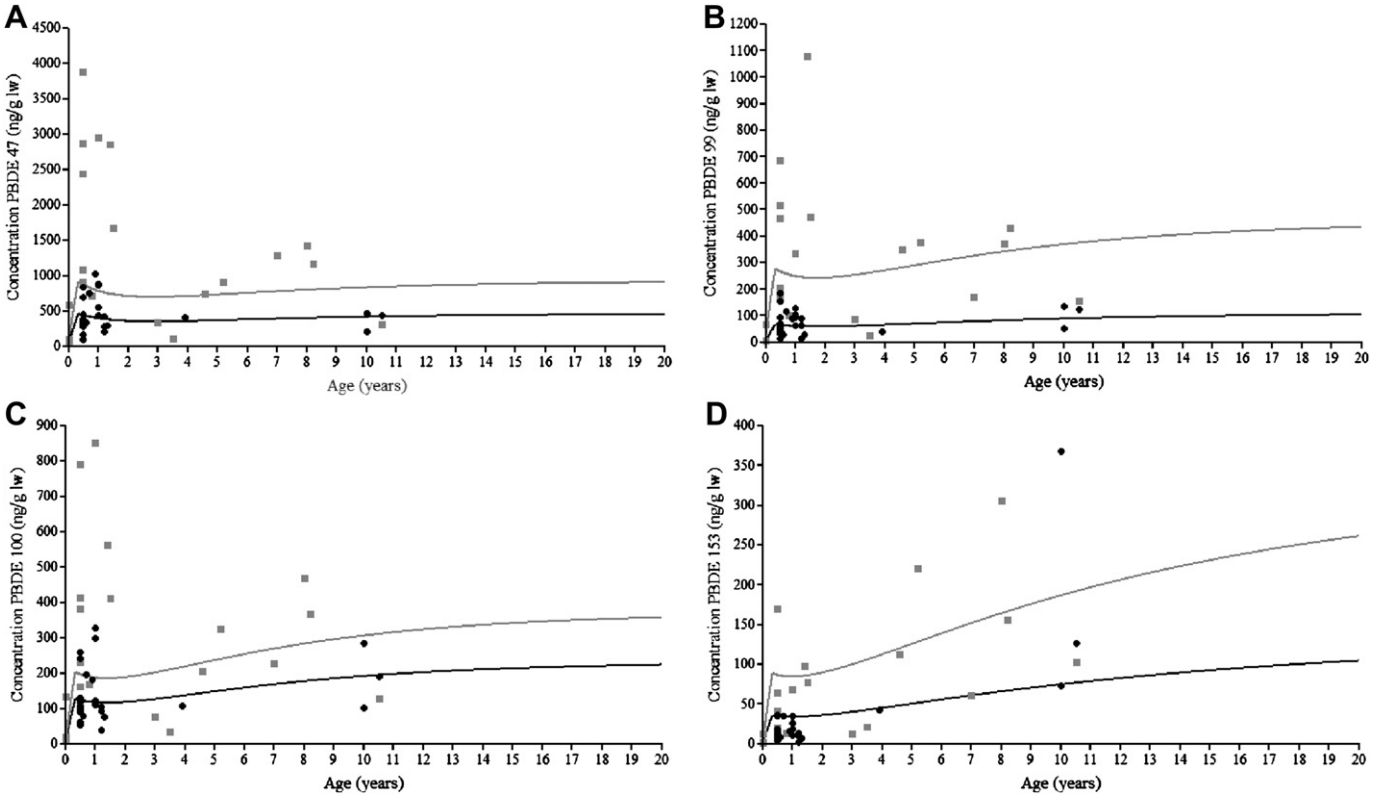

Fig. 2. Time trends in age-dependent bioaccumulation of (A) PBDE 47, (B) PBDE 99, (C) PBDE 100 and (D) PBDE 153 in blubber of male harbour porpoises from the North Sea. All concentrations are expressed in ng/g lw. ■ = individual data for male harbour porpoises from the North Sea from 1990–2001, ● = individual data for male harbour porpoises from the North Sea from 2002–2008, ▬ = model prediction for male harbour porpoises from 1990–2001, ▬ = model prediction for male harbour porpoises from 2002–2008.

technology, physiology of the organism and biochemistry of the compound of interest. As such, PBPK modeling is certainly in line with the spirit of reducing or eliminating animal experimentation by the REACH (Registration, Evaluation, Authorization, and Restriction of Chemical substances) legislation in the EU.

4.1. Goal 1: computational toxicology approach: development of PBPK models for PBDEs

The search for reliable parameters necessary to develop PBPK models for marine mammals is a challenging task. Typical experimental model organisms, such as rodents, often have a different physiology from marine mammals and thus the available parameters from other species are not always suitable for marine mammals.

4.1.1. Elimination half-lives

In PBPK models for PCBs in harbour porpoises, the elimination half-lives were much higher than those reported in the literature, probably due to their larger lipid stores of marine mammals compared to rodents or humans. Because of the size differences between humans and rodents, the longer human elimination half-lives were preferred at the first modeling attempt (Geyer et al., 2004). However, model simulations with these half-lives underestimated the Black Sea dataset. To solve this, the fish intake rate (e.g. PBDE concentration in the prey) or the elimination half-lives had to be increased.

The fish intake rate was calculated based on the PBDE concentrations in milk of Black Sea porpoises. The difference in PCB 153 concentrations in fish and milk (116 times; Weijs et al., 2010a) was used to estimate the fish PBDE concentrations. Although the validity of these input concentrations cannot be checked, the calculated milk:fish factor of 116 is similar to the milk:fish ratios of several PCBs (Weijs et al., 2011) which have comparable tissue/blood partition coefficients as the PBDEs in the present study (Table 1; Weijs et al., 2011). Since there was no further available information to calculate new fish intake concentrations, the only solution was thus to increase the elimination half-lives.

The elimination half-lives were originally lowest, so faster elimination, for PBDE 100 followed by PBDE 99, 47 and 153, respectively. The elimination half-lives found by the software after fitting to the Black Sea data were lowest for PBDE 47, followed by PBDE 100, 99 and 153 (Table 1). These findings are in accordance with the results of Lupton et al. (2009) who reported that PBDE 153 was not metabolized by human liver microsomes, but are in contrast with the same study which found that the relative rate of PBDE 99 metabolism was slightly faster than that of PBDE 47 in humans. Lupton et al. (2009) investigated only the metabolic biotransformation of PBDEs, whereas the elimination half-lives in the current models also include fecal and urinary excretion. According to our results, PBDE 153 is more persistent than PBDE 47 in marine mammals, although the concentrations of PBDE 47 are much higher than of PBDE 153 due to a higher input (higher concentrations in prey fish) caused by a higher solubility and bioavailability of PBDE 47. The differences in behavior of PBDEs among species (e.g. rodents, humans) and the lack of information about elimination of PBDEs in marine mammals complicate the search for appropriate elimination half-lives. Nevertheless, within the limits of the PBPK models and the input concentrations used, the current elimination half-lives are the most viable values.

The elimination half-life in this study includes elimination through metabolic breakdown and fecal/urinary excretion although the urinary excretion will be very low due to the high lipophilic nature of the four PBDEs. As an overall elimination half-life, it is impossible to distinguish between these three processes in the models. Nevertheless, with the currently available information, the

three processes are not equally important and are highly species-specific. Since there were no fecal or urine samples of Black Sea porpoises available for analysis, it is impossible to estimate the levels of PBDEs in their feces or urine. Urinary elimination of the parent PBDE 47, one of the most persistent PBDEs, has been described in mice as an important elimination pathway (Örn and Klasson-Wehler, 1998; Staskal et al., 2005). However, other studies have shown that urinary elimination of PBDEs plays only a minor role in rats (Hakk and Letcher, 2003; Emond et al., 2010). The lack of consistency between elimination pathways in mice and rats makes the extrapolation to marine mammals difficult.

PBDEs can be metabolically biotransformed into HO-PBDEs metabolites, but can also be debrominated into lower brominated PBDEs or form bromophenols through a cleavage of the ether bridge (Qiu et al., 2007). Debromination of PBDEs in common sole was more important than the formation of HO-PBDEs (Munschy et al., 2010). This was also confirmed for PBDE 99 for common carp and Chinook salmon, but the end product of debromination differed among species (PBDE 47 for common carp and PBDE 49 for Chinook salmon) (Browne et al., 2009). Contrastingly, human liver cells metabolized PBDEs *in vitro* primarily to oxidative metabolites rather than through reductive debromination (Lupton et al., 2009; Stapleton et al., 2009). McKinney et al. (2006) reported insignificant metabolic biotransformation of PBDE 47, 99, 100 and 153 in beluga whales, whereas these compounds were significantly depleted by rat microsomes. De Boer et al. (1998) found no indication for biotransformation of PBDE 47 and 99 in a white beaked dolphin, a sperm whale and a harbour seal. In addition, Weijs et al. (2009c) showed that HO-PBDEs were not detected in serum of harbour porpoises, even though blood is the preferred storage medium because of their higher affinity for plasma proteins than for lipids. This suggests that metabolic biotransformation of PBDEs into HO-PBDEs is probably not an important elimination pathway in marine mammals. Model simulations excluding elimination processes predicted curves with much steeper upward slopes which are obviously not consistent with the dataset from the Black Sea (models not shown). Differences between those models without elimination half-lives and the current models indicate the existence of elimination processes in porpoises. However, the models are not capable of pointing at a specific elimination route.

4.1.2. Brain PBDE concentrations in relation to lipid composition and blood/brain barrier

Similar to the PBPK models for PCBs in porpoises (Weijs et al., 2011), the blood/brain partition coefficients (PB) calculated with the method from Parham et al. (1997) and the lipid percentages of the tissues (Weijs et al., 2010a,b) were too high, leading to overestimations of the real data (Fig. 1D and Supporting Information Fig. S1-3D). *In vitro* studies have shown that the affinity of PCBs for triglycerides, the major group of lipids in the blubber, is higher than for phospholipids which are more abundant in the brain (Sandermann, 2003). Therefore, the lower PBs for PBDEs can be due to the different lipid composition of the brain compared to all other tissues. On the other hand, it has also been reported that the blood/brain barrier (BBB) is capable of blocking molecules larger than 180 Da (Doolittle et al., 1998) from entering the brain. Since the investigated PBDEs have molecular weights higher than 180 Da (Burreau et al., 1997), both options can be used to explain the lower PBs. In the PBPK models for PCBs, the PB of PCB 99 was comparable to PCB 180 despite their different molecular sizes indicating a higher influence of the lipid composition of the brain (Weijs et al., 2011). However, the role of the brain lipid composition or the presence of a BBB on the uptake of PBDEs in the brain is less predictable, as the PBs are different for each PBDE congener.

PBDE 47 has a lower PB than PBDE 153, although their partitioning is similar in other tissues (Table 1). The lower PB of PBDE 47 suggests that the brain is much more efficient in blocking PBDE 47 (molecular weight: 485, Effective Cross Section (ECS): 8.1 Å) from entering the brain than PBDE 153 (molecular weight: 646, ECS: 9.6 Å; Burreau et al., 1997). On the other hand, PCB 153 is also a smaller molecule than PBDE 153 (Burreau et al., 1997), but has the same PB as PBDE 153 (Table 1; Weijs et al., 2010a). Therefore, it appears that the brain lipid composition and not the BBB, is important to explain the distribution of PBDEs in the brain. Contrastingly, PBDE 99 has a lower PB than PCB 99 (Weijs et al., 2011), but the same partition coefficients for the other tissues.

The lower concentrations of PBDEs in the brain compared to blubber or other tissues of porpoises are not uncommon and they have been reported as well in polar bears (Letcher et al., 2009) and striped dolphins (Isobe et al., 2009). However, none of these studies included enough information on the lipid composition of the brain to present this as an alternative explanation next to the presence of a BBB.

4.1.3. Assimilation efficiency of milk

Levels of PBDEs in harbour porpoise calves depend on the concentrations of the PBDEs in the milk and on the assimilation efficiencies. The concentrations of the individual PBDEs used in the present study were the average of 7 milk samples of harbour porpoise mothers from the Black Sea in 1997–1998. The assimilation efficiencies for the uptake of PBDEs through milk in harbour porpoise calves are not reported in the literature so these parameters were fitted in order to achieve good model predictions. The resulting assimilation efficiencies for milk were lower than the assimilation efficiencies for fish. This can indicate that calves are somewhat protected against the PBDEs delivered by the milk of their mothers as they seem to absorb on average only a third of the PBDEs present in the ingested milk (Table 1). To investigate this explanation, PBDEs in feces or urine samples of harbour porpoise calves should be analyzed as the concentrations are expected to be proportionally much higher than in feces or urine samples of juvenile or adult harbour porpoises. Unfortunately, samples of feces and urine were not available for this study in any animal. Nevertheless, even though the calves only absorb a third of the PBDEs present in the ingested milk, the concentrations in the milk are still high enough to cause elevated levels in the body of the calves.

4.1.4. Sensitivity analysis

For the sensitivity analysis, the changes in blood curves were considered. These changes are directly influenced by the blood parameters which explain the high sensitivity coefficients found for parameters like the density of blood and the lipid percentage of blood. Obviously, the combination of input and output has a large impact on the bioaccumulation of a chemical in an organism. However, as mentioned earlier, the calculated fish concentrations and fitted elimination half-lives are the best approach available as the information about both parameters in the literature is non-existent. The blubber in marine mammals is the main compartment for storage of lipophilic compounds. Anything that can compromise that, such as the blood/blubber partition coefficient, has logically consequences for the distribution of the chemical in the entire body. The concentrations of PBDE 47 in tissues of the fetus are much higher than the concentrations of the other three PBDEs in the fetus which might explain why the PBDE 47 model is more sensitive to small changes in dietary milk input than PBDE 99, 100 and 153.

4.2. Goal 2: assessing temporal trends for PBDEs

Concentrations of PBDEs investigated for North Sea porpoises decreased from 1990 until 2008, but not at the same rate. There was a 4.2 times difference between levels of PBDE 99 in porpoises in

1990-2001 and levels of PBDE 99 in animals from 2002–2008 (Fig. 2B). For PBDE 153, there is a 2.5 times difference between the two time periods (Fig. 2D) followed by a 2.0 and 1.6 times difference for PBDE 47 and 100, respectively (Fig. 2A, C). The two time periods were not the same as for PCBs where the groups were divided from 1990–2000 and from 2001–2008 (Weijs et al., 2011). Since the production of the Penta-BDE mixture containing the investigated congeners has been banned since 2004, a difference between PBDE levels in animals from 1990–2004 and from 2005–2008 would seem plausible. However, harbour porpoises are top predators in the aquatic food chains and it takes some time to see the result of changes in the production or release of chemicals throughout the food chain. The results show that PBDE concentrations were already decreasing before PBDEs were banned. However, this conclusion should be taken with caution because it might be that the two groups are divided differently with larger sample sizes per year. Although there are reports of increasing PBDE trends in fish and marine mammals (She et al., 2002; Lebeuf et al., 2004), there are also studies reporting the absence of temporal trends (Stapleton et al., 2006) or even decreasing trends in guillemot eggs (Sellström et al., 2003) and pike (Kierkegaard et al., 2004).

Harbour porpoises live for about 20 years and are capable of producing a calf each year. Because of the high lipid percentage in the milk, the calves receive high loads of pollutants. In addition, the low ability for eliminating these compounds in calves, juveniles and adults ensure that these high loads are retained in the body of the individual animals and in the population in general. Over 18 years, PBDE levels have decreased only slowly in harbour porpoises, which has obviously health implications for the viability of the population.

5. Conclusions

This is the first study to assess the kinetics of PBDEs in a marine mammal species through PBPK modeling. PBPK models combine physiological information of the species and biochemical information of the pollutant and can connect the typical biomonitoring data with the results of *in vitro* studies in a non-invasive manner. The parameterization and validation of the PBPK models were executed using PBDE data found in the literature from harbour porpoises from the Black Sea and North Sea. Although some parameters from other species were proven to be inadequate for these models, the final parameterization was carried out by performing computer optimization using data from Black Sea porpoises. Since the models with the optimized parameters were capable of visualizing the slow decrease in PBDE levels in North Sea porpoises from 1990 until 2008, it proves that the use of these parameters is justified for all harbour porpoises. From this perspective, the PBPK models for PBDEs can be used as a framework to improve our knowledge about the kinetics of PBDEs in harbour porpoises and to test future exposure scenarios.

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Appendix. Supplementary data

A description of the sensitivity analyses, 1 table with PBDE results in milk samples, 3 figures, 4 tables with the results of the sensitivity analyses and a reference list is available in Supporting information, at doi:10.1016/j.envpol.2011.12.037.

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