Toxicology and Applied Pharmacology 256 (2011) 136–145

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

A non-invasive approach to study lifetime exposure and bioaccumulation of PCBs in protected marine mammals: PBPK modeling in harbor porpoises

Liesbeth Weijs [a,b,*], Adrian Covaci [a,b], Raymond S.H. Yang [c], Krishna Das [d], Ronny Blust [a]

[a] Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[b] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[c] Quantitative and Computational Toxicology Group, Department of Environmental and Radiological Health Sciences, Colorado State University, 1680 Campus Delivery, Fort Collins, CO 80523, USA
[d] Laboratory for Oceanology-MARE Center, University of Liège, 4000 Liège, Belgium

ARTICLE INFO

Article history:
Received 12 April 2011
Revised 21 July 2011
Accepted 25 July 2011
Available online 6 August 2011

Keywords:
Harbor porpoises
Metabolism
PBPK models
PCBs
Time trends

ABSTRACT

In the last decade, physiologically based pharmacokinetic (PBPK) models have increasingly been developed to explain the kinetics of environmental pollutants in wildlife. For marine mammals specifically, these models provide a new, non-destructive tool that enables the integration of biomonitoring activities and *in vitro* studies. The goals of the present study were firstly to develop PBPK models for several environmental relevant PCB congeners in harbor porpoises (*Phocoena phocoena*), a species that is sensitive to pollution because of its limited metabolic capacity for pollutant transformation. These models were tested using tissue data of porpoises from the Black Sea. Secondly, the predictive power of the models was investigated for time trends in the PCB concentrations in North Sea harbor porpoises between 1990 and 2008. Thirdly, attempts were made to assess metabolic capacities of harbor porpoises for the investigated PCBs. In general, results show that parameter values from other species (rodents, humans) are not always suitable in marine mammal models, most probably due to differences in physiology and exposure. The PCB 149 levels decrease the fastest in male harbor porpoises from the North Sea in a time period of 18 years, whereas the PCB 101 levels decrease the slowest. According to the models, metabolic breakdown of PCB 118 is probably of lesser importance compared to other elimination pathways. For PCB 101 and 149 however, the presence of their metabolites can be attributed to bioaccumulation of metabolites from the prey and to metabolic breakdown of the parent compounds in the harbor porpoises.

Introduction

The global awareness of the major impact of anthropogenically produced pollutants on the environment makes risk assessment in wildlife a topic of great interest. Assessing toxicity in organisms requires correlations between the concentration of a chemical in an organism and the response induced by that specific pollutant (Walker et al., 2006). Typically, this approach leads to some practical problems for marine mammals. Marine mammals are long-lived mammals that occupy the top positions in aquatic food webs around the world (Ross, 2000) and that can transfer considerable amounts of pollutants to their offspring through lactation because of their lipid rich milk (Debier et al., 2003). They have limited capacities for eliminating pollutants and have experienced effects on several health endpoints (e.g. Mos et al., 2007; Reijnders, 1986; Ross et al., 1996). Because of this, marine mammals are sensitive to pollution and therefore relevant study organisms (Ross, 2000). However, due to their protected status, *in vivo* toxicological research or exposure experiments in marine mammals are undesirable and prohibited. As a consequence, due to the more advanced techniques and procedures, risk assessment in marine mammals occurs more and more through *in vitro* studies (e.g. Dufresne et al., 2010; Li et al., 2003; McKinney et al., 2006), probably the only ethical possibility to investigate the effects of pollution in these animals. However, such work focuses mainly on one target tissue or type of cells. It thus fails to provide an integrative picture and to understand the interactions between several tissues.

Computer models or *in silico* studies may be able to provide a solution. The type of models used depends on the questions that need to be addressed or on the availability of data required to develop the models. Physiologically based pharmacokinetic (PBPK) models give information about the absorption, elimination and distribution of a pollutant in an organism by integrating physiology of the organism and biochemistry of the specific pollutant (Clewell and Clewell, 2008; Reddy et al., 2005). Traditionally, these models were used to describe the kinetics of chemicals in rodents (Corley et al., 1990; Reitz et al., 1988). Recently, the bioaccumulation of environmental pollutants, such as polychlorinated biphenyls (PCBs) in wildlife and humans has

* Corresponding author at: Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, Building U, 5th Floor, 2020 Antwerp, Belgium. Fax: +32 3 265 34 97.
E-mail address: liesbeth.weijs@ua.ac.be (L. Weijs).

0041-008X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2011.07.020

received increasing attention (Hickie et al., 1999; Maruyama and Aoki, 2006; Redding et al., 2008; Sonne et al., 2009; Verner et al., 2008; Weijs et al., 2010b).

Harbour porpoises (*Phocoena phocoena*) are small cetaceans living in the Northern Hemisphere. They are apex predators, have long life spans and are assumed to have limited metabolic capacities for the breakdown of pollutants compared to other marine mammals, such as harbor seals (Weijs et al., 2009a, 2009b). During the last decade, populations of harbor porpoises in northern Europe were moving more south, probably following the fish migrations, towards the relatively smaller and more land locked North Sea (SCANS II, 2006), thereby exposing themselves to higher levels of pollutants that are present in that area due to run-off of the highly industrialized surrounding countries. Together with their potential limited metabolic capacities, there is a clear need for information regarding the kinetics and effects of pollutants in their bodies to ensure proper protection and viable populations worldwide.

Recently, models were developed for the lifetime distribution and kinetics of PCB 153 in harbor porpoises which is the most persistent PCB in marine mammals (Weijs et al., 2010b). However, PCB 153 is not the only threat for these animals as it is only one congener in the PCB mixtures (Aroclor) which were commercially available and widely used before their ban in the 1970s. The goals of the present study were therefore, 1) to develop PBPK models for PCBs other than PCB 153 in harbor porpoises, 2) to investigate the temporal trends of PCBs using these models, 3) to gather more information about the metabolic breakdown of some PCBs.

The PCBs other than PCB 153 were selected according to the chlorine substitution pattern on the *ortho*, *meta* and *para* positions (Wolkers et al., 1998) implying that they are all metabolized by different subsystems of the cytochrome P450 enzyme complex. Congeners PCB 180 (group I), PCB 101 (group II), PCB 118 (group IIIa) and PCB 170 and PCB 99 (group IIIb) were chosen because of their persistence in marine mammals. A model for PCB 149 (group II) was developed as well because of its typically higher concentrations in cetaceans compared to pinnipeds (Boon et al., 1997).

Materials and methods

PBPK models were constructed for six PCB congeners and were based on our earlier published model for PCB 153 in male harbor porpoises (Weijs et al., 2010b). Accordingly, all models consist of 5 compartments: liver, blubber, kidneys, brain and rest of the body (Fig. 1), all connected through blood. For the 'rest of the body'-compartment, parameters and data of muscle tissue of harbor porpoises were used. All tissues were considered to be flow-limited similar as in Weijs et al. (2010b) and for humans in Redding et al. (2008). Exposure was assumed to be through fish and milk consumption only. Dermal uptake was neglected because lipophilic compounds do not dissolve readily in sea water. Moreover, Hickie et al. (1999) found that dermal exposure only played a negligible role for the bioaccumulation of PCBs in beluga whales. The uptake of PCBs through the fish or milk diet was set to the liver as this compartment was the only tissue of the gastrointestinal tract represented in the models. All models were coded using Berkeley Madonna (version 8.3.14) and are available on request to the corresponding author.

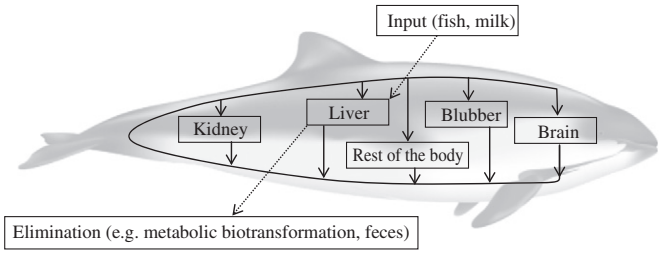

Fig. 1. Conceptual representation of the PBPK models for selected PCBs in male harbor porpoises.

Parameters. All models were developed using parameters from the literature or obtained through fitting to the data if clearly indicated. The same physiological parameters and equations of the male harbor porpoises as in Weijs et al. (2010b) were used. Biochemical parameters were adjusted according to the specific PCB (Table 1). Blood/tissue partition coefficients were calculated using the approach from Parham et al. (1997) and the average lipid percentage of the tissues which can be found in Weijs et al. (2010a). However, blood/brain partition coefficients (PB) calculated with this approach overestimated the levels of the PCBs in the brain of the Black Sea harbor porpoises and were therefore fitted to the data of the brain. Both calculated and predicted PBs can be found in Table 1. Other parameters that were fitted (elimination half-lives and assimilation efficiency for the milk or AE2; see Table 1) 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. In general, the following order in the fitting process was followed: 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 and PBs only had an impact on the curve of the brain compartment and were therefore fitted last. Overall, fitting or estimating several parameter values through modeling generally reduces the reliability

Table 1
Compound specific parameters for several PCBs. PCB congeners were selected based on the groups from Wolkers et al. (1998). The original values of the parameters are given between brackets for parameters that were fitted to the data.

	PCB 180	PCB 101	PCB 149	PCB 118	PCB 99	PCB 170
Group[a]	I	II	II	IIIa	IIIb	IIIb
log (K_{fp})[b]	2.41682	1.91643	1.85712	2.23748	2.41682	2.41682
PF[c]	380.2	101.4	88.5	251.6	380.2	380.2
PL[c]	9.0	2.4	2.1	6.0	9.0	9.0
PK[c]	5.3	1.4	1.2	3.5	5.3	5.3
PB[c]	6.3 (15.2)	1.3 (4.0)	1.4 (3.5)	3.5 (10.0)	6.3 (15.2)	6.1 (15.2)
PR[c]	9.2	2.5	2.2	6.1	9.2	9.2
AE 1 (%)[d]	91	98	90	99	90	90
AE 2 (%)[e]	54	66	54	70	55	46
CFetusF[f]	53.6	87.1	99.3	113.1	61.0	18.0
CFetusL[f]	33.0	37.4	49.4	43.8	26.8	10.4
CFetusK[f]	32.9	48.6	58.2	52.1	36.0	ND
CFetusB[f]	8.5	11.7	13.6	13.7	8.2	ND
Half life[g]	521 (9.9)	6.1 (5.7)	80.00 (5.7)	9.6	334 (5.7)	21.0 (3.9)

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] Groups based on the chlorine substitution pattern on the *ortho*, *meta* and *para* positions according to Wolkers et al. (1998).

[b] Adipose tissue to plasma partition coefficients from Parham et al. (1997).

[c] 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., 2010a). For the 'rest of the body'-compartment, the average lipid content of muscle was used (Weijs et al., 2010a).

[d] Assimilation efficiency for the fish diet or the percentage of PCB absorbed by the juveniles and adults after ingestion of the fish prey. Values taken from Thomas et al. (2005), average net absorption for all congeners measured was >89%, so for PCB 99, PCB 149 and PCB 170, an assimilation efficiency of 90% was assumed.

[e] Assimilation efficiency for the milk diet or the percentage of PCB absorbed by the calves after milk ingestion. Values were fitted to the Black Sea dataset.

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

[g] Values between brackets are the original elimination half-lives used in the first modeling attempt. Other values are fitted to the dataset for validation and are used in all other models (models for goals 2 and 3) of this study.

of the model outcome as these parameters are more mathematically justified than biologically inspired. Therefore, this procedure was kept to a minimum and only employed in cases where there were no parameters available in the literature or where the parameters (the models) did not match the real-life data from the harbor porpoises.

Datasets. Physiological parameters were kept rather general so that the models would be species-specific instead of population-specific. In that way, it is assumed that the models can be used for all male harbor porpoises. The models include also five compartments which allows to use other data than only PCB levels in blubber. All this is reflected here as there are several datasets used for the different applications:

Black Sea. This dataset was used to parameterize the PCB models (goal 1), similar to the PCB 153 model in male harbor porpoises (Weijs et al., 2010b). All animals (9 juveniles, 11 adults) were by-caught (n = 17) or found stranded (n = 3) in 1998 in the Black Sea. Carcasses were in good condition or only moderately composed and none of the animals was severely emaciated. Levels in blubber, liver, kidney, brain and muscle are discussed in Weijs et al. (2010a). Levels of one neonate/fetus (Table 1) were used as well together with the levels of PCBs in milk of Black Sea harbor porpoises (Table S1).

North Sea. This dataset consists of blubber and liver PCB concentrations. The blubber data were used to investigate the predictability of the PBPK model for the PCBs in time (goal 2), the liver data were used to better understand the importance of metabolic breakdown of some PCB parent compounds (goal 3). One part of the animals included in this dataset were found stranded or were by-caught on the Belgian coast of the North Sea in 1999–2004. The PCB results in blubber (n = 20) were used for goal 2 and can be found in Weijs et al. (2009a and b). The PCB, methylsulfone-PCB ($MeSO_2$-PCB) and hydroxylated PCB metabolites (HO-PCB) levels in liver (n = 10) were used for goal 3 and were discussed in Covaci et al. (2002) (PCBs), in Chu et al. (2003) ($MeSO_2$-PCBs) and were from Weijs (unpublished data) (HO-PCBs) (Table 2). The other part of the animals included in this dataset (n = 26) were found alive on the coasts of Belgium and The Netherlands, but died during rehabilitation in SOS Dolfijn, Harderwijk, The Netherlands in 1990–2006. Levels (sum of PCBs) can be found in Weijs et al. (2010c). Data of PCBs in blubber were used for goal 2 whereas data of PCBs in liver were not used for goal 3 since PCB metabolites were not targeted.

Table 2
Hydroxylated (HO) and methylsulfone ($MeSO_2$)-metabolites of the parent PCB compounds in liver of harbor porpoises from the North Sea (n = 10; expressed in ng/g lw). Parent PCB compounds are discussed in Covaci et al. (2002), $MeSO_2$-PCBs in Chu et al. (2003), HO-PCBs from Weijs, unpublished data.

	PCB 180	PCB 101	PCB 149	PCB 118	PCB 99	PCB 170
Harbor porpoises						
HO-PCB	ND-3.8[a]	NT	NT	ND-5.9[b]	NT	ND-0.3[a]
$MeSO_2$-PCB	NT	21.7–1176.1	7.6–618.9	NT	NT	NT
Fish						
HO-PCB[c]	ND	NT	NT	ND	NT	ND
$MeSO_2$-PCB[d]	NT	0.9–1.4	1.0–2.7	NT	NT	NT

ND — Not Detected; NT — Not Targeted.
HO-PCBs for PCB 180 is the sum of 3-HO-PCB 180 and 4-HO-PCB 172; HO-PCBs for PCB 118 is the sum of 4-HO-PCB 120 and 3-HO-PCB 118; HO-PCBs for PCB 170 is only 4-HO-PCB 172.
$MeSO_2$-PCBs for PCB 101 is the sum of 3-$MeSO_2$-PCB 101 and 4-$MeSO_2$-PCB 101; $MeSO_2$-PCBs for PCB 149 is the sum of 3-$MeSO_2$-PCB 149 and 4-$MeSO_2$-PCB 149.
[a] ND in 9 out of 10 samples.
[b] ND in 5 out of 10 samples.
[c] Investigated in flounder (*Platichthys flesus*), cod (*Gadus morhua*), dab (*Limanda limanda*), and whiting (*Merlangius merlangus*) caught in 2008 in the North Sea (Weijs, unpublished data).
[d] Investigated in plaice (*Pleuronectes platessa*), sole (*Solea solea*), pout (*Trisopterus luscus*) and whiting (*Merlangius merlangus*) caught in 2001 in the North Sea Covaci (unpublished data).

Results

Of the 6 PCBs selected, only PCBs 101, 118 and 149 were involved in all goals. Of these three PCBs, PCB 101 was randomly selected to be shown in the manuscript (Figs. 2, 4) whereas figures of all other PCBs can be found in Supporting Information (Fig S1–S7). PCBs 180, 99 and 170 could not be used for goal 3 because metabolites of those congeners were not targeted (for PCB 99) or were only detected in low concentrations in 1 out of 10 samples (for PCBs 180 and 170) (Table 2).

Goal 1: Models for PCBs other than PCB 153

Elimination half-lives, in the present study defined as the time at which 50% of the chemical is eliminated (e.g. by metabolic transformation, fecal excretion) from the body, are important parameters. For most PCBs, the literature provides several half-lives dependent on the investigated species (e.g. humans, rodents) and circumstances (e.g. occupational exposure, long term exposure). Initially, all models were run with the longest elimination half-lives available in the literature (see elimination half-lives between brackets in Table 1). However, because the curves did not reflect the Black Sea dataset for 5 out of 6 PCBs, new elimination half-lives (Table 1) were estimated by fitting the models to the Black Sea data (*i.e.* model parameterization). Models for all 6 PCB congeners had two things in common: 1) the blood/brain partition coefficients (PB), originally calculated according to Parham et al. (1997), were not consistent with the results of the Black Sea dataset and were therefore fitted to the brain data, 2) an additional assimilation coefficient for the milk diet (AE2) was added to the models of all PCB congeners because initial modeling attempts suggested that the models overestimated the validation dataset from the Black Sea for the youngest animals when using the same assimilation efficiency as the fish diet (Table 1).

Tanabe et al. (1997) reported concentrations of PCB 118, 170 and 180, but not of PCB 101, 99 and 149, in fish prey of harbor porpoises from the Black Sea from 1993. Therefore, levels of PCB 101, 99 and 149 in the fish diet were estimated using the average concentration of the respective congener in the milk diet (Table S1). In the PBPK model for PCB 153 bioaccumulation in harbor porpoises, there was a 116 times difference between the fish diet (1.1 ng/g ww; Tanabe et al., 1997) and milk diet (127.6 ng/g ww; Weijs et al., 2010b). The factor 116 has been used here as an estimate leading to a fish diet of 0.3 ng/g ww for PCB 101, of 0.5 ng/g ww for PCB 99 and of 0.7 ng/g ww for PCB 149 .

PCB 101

Levels of PCB 101 in all compartments increase little with age. In each tissue, the highest concentrations can be found for animals younger than 1 year, especially the animals that are drinking milk or animals that have just switched from a milk diet to a fish diet. The elimination half-life, PB and AE2 that fitted best to the dataset of the harbor porpoises from the Black Sea are given in Table 1 and model results can be found in Fig. 2 (A–E).

PCB 149

Because metabolic half-lives for PCB 149 are scarce in the literature, even for typical model species such as rodents, the first modeling exercise used a metabolic half life of 5.7 years, similar as the metabolic half life of PCB 101 (also group II; Wolkers et al., 1998) which resulted in model predictions that underestimated the Black Sea dataset by far. Therefore, the Black Sea data were used to find a better fit of the curves,

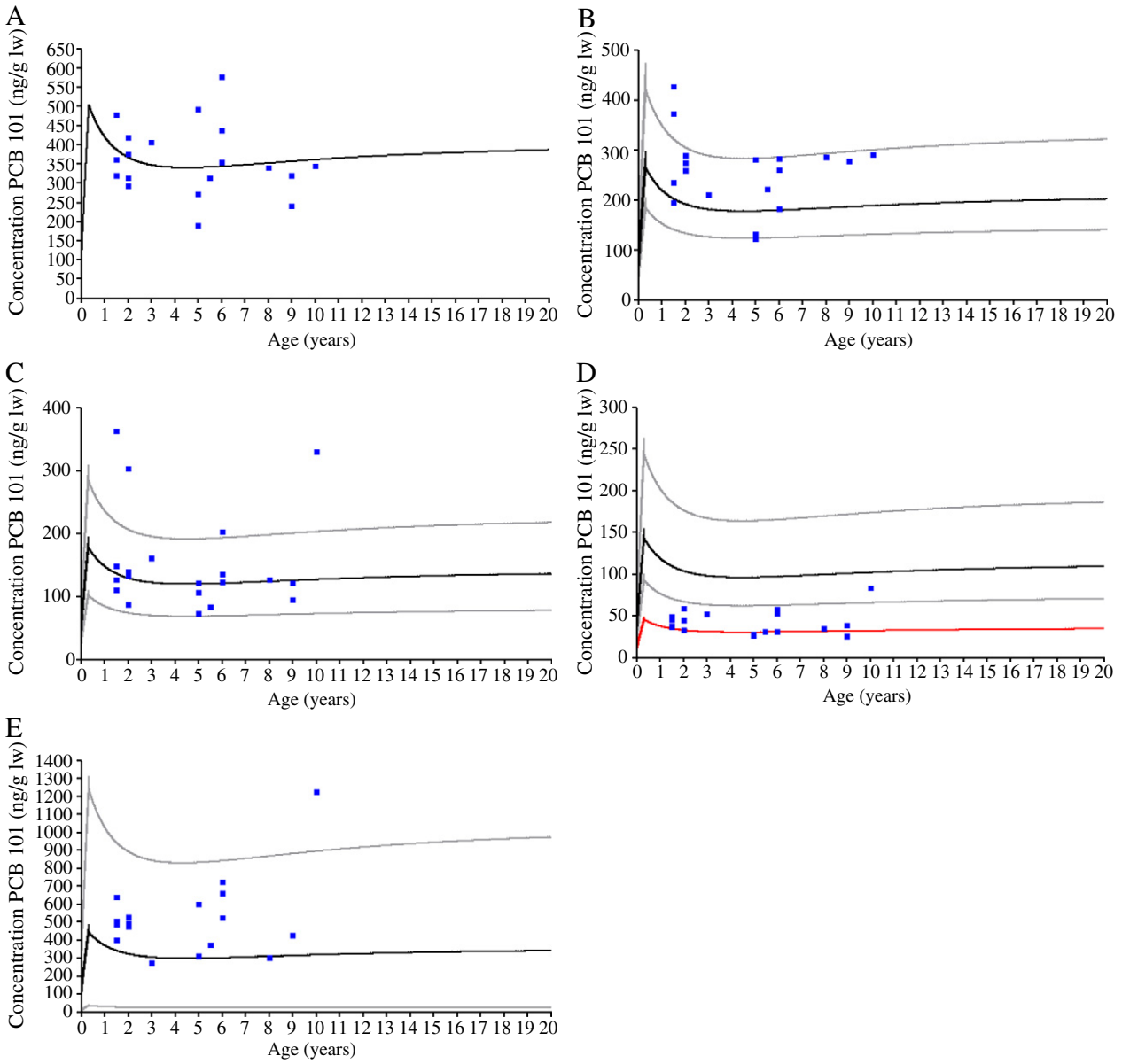

Fig. 2. Age-dependent bioaccumulation of PCB 101 (expressed in ng/g lw) in (A) blubber, (B) liver, (C) kidney, (D) brain and (E) muscle (rest of the body) of male harbor porpoises from the Black Sea. ■ = Individual data from male harbor porpoises from the Black Sea from 1998 (Weijs et al., 2010a), — = 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.

resulting in an estimated elimination half-life value of about 80 years (Table 1; Fig S1 A–E).

PCB 99

The longest elimination half-lives for PCB 99 in the literature are not compatible with the Black Sea dataset for validation, so the software was asked again to find an elimination half-life so that the curves would fit to the Black Sea dataset, resulting in an estimated elimination half-life of 334 years ((Table 1; Fig S2 A–E).

PCB 118

In contrast with all other PCBs in the present study, the Black Sea data are more scattered for PCB 118 than for all other PCBs considered so far. Nevertheless, the model, developed with the parameters from Table 1 and a concentration of PCB 118 in the fish diet as measured by Tanabe et al. (1997), is a fairly good reflection of the real life data from the Black Sea (Fig S3 A–E).

PCB 170

The curves were again underestimating the Black Sea data by far using the elimination half-life from the literature. In contrast to PCB 99, levels of PCB 170 were measured in fish prey of harbor porpoises from the Black Sea at a concentration of 0.18 ng/g ww (Tanabe et al., 1997). The only parameter that can be adjusted was thus the elimination half-life. This was again fitted to the Black Sea data by the model giving a value of 21 years (Table 1; Fig S4 A–E).

PCB 180

The concentration of PCB 180 in the fish diet from Tanabe et al. (1997) and an elimination half-life of 9.9 years, gives slightly increasing curves with age for all compartments, whereas they should be increasing much more according to the Black Sea data. As for PCB 99, the model was used to find a value for the elimination half-life so that the curves would fit better to the Black Sea dataset. Similar to PCB 99, the resulting elimination half-life was very high, namely 521 years (Fig S5 A–E).

Goal 2: Assessing temporal trends for PCBs

Using the blubber data from the North Sea dataset, temporal trends were investigated. The entire dataset (n = 46) covers data from 1990 until 2008. The models used are exactly the same as developed and parameterized in goal 1, except for the input parameters. These input parameters, namely the concentration of the specific PCB in the fish and milk diet, were found by Reverse Dosimetry Modeling meaning that they were adjusted in order to find curves that would fit to the North Sea data (Redding et al., 2008). The ratio of the concentration in milk to the concentration in fish was kept the same as in the models that were parameterized using Black Sea data (so 116 times difference for PCB 101, 99 and 149; 84 times difference for PCB 118, 107 times difference for PCB 170 and 203 times difference for PCB 180; see Goal 1). Overall, although the data of each year were added to the models separately, results revealed that they could easily be divided into 2 groups, from 1990 until 2000 and from 2001 until 2008. Within each group, the levels of the PCBs increased insignificantly in time. Between the two groups, there was a difference between the groups with lower concentrations in the second group (2001–2008) compared to the first group (1990–2001). The difference in concentrations in the diet ranged from a factor 1.9 to 3.5 with 1.9, 2.5, 2.8, 2.8, 3.3 and 3.5 for PCB 101, PCB 99, PCB 118, PCB 180, PCB 170 and PCB 149, respectively (Fig. 3 A–F).

Goal 3: Metabolism and elimination of PCBs

Attempts were made to link the concentrations of PCB metabolites to the models of the parent PCB congeners. This was done using data of parent PCBs (Covaci et al., 2002), $MeSO_2$-PCBs (Chu et al., 2003) and HO-PCBs (Weijs, unpublished data) in liver of harbor porpoises from the North Sea (n = 10). This method was applied for PCB 101, 118 and 149, but not for PCB 99, 170 and 180 because the metabolites were not targeted or were present in low concentrations in only 1 out of 10 samples (Table 2). Theoretically, under the assumption that the diet or prey (and not seawater or air) is the only source of PCBs and/or metabolites for marine mammals, there are three different possible situations (Letcher et

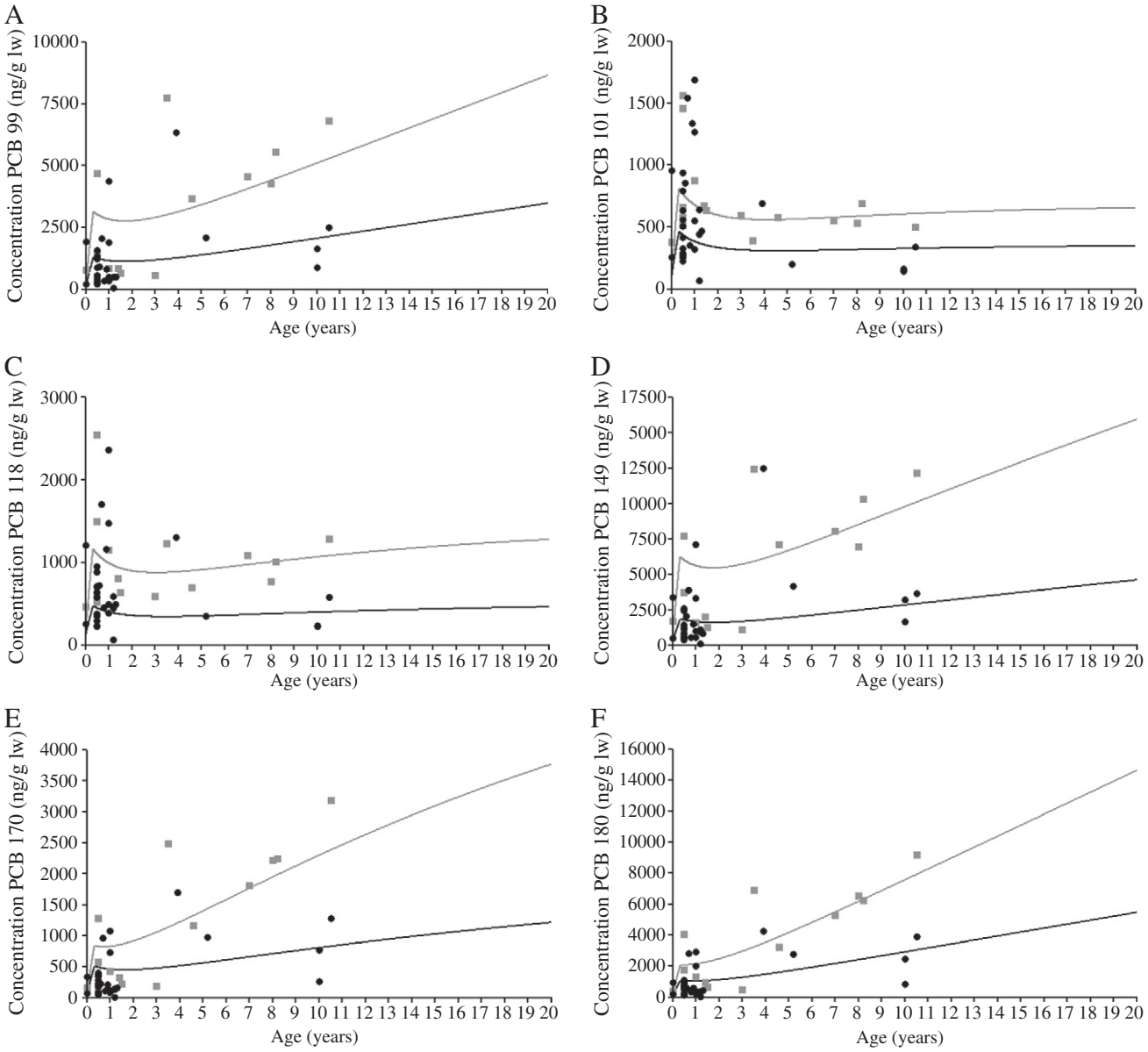

Fig. 3. Time trends in age-dependent bioaccumulation of (A) PCB 99, (B) PCB 101, (C) PCB 118, (D) PCB 149, (E) PCB 170 and (F) PCB 180 in blubber of male harbor porpoises from the North Sea. All concentrations are expressed in ng/g lw. ■ = individual data for male harbor porpoises from the North Sea from 1990 to 2000, ● = individual data for male harbor porpoises from the North Sea from 2001 to 2008, —— = model prediction for male harbor porpoises from 1990 to 2000, —— = model prediction for male harbor porpoises from 2001 to 2008.

al., 1998): 1) the precursor PCB is present in the prey, the metabolites are not, 2) the metabolites are present in the prey, the precursor PCB is not, and 3) the precursor PCB and its metabolites are present in the prey.

PCB 118, 101 and 149 were all present in the prey (Table 2), making the second situation impossible. PCB 118 belongs to the first situation as potential HO-metabolites of PCB 118 were targeted, but not detected in the prey (Table 2; Weijs, unpublished data). The presence of HO-metabolites of PCB 118 is thus solely due to PCB metabolism in the harbor porpoises. PCB 101 and 149 as well as their $MeSO_2$-metabolites were found in the prey, so both PCB 101 and 149 can be assigned to the third situation.

PCB 118

The difference between the model without elimination (green curve; Fig S6) and the model with elimination (characterized by an elimination half-life of 9.6 years) (Table 1; black curve; Fig S6) gives the concentration of PCB 118 eliminated from the body by metabolic breakdown or fecal excretion (gray curve; Fig S6). The levels of potential HO-metabolites of PCB 118 are only a minor fraction of this, indicating that metabolic breakdown is of lesser importance compared to fecal excretion of PCB 118.

PCB 149

There is an increase in concentrations of metabolites of PCB 149, similar as predicted by the model (gray curve; Fig S7). However, the origin of the metabolites in this study, either from the prey through bioaccumulation or from metabolic breakdown in harbor porpoises, remains unknown.

PCB 101

Similar as PCB 149 and as predicted by the model (gray curve; Fig. 4), there is also an increase in concentrations of metabolites of PCB 101. For the animals younger than 3 years, the concentrations of metabolites are situated under the gray curve, so for these animals, the origin of the metabolites remains unknown. For the animals older than 3 years, there is a sudden increase in concentrations of the metabolites as well as in the metabolites/parent compound percentages. At higher age, these animals clearly accumulate more metabolites as can be produced through metabolic breakdown. The difference in metabolites/parent compound percentages between the younger animals (<3 years) and the older animals (>3 years) suggests that the capacity for metabolic breakdown of PCB 101 is greatly enhanced or induced at higher ages as it would be difficult to explain this steep increase simply by bioaccumulation.

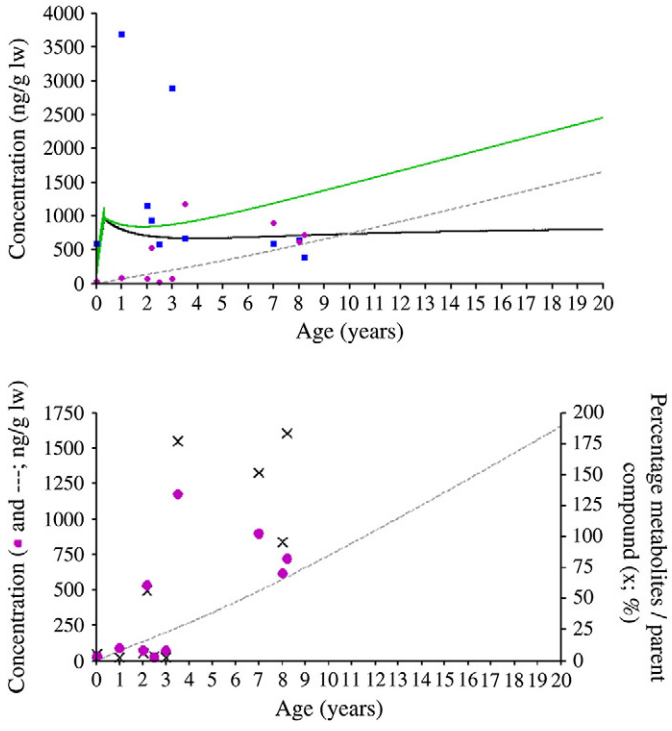

Fig. 4. Age-dependent elimination of PCB 101 in liver of male harbor porpoises from the North Sea. All concentrations are expressed in ng/g lw. ■ = individual data of PCB 101 in livers of male harbor porpoises from the North Sea, ● = individual data of $MeSO_2$-PCB metabolites of PCB 101 (Table 2), —— = model predictions with elimination half-life of 6.1 years (Table 1), —— = model predictions without elimination, --- = difference between —— and —— (thus the concentration that is eliminated), × = percentage of concentration of PCB 101-metabolites/concentration of PCB 101.

Discussion

The significance of this work is the development and demonstration of a workable, non-invasive, computational approach to study lifetime exposure and bioaccumulation of PCBs in protected marine mammals, such as the harbor porpoises. For protected marine mammals, biomonitoring data on stranded or dead animals are generally the only experimental information available. In the past, such data were reported as survey results in the literature and that was the end of the study. With the application of PBPK modeling, however, the utility of such biomonitoring results is greatly expanded to provide further insights into the pharmacokinetics of the interested chemicals in these marine mammals and to help with the interpretation of their toxic effects as revealed by results of *in vitro* studies. This approach is certainly in line with the spirit of reducing or eliminating animal experimentation advanced by REACH (Registration, Evaluation, Authorization, and restriction of Chemical substances) in the European Union (EU).

PBPK modeling integrates computational technology and all the physiological and biochemical information on the chemical(s) of interest in the species of interest. Thus, it can be used as well in other species in which *in vivo* experiments are often unethical, such as humans. This report, as well as other similar work (Weijs et al., 2010a; Weijs et al., submitted for publication), demonstrates that lifetime modeling including special physiological states can be effectively implemented for an entire class of chemicals. Although PBPK models for some selected chemicals in humans already exist, only few studies compare models for several PCBs. It is through comparisons between highly comparable and similar compounds, such as PCBs, that the knowledge about the kinetics of these compounds and the influence of their biochemical properties on the kinetics can progress. This is the first study that compares the kinetics of several PCBs, assesses their temporal trends and attempts to unravel metabolic pathways in a marine mammal species through PBPK modeling. Although the use of more datasets in the future will make the models stronger and more robust, the datasets used now take nothing away from the quality of the models, nor from their applications (goals 2 and 3) or conclusions.

Goal 1: Usefulness of models for other PCBs

PBPK models in the present study rely partly on biochemical parameters found in the literature from *in vitro* or *in vivo* experiments with other species and partly on parameters estimated by using data of PCBs in relevant tissues of harbor porpoises from the Black Sea. In the wild, the health condition of marine mammals ranges from healthy to emaciated and severely ill which can influence the kinetics of pollutants in their body as such. However, the Black Sea data used to evaluate the models consisted of more by-caught (considered healthy) animals than stranded (potentially sick) ones. In addition, as the models are not entirely based on the datasets in the present study, the model outcomes or conclusions drawn from the models are not influenced by including data of some ill animals.

Elimination half-life

In Weijs et al. (2010b), the models with a metabolic half-life of 27.5 years for bioaccumulation of PCB 153 in male and female harbor

porpoises reflected nicely the Black Sea data. The half-life of 27.5 years was taken from a human model (Verner et al., 2008) and was among the highest for PCB 153 available in the literature. Because of the usefulness of the human elimination half-life in the PBPK model of PCB 153 in harbor porpoises (Weijs et al., 2010b) and the resemblance of porpoises and humans (in body size, body weight and body composition), the same approach was followed in the present study. Thus, the highest elimination half-life values from the literature were used first, but the model was asked to estimate new half-lives in cases where the curves initially differed from the Black Sea data. Except for PCB 118, these new values were slightly higher (for PCB 101) to much higher (for PCB 170, 149). For PCB 180 and 99, the new half-lives even exceeded 300 years (Table 1). Although these extremely high half-lives may seem impossible, they are most likely an artifact caused by the relatively short life-spans of the harbor porpoises and thus the limited number of data points that were available in the fitting-process. For animals that live longer, such as killer whales, elimination half-lives for PCB 180 and 99 would probably be lower because of the higher number of data points at higher ages that can be included in the fitting-process. Nevertheless, for harbor porpoises that only live for about 20 years, the elimination half-lives of PCB 180 and 99 mean that both PCBs are barely eliminated during their entire life spans. In the present study, the elimination half-life only deals with chemicals absorbed by the organism. Assuming that 46% (for calves that drink milk) and 9% (for porpoises that eat fish) of the ingested amount of PCB 180 and 45% (for calves that drink milk) and 10% (for porpoises that eat fish) of the ingested amount of PCB 99 are not part of the model because these percentages were not absorbed by the animals (Table 1), it is noteworthy that it is still possible to find PCB 180 and PCB 99 in feces of harbor porpoises as also found in feces of bottlenose dolphins (Marsili et al., 1995) and right whales (Weisbrod et al., 2000).

It has been suggested that the amount of fat influences the elimination half-life value of chemicals in an organism as the chemical is no longer available for metabolic transformation or fecal/urinary elimination (Grandjean et al., 2008). Compared to rodents for example, marine mammals have much larger fat deposits to be able to maintain a constant body temperature in cold waters, to enhance locomotion and to provide energy in the form of lipids in times of food scarcity (Koopman, 2007). Obviously, this has a major impact on the elimination half-lives which were found to be much higher than those reported in the literature for humans or rodents. Elimination half-lives in the literature are often calculated after experiments in which the organisms were exposed to high doses of pollutants for only a short term (*e.g.* Lee et al., 2002; Maruyama and Aoki, 2006; Reitz et al., 1988). However, information about elimination half-lives after continuous exposure for years is scarce. Collectively, therefore, larger fat reserves and lifetime exposure, may lead to half-lives that are expected to be much higher as found in exposure experiments in the literature.

In the models, changes in the input (concentration of the compound in fish or milk) or elimination half-life can give the same model results. Concentrations of all PCBs were measured in seven milk samples from animals from the Black Sea and concentrations of PCB 118, PCB 170 and PCB 180 in their prey were taken from Tanabe et al. (1997), both of which were reliable sources for intake levels of the respective PCB congeners. For PCB 118, 170 and 180, the only appropriate parameter to adjust in the models is thus the elimination half-life.

For PCB 99, 101 and 149, the levels in the fish prey were not available in Tanabe et al. (1997), so these levels were estimated. Since milk concentrations were available for all PCBs, there were actually two different ways to estimate the fish levels for PCB 99, 101 and 149:

1) The first way was to use the milk-to-fish ratio of PCB 153 (Weijs et al., 2010b), PCB 118, 170 or 180 and the milk levels of PCB 99, 101 and 149;
2) The second way was to look at the PCB patterns in fish in general and the fish data that we have for PCB 153, 118, 170 and 180 from Tanabe et al. (1997), *e.g.* the proportion of PCB 149 to PCB 153 in North Sea fish is probably the same in Black Sea fish (and the levels of PCB 153 in Black Sea fish can be found in Tanabe et al., 1997).

In the present study, both ways were used: the first way was used to estimate the fish levels and the second way to check whether these fish levels were realistic. The following discussion provides more specifics to the estimations of the fish levels of PCB 99, 101 and 149. In the PCB 153 model (Weijs et al., 2010b), there is a 116 times difference between the PCB 153 concentration in fish and milk which was used to estimate the levels in fish of PCB 99, 101 and 149. This factor differs little from the 84, 107 and 203 times differences between the concentration of PCB 118, 170 and 180, respectively, in fish and milk in the current PCB 118, 170 and 180 models. To test whether the calculated fish levels of PCB 99, 101 and 149 were comparable with real concentrations in fish, the patterns of PCBs reported in fish were taken into account as well. In the North Sea, levels of PCB 149 in several fish species may reach relatively high concentrations which are between 30 and 50% of the levels of PCB 153 (Voorspoels et al., 2004). The concentration used in the present study for PCB 149, which is 0.7 ng/g ww or 64% of 1.1 ng/g ww (input used in the PCB 153 model (Weijs et al., 2010b), seems therefore already high enough. Consequently, an increase in elimination half-life in the PCB 149 model was assumed to be more logic than an increase in dietary input of fish. Similar, in fish species from the North Sea, PCB 99 is between 24 and 35% of the concentration of PCB 153 (Voorspoels et al., 2004). This would give a concentration of PCB 99 between 0.26 and 0.39 ng/g ww. Therefore, the value of 0.51 ng/g ww, calculated as a 116 times difference compared to the value in milk, is already high enough and should not be any higher just to get a steep increase in the curves. So also for PCB 99, a higher elimination half-life is preferred rather than an increase in input concentration. In the present study, concentrations in fish were used as a constant thereby probably neglecting a potential decrease in fish levels over time. Tanabe et al. (1997) report on PCBs in harbor porpoises and their prey (European anchovy and whiting) in the Black Sea from 1993. Since there are no available prey or fish data for harbor porpoises in the current dataset from 1998, the fish data from 1993 were used for further calculations. To our knowledge, there are no reports of gradual decreases in fish concentrations from the Black Sea. However, as concentrations of PCBs in fish probably change over time, it is worthwhile to mention that future models would benefit from having fish data reflecting these changes.

Blood/brain partition coefficients

Tissue/blood partition coefficients were calculated with the method of Parham et al. (1997). This method is based on the lipid percentage of the respective tissues (Weijs et al., 2010a) which is the most logical background for the partitioning of lipophilic compounds. All calculated tissue/blood partition coefficients worked nicely for all PCBs, except for the blood/brain partition coefficients. Unlike the blood–brain barriers (BBB) in fish, mammalian BBBs have been suggested to act as a potential protective shield by blocking some chemicals from entering the brain. Bachour et al. (1998) found a uniformly distribution of PCBs in several tissues, including the brain, of fish (rainbow trout), whereas there were significantly lower concentrations of PCBs in the brain of mammals (fox, human) compared to all other tissues investigated. However, next to the presence of a BBB, there is also the possibility that the lipid composition of the brain affects the accumulation of PCBs. The brain is mainly made up of polar phospholipids and sphingolipids whereas it has only a minor portion of triglycerides in contrast with other tissues. In an exposure experiment with chicken embryos, Maervoet et al. (2005) found that concentrations of PCBs (PCB 77, 153 and 180) remained relatively stable in the brain at the latest stage before hatching, while the levels increased exponentially in other tissues such as the liver. Considering that the BBB was still incomplete at that

time (the BBB is only fully completed after hatching), the same study concluded that the specific lipid composition of the brain is less attractive for lipophilic compounds such as PCBs and thus responsible for the lower concentrations of PCBs found in the brain. *In vitro* studies have also proven that PCBs have a much higher affinity for triglycerides than for phospholipids which is indicative for a lesser bioaccumulation in the brain (Sandermann, 2003).

Therefore, a different lipid composition might indeed be the reason why concentrations of PCBs are lower in the brain than in any other tissue. However, it is probably not the only explanation. The lipid composition of the brain of fish (Atlantic herring) is comparable to the lipid composition of the brain of mammals with a higher proportion of polar lipids in mature animals (Mourente and Tocher, 1992). Together with the results of Bachour et al. (1998), this would mean that there is indeed something like a BBB that results in lower concentrations in the brain of mammals compared to other tissues. In the present study, lipids were determined gravimetrically and a hexane/acetone mixture was used for extraction (Weijs et al., 2010a). This method allows to measure triglycerides, cholesterol and less polar phospholipids, but not polar phospholipids or sphingolipids. The lipid percentages were then used to calculate the blood/tissue partition coefficients (Parham et al., 1997) which were for the blood/brain partition coefficients, in all models, too high compared to the Black Sea dataset.

The adjusted (fitted) blood/brain partition coefficients are, for all PCB congeners, 2.5 to 3 times lower than the partition coefficients originally calculated with the method of Parham et al. (1997). The fact that this is independent of the molecular sizes of the molecules (PCB 99 has a comparable blood/brain partition coefficient as PCB 180), is in favor of the theory that the lipid composition of the brain determines the accumulation of PCBs in the brain. This is also supported by the lower concentrations of the PCBs found in the brain of the fetus compared to blubber, liver or kidney (Table 1). In humans, the BBB is incompletely developed at birth (Anthony et al., 1996). If this is true for marine mammals as well, than the lower PCB concentrations in the brain of the fetus can only be caused by the different lipid composition. However, the influence of the BBB cannot be ruled out as there are studies that have reported that the BBB excludes effectively substances with molecular weights greater than 180 Da (Doolittle et al., 1998), while molecular weights of penta-PCBs (such as PCB 99) and hepta-PCBs (such as PCB 180) are between 325 and 400 Da. Lower concentrations of PCBs in the brains of pilot whales and harbor porpoises compared to levels in other tissues were also reported by Tilbury et al. (1999) and Tilbury et al. (1997).

Assimilation efficiency for calves

Debier et al. (2003) and Beckmen et al. (1999) reported that there is a selective gastrointestinal uptake of PCBs from milk, but assimilation efficiencies for individual PCBs from milk remained unknown. Hickie et al. (1999) used higher assimilation efficiency from milk than from fish in the models of beluga whales, but did not discuss PCBs separately. In the present study, the model outputs for the youngest animals overestimated the dataset used for validation when using identical assimilation efficiencies as for the fish. There are basically two parameters that can influence the input of PCBs through milk, namely the assimilation efficiency for milk and the concentration of the PCB in the milk. The concentrations of the individual PCBs in milk were analyzed in 7 milk samples. The only parameter that could be changed was thus the assimilation efficiency from the milk. The resulting assimilation efficiencies for the milk range from almost 50 to 70%, which differs from to the assimilation efficiencies for the fish (90–99%). As unabsorbed PCBs would end up in the feces, concentrations of PCBs in feces of calves should be higher relative to PCBs in feces of older animals. However, fecal samples of harbor porpoises were not available, so this explanation could not be checked.

Goal 2: Assessing temporal trends for parent PCBs

Concentrations of all PCBs investigated in harbor porpoises from the North Sea decreased over a time period of 18 years (1990–2008) although not at the same rate (Fig. 3 A–F). Considering the levels of the PCB congeners in liver of harbor porpoises of 5 years of age for example, levels of PCB 149 decreased the fastest (from 6675 ng/g lw in 1990–2000 to 1943 ng/g lw in 2001–2008; Fig. 3D) whereas levels of PCB 101 decreased the slowest (from 561 ng/g lw in 1990–2000 to 309 ng/g lw in 2001–2008; Fig. 3B). This decrease in PCB levels in harbor porpoises over time is most likely due to a decline in PCBs in the fish, thus a decline in input concentrations for the porpoises over time. In Europe, declining PCB levels over time were reported in several fish species (Skåre et al., 1985; Szlinder-Richert et al., 2009). Decreasing levels over time were also reported for several marine mammal species (harbor porpoises-Law et al., 2010; beluga whales-Lebeuf et al., 2007; Baikal and Caspian seals-Tanabe et al., 2003; polar bears-Dietz et al., 2004) although there were also reports about increasing PCB concentrations over time (sea lions-Borrell et al., 2010; northern fur seals-Kajiwara et al., 2004). Despite these decreasing PCB trends in the North Sea, the area is still a highly polluted area for several years to come.

Goal 3: Metabolism/elimination

Metabolic breakdown and/or elimination pathways are typically difficult to study in living marine mammals, however, *in vitro* studies in these animals exist. In an *in vitro* hepatic microsomal assay with cells from beluga whales, McKinney et al. (2006) found a slow, but significant metabolic biotransformation of PCB 118 ($98 \pm 1\%$ remaining), whereas PCB 101 and PCB 105 were not depleted. For PCB 118, the depletion of 2% is roughly 10 times higher than the ratio between PCB 118 metabolites and the parent compound which ranged from 0 to 0.26% (Fig S6) in the present study. This discrepancy might be due to the duration of exposure; the assay was only 90 min whereas animals in the wild are continuously exposed. PCB 118 is a molecule without vicinal *meta, para* H-atoms, and is as such metabolized by the cytochrome P450 monooxygenase isoform CYP1A, which is present in marine mammals (Hirakawa et al., 2007; Miller et al., 2005; Routti et al., 2008; Tilley et al., 2002; Wilson et al., 2010). Yordy et al. (2010) found that concentrations of PCBs relying on CYP1A metabolism did not increase with age in bottlenose dolphins, thus assuming a metabolic pathway for these congeners in cetaceans. In the present study, HO-metabolites of PCB 118 were detected in 2 out of 6 young animals (age <3 years) and in 3 out of 4 older animals (age >3 years). This might be an indication for an age-dependent induction of the CYP1A system although this needs further investigation with a higher sample size.

In contrast to what was found by McKinney et al. (2006), PCB 101 is probably metabolized in liver of harbor porpoises as has been reported for gray seals (Li et al., 2003). PCB 101 has vicinal *meta, para* H-atoms, 2 Cl-atoms at both *ortho*-positions and is metabolized by CYP2B and 3A family enzymes which are present in marine mammals (Li et al., 2003; Routti et al., 2008). On the other hand, metabolites of this compound have been found in the prey of the harbor porpoises as well (Table 2). According to the PCB 101 model in the present study and the PCB 101-metabolites detected in the harbor porpoises, the animals, especially at older ages, have more PCB 101-metabolites than they can produce through metabolic breakdown (Fig. 4). It is also apparent that the levels of the PCB 101-metabolites are much higher for older animals (>3 years) than for younger animals (<3 years). Dietary accumulation of PCB 101-metabolites should be a continuous process shown as a gradual increase in metabolite levels. Fig. 4, however, shows a more sudden increase in metabolites for older animals compared to younger animals suggesting that there might be an age-dependent biotransformation of PCB 101 additional to a

continuous bioaccumulation of PCB 101 metabolites from the fish with age.

The increase in levels of PCB 149 metabolites is more gradual or continuous (Fig S7) than for the PCB 101 metabolites (Fig. 4). In the past, PCB 149 has not been the focus of many studies as it is not very common in marine mammals other than cetaceans. In harbor porpoises from the North Sea, PCB 149 is consistently measured as the third congener after PCBs 153 and 138 (Weijs et al., 2009a). In other cetacean species, PCB 149 is also considered as one of the most persistent PCB congeners. In theory, PCB 149 is metabolized by CYP2B/3A enzyme systems, but that probably does not play an important role taken the persistence of PCB 149 in cetaceans into account. Yordy et al. (2010) found a significant relationship between the age of bottlenose dolphins and the concentration of PCBs subjected to CYP2B/3A enzymes. In the present study, the continuous increase in PCB 149-metabolites (Fig S7) can be explained by dietary bioaccumulation, although possible metabolic biotransformation of PCB 149 cannot be completely excluded based on the PCB 149 model alone.

Conclusions

Understanding the bioaccumulation of pollutants and associated risks in marine mammals is a challenging problem. *In silico* or computer-based models take the animal as a whole, but often lack important, detailed information about distribution or uptake processes. This information can be taken from studies with other organisms such as rodents, but that does not always seem to work because of the physiological differences between marine mammals and rodents. In the present study, the estimated elimination half-lives of PCBs in marine mammals were higher to much higher compared to the elimination half-lives of PCBs taken from the literature, probably because of the greater lipid deposits in marine mammals. For the brain, not only the lipid percentage plays an important role in the distribution of chemicals, also the lipid composition, together with a potential influence of the blood–brain barrier, are determining factors for the bioaccumulation of lipophilic contaminants. Although more physiological background information of marine mammals (*e.g.* lipid composition of the tissues, enzyme-mediated metabolic transformation) would enhance the extrapolation of the models from one chemical to the other, PBPK models are certainly useful. Once they have been developed, these models can be used for a broad range of applications, such as visualizing temporal trends or shedding some light on possible metabolic breakdown capacities and as such, they go further than simply describing the results of typical biomonitoring studies. As developed and used in the present study, PBPK models are non-invasive and non-destructive which is the only possible approach to study the kinetics of chemicals in marine mammals.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

Liesbeth Weijs and Adrian Covaci acknowledge financial support from the Scientific Research Foundation — Flanders (FWO). Krishna Das is a FRS – FNRS Research Associate. Ursula Siebert, Alexei Birkin and Ludo Holsbeek are acknowledged for performing the necropsies and for providing the samples of the harbor porpoises from the Black Sea. Thierry Jauniaux and SOS Dolfijn, Dolfinarium Harderwijk, The Netherlands are acknowledged for providing the samples of the animals from the North Sea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.taap.2011.07.020.

References

Anthony, D.C., Montine, T.J., Graham, D.G., 1996. Toxic responses of the nervous system. In: Klaassen, C.D., Amdur, M.O., Doull, J. (Eds.), Casarett & Doull's Toxicology: The Basic Science of Poisons. The McGraw-Hill Companies, Inc, pp. 463–486.

Bachour, G., Failing, K., Georgii, S., Elmadfa, I., Brunn, H., 1998. Species and organ dependence of PCB contamination in fish, foxes, roe deer, and humans. Arch. Environ. Contam. Toxicol. 35, 666–673.

Beckmen, K.B., Ylitalo, G.M., Towell, R.G., Krahn, M.M., O'Hara, T.M., Blake, J.E., 1999. Factors affecting organochlorine contaminant concentrations in milk and blood of northern fur seal (*Callorhinus ursinus*) dams and pups from St. George Island, Alaska. Sci. Total Environ. 231, 183–200.

Boon, J.P., van der Meer, J., Allchin, C.R., Law, R.J., Klungsøyr, J., Leonards, P.E.G., Spliid, H., Storr-Hansen, E., Mckenzie, C., Wells, D.E., 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. Arch. Environ. Contam. Toxicol. 33, 298–311.

Borrell, A., Garcia-Solà, A., Aguilar, A., Garcia, N.A., Crespo, E.A., 2010. Organochlorine residues in South American sea lions, *Otaria flavescens* (Shaw, 1800): bioaccumulation and time trends. Bull. Environ. Contam. Toxicol. 84, 731–737.

Bossart, G.D., Reidarson, T.H., Dierauf, L.A., Duffield, D.A., 2001. Clinical Pathology, In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine, Second Edition. CRC Press LCC, Florida, USA, pp. 383–436.

Chu, S., Covaci, A., Haraguchi, K., Voorspoels, S., Van de Vijver, K., Das, K., Bouquegneau, J.M., De Coen, W., Blust, R., Schepens, P., 2003. Levels and enantiomeric signatures of methyl sulfonyl PCB and DDE metabolites in livers of harbor porpoises (*Phocoena phocoena*) from the Southern North Sea. Environ. Sci. Technol. 37, 4573–4578.

Clewell, R.A., Clewell III, H.J., 2008. Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regul. Toxicol. Pharmacol. 50, 129–143.

Corley, R.A., Mendrala, A.L., Smith, F.A., Staats, D.A., Gargas, M.L., Conolly, R.B., Andersen, M.E., Reitz, R.H., 1990. Development of a physiologically based pharmacokinetic model for chloroform. Toxicol. Appl. Pharmacol. 103, 512–527.

Covaci, A., Van de Vijver, K., De Coen, W., Das, K., Bouquegneau, J.M., Blust, R., Schepens, P., 2002. Determination of organohalogenated contaminants in liver of harbour porpoises (*Phocoena phocoena*) stranded on the Belgian North Sea coast. Mar. Pollut. Bull. 44, 1157–1169.

Debier, C., Pomeroy, P.P., Dupont, C., Joiris, C., Comblin, V., Le Boulengé, E., Larondelle, Y., Thomé, J.P., 2003. Dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*: differences in PCB profile between compartments of transfer and changes during the lactation period. Mar. Ecol. Prog. Ser. 247, 249–256.

Dietz, R., Riget, F.F., Sonne, C., Letcher, R., Born, E.W., Muir, D.C.G., 2004. Seasonal and temporal trends in polychlorinated biphenyls and organochlorine pesticides in East Greenland polar bears (*Ursus maritimus*), 1990–2001. Sci. Total Environ. 331, 107–124.

Doolittle, N.D., Petrillo, A., Bell, S., Cummings, P., Eriksen, S., 1998. Blood-brain barrier disruption for the treatment of malignant brain tumors: the national program. J. Neurosci. Nurs. 30, 81–90.

Dufresne, M.M., Frouin, H., Pillet, S., Lesage, V., De Guise, S., Fournier, M., 2010. Comparative sensitivity of harbour and grey seals to several environmental contaminants using *in vitro* exposure. Mar. Pollut. Bull. 60, 344–349.

Grandjean, P., Budtz-Jørgensen, E., Barr, D.B., Needham, L.L., Weihe, P., Heinzow, B., 2008. Elimination half-lives of polychlorinated biphenyl congeners in children. Environ. Sci. Technol. 42, 6991–6996.

Hickie, B.E., Mackay, D., de Koning, J., 1999. Lifetime pharmacokinetic model for hydrophobic contaminants in marine mammals. Environ. Toxicol. Chem. 18, 2622–2633.

Hirakawa, S., Iwata, H., Takeshita, Y., Kim, E.Y., Sakamoto, T., Okajima, Y., Amano, M., Miyazaki, N., Petrov, E.A., Tanabe, S., 2007. Molecular characterization of cytochrome P450 1A1, 1A2, and 1B1, and effects of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners on their hepatic expression in Baikal seal (*Pusa sibirica*). Toxicol. Sci. 97, 318–335.

Kajiwara, N., Ueno, D., Takahashi, A., Baba, N., Tanabe, S., 2004. Polybrominated diphenyl ethers and organochlorines in archived Northern fur seal samples from the Pacific coast of Japan, 1972–1998. Environ. Sci. Technol. 38, 3804–3809.

Koopman, H.N., 2007. Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of Odontocetes. Mar. Biol. 151, 277–291.

Law, R.J., Bersuder, P., Barry, J., Deaville, R., Reid, R.J., Jepson, P.D., 2010. Chlorobiphenyls in the blubber of harbour porpoises (*Phocoena phocoena*) from the UK: Levels and trends 1991–2005. Mar. Pollut. Bull. 60, 470–473.

Lebeuf, M., Noël, M., Trottier, S., Measures, L., 2007. Temporal trends (1987–2002) of persistent, bioaccumulative and toxic (PBT) chemicals in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada. Sci. Total Environ. 383, 216–231.

Lee, S.K., Ou, Y.C., Yang, R.S.H., 2002. Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. Toxicol. Sci. 65, 26–34.

Letcher, R.J., Norstrom, R.J., Muir, D.C.G., 1998. Biotranformation versus bioaccumulation: sources of methyl sulfone PCB and 4,4′-DDE metabolites in the polar bear food chain. Environ. Sci. Technol. 32, 1656–1661.

Li, H., Boon, J.P., Lewis, W.E., van den Berg, M., Nyman, M., Letcher, R.J., 2003. Hepatic microsomal cytochrome P450 enzyme activity in relation to *in vitro* metabolism/inhibition of polychlorinated biphenyls and testosterone in Baltic grey seal (*Halichoerus grypus*). Environ. Toxicol. Chem. 22, 636–644.

Maervoet, J., Beck, V., Roelens, S.A., Covaci, A., Voorspoels, S., Geuns, J.M.C., Darras, V.M., Schepens, P., 2005. Uptake and tissue-specific distribution of selected polychlorinated biphenyls in developing chicken embryos. Environ. Toxicol. Chem. 24, 597–602.

Marsili, L., Gaggi, C., Bortolotto, A., Stanzani, L., Franchi, A., Renzoni, A., Bacci, E., 1995. Recalcitrant organochlorine compounds in captive bottlenose dolphins (*Tursiops truncatus*): biomagnification or bioaccumulation? Chemosphere 31, 3919–3932.

Maruyama, W., Aoki, Y., 2006. Estimated cancer risk of dioxins to humans using a bioassay and physiologically based pharmacokinetic model. Toxicol. Appl. Pharmacol. 214, 188–198.

McKinney, M.A., De Guise, S., Martineau, D., Béland, P., Arukwe, A., Letcher, R.J., 2006. Biotransformation of polybrominated diphenyl ethers and polychlorinated biphenyls in beluga whale (*Delphinapterus leucas*) and rat mammalian model using an *in vitro* hepatic microsomal assay. Aquat. Toxicol. 77, 87–97.

Miller, K.A., Assunção, M.G.L., Dangerfield, N.J., Bandiera, S.M., Ross, P.S., 2005. Assessment of cytochrome P450 1A in harbour seals (*Phoca vitulina*) using a minimally-invasive biopsy approach. Mar. Environ. Res. 60, 153–169.

Mos, L., Tabuchi, M., Dangerfield, N., Jeffries, S.J., Koop, B.F., Ross, P.S., 2007. Contaminant-associated disruption of vitamin A and its receptor (retinoic acid receptor α) in free-ranging harbour seals (*Phoca vitulina*). Aquat. Toxicol. 81, 319–328.

Mourente, G., Tocher, D.R., 1992. Lipid class and fatty acid composition of brain lipids from Atlantic herring (*Clupea harengus*) at different stages of development. Mar. Biol. 112, 553–558.

Parham, F.M., Kohn, M.C., Matthews, H.B., DeRosa, C., Portier, C.J., 1997. Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. I. Tisue:blood partition coefficients. Toxicol. Appl. Pharmacol. 144, 340–347.

Redding, L.E., Sohn, M.D., McKone, T.E., Chen, J.W., Wang, S.L., Hsieh, D.P.H., Yang, R.S.H., 2008. Population physiologically based pharmacokinetic modeling for the human lactational transfer of PCB-153 with consideration of worldwide human biomonitoring results. Environ. Health Perspect. 116, 1629–1634.

Reddy, M., Yang, R.S.H., Clewell III, H.J., Andersen, M.E., 2005. Physiologically Based Pharmacokinetics: Science and Applications. John Wiley & Sons. 420 pp.

Reijnders, P.J.H., 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324, 456–457.

Reitz, R.H., McDougal, J.N., Himmelstein, M.W., Nolan, R.J., Schumann, A.M., 1988. Physiologically based pharmacokinetic modeling with methylchloroform: implications for interspecies, high dose/low dose, and dose route extrapolations. Toxicol. Appl. Pharmacol. 95, 185–199.

Ross, P.S., 2000. Marine mammals as sentinels in ecological risk assessment. Hum. Ecol. Risk Assess. 6, 1–18.

Ross, P.S., De Swart, R.L., Timmerman, H.H., Reijnders, P.J.H., Vos, J.G., Van Loveren, H., Osterhaus, A.D.M.E., 1996. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. Aquat. Toxicol. 34, 71–84.

Routti, H., Letcher, R.J., Arukwe, A., Van Bavel, B., Yoccoz, N.G., Chu, S., Gabrielsen, G.W., 2008. Biotransformation of PCBs in relation to phase I and phase II xenobiotic-metabolizing enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Environ. Sci. Technol. 42, 8952–8958.

Sandermann Jr., H., 2003. Differential lipid affinity of xenobiotics and natural compounds. FEBS Lett. 554, 165–168.

SCANS II, 2006. Small Cetaceans in the European Atlantic and North SeaFinal report available at: http://biology.st-andrews.ac.uk/scans2/inner-furtherInfo.html2006.

Skåre, J.U., Stenersen, J., Kveseth, N., Polder, A., 1985. Time trends of organochlorine chemical residues in seven sedentary marine fish species from a Norwegian fjord during the period 1972–1982. Arch. Environ. Contam. Toxicol. 14, 33–41.

Sonne, C., Gustavson, K., Rigét, F.F., Dietz, R., Birkved, M., Letcher, R.J., Bossi, R., Vorkamp, K., Born, E.W., Petersen, G., 2009. Reproductive performance in East Greenland polar bears (*Ursus maritimus*) may be affected by organohalogen contaminants as shown by physiologically-based pharmacokinetic (PBPK) modeling. Chemosphere 77, 1558–1568.

Szlinder-Richert, J., Barska, I., Mazerski, J., Usydus, Z., 2009. PCBs in fish from the southern Baltic Sea: levels, bioaccumulation features, and temporal trends during the period from 1997 to 2006. Mar. Pollut. Bull. 58, 85–92.

Tanabe, S., Madhusree, B., Oztürk, A.A., Tatsukawa, R., Miyazaki, N., Ozdamar, E., Aral, O., Samsun, O., Oztürk, B., 1997. Isomer-specific analysis of polychlorinated biphenyls in harbour porpoise (*Phocoena phocoena*) from the Black Sea. Mar. Pollut. Bull. 34, 712–720.

Tanabe, S., Niimi, S., Binh Minh, T., Miyazaki, N., Petrov, E.A., 2003. Temporal trends of persistent organochlorine contamination in Russia: a case study of Baikal and Caspian seal. Arch. Environ. Contam. Toxicol. 44, 533–545.

Thomas, G.O., Moss, S.E.W., Asplund, L., Hall, A., 2005. Absorption of decabromodiphenyl ether and other organohalogen chemicals by grey seals (*Halichoerus grypus*). Environ. Pollut. 133, 581–586.

Tilbury, K.L., Stein, J.E., Meador, J.P., Krone, C.A., Chan, S.L., 1997. Chemical contaminants in harbor porpoise (*Phocoena phocoena*) from the North Atlantic coast: tissue concentrations and intra-and interorgan distribution. Chemosphere 34, 2159–2181.

Tilbury, K.L., Adams, N.G., Krone, C.A., Meador, J.P., Early, G., Varanasi, U., 1999. Organochlorines in stranded pilot whales (*Globicephala melaena*) from the coast of Massachusetts. Arch. Environ. Contam. Toxicol. 37, 125–134.

Tilley, R.E., Kemp, G.D., Teramitsu, I., Hall, A.J., 2002. Isolation of two cytochrome P450 cDNAs, CYP1A1 and CYP1A2, from harp seal (*Phoca groenlandica*) and grey seal (*Halichoerus grypus*). Comp. Biochem. Physiol. C 132, 181–191.

Verner, M.A., Charbonneau, M., Lopez-Carrillo, L., Haddad, S., 2008. Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: a new tool in breast cancer epidemiologic studies. Environ. Health Perspect. 116, 886–892.

Voorspoels, S., Covaci, A., Maervoet, J., De Meester, I., Schepens, P., 2004. Levels and profiles of PCBs and OCPs in marine benthic species from the Belgian North Sea and the Western Scheldt Estuary. Mar. Pollut. Bull. 49, 393–404.

Walker, C.H., Hopkin, S.P., Sibly, R.M., Peakall, D.B., 2006. Chapter 6: Toxicity testing, In: Walker, C.H., Hopkin, S.P., Sibly, R.M., Peakall, D.B. (Eds.), Principles of Ecotoxicology, Third Edition. CRC Press, Taylor & Francis Group, USA, pp. 87–108.

Weijs, L., Dirtu, A.C., Das, K., Gheorghe, A., Reijnders, P.J.H., Neels, H., Blust, R., Covaci, A., 2009a. Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 1. Accumulation patterns in harbour seals and harbour porpoises. Environ. Pollut. 157, 437–444.

Weijs, L., Dirtu, A.C., Das, K., Gheorghe, A., Reijnders, P.J.H., Neels, H., Blust, R., Covaci, A., 2009b. Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 2. Biomagnification in harbour seals and harbour porpoises. Environ. Pollut. 157, 445–451.

Weijs, L., Das, K., Neels, H., Blust, R., Covaci, A., 2010a. Occurrence of anthropogenic and naturally-produced organohalogenated compounds in tissues of Black Sea harbour porpoises. Mar. Pollut. Bull. 60, 725–731.

Weijs, L., Yang, R.S.H., Covaci, A., Das, K., Blust, R., 2010b. Physiologically based pharmacokinetic models for lifetime exposure to PCB 153 in male and female harbour porpoises (*Phocoena phocoena*): model development and validation. Environ. Sci. Technol. 44, 7023–7030.

Weijs, L., van Elk, N., Das, K., Neels, H., Blust, R., Covaci, A., 2010c. Persistent organic pollutants and methoxylated PBDEs in harbour porpoises from the North Sea from 1990 until 2008: young wildlife at risk? Sci. Total Environ. 409, 228–237.

Weijs, L., Covaci, A., Yang, R.S.H., Das, K., Blust, R. Computational Toxicology: Physiologically based pharmacokinetic models (PBPK) for lifetime exposure and bioaccumulation of polybrominated diphenyl ethers (PBDEs) in marine mammals. Environ. Pol. (submitted for publication).

Weisbrod, A.V., Shea, D., Moore, M.J., Stegeman, J.J., 2000. Organochlorine exposure and bioaccumulation in the endangered Northwest Atlantic right whale (*Eubalaena glacialis*) population. Environ. Toxicol. Chem. 19, 654–666.

Wilson, J.Y., Moore, M.J., Stegeman, J.J., 2010. Catalytic and immunochemical detection of hepatic and extrahepatic microsomal cytochrome P450 1A1 (CYP1A1) in white-sided dolphin (*Lagenorhynchus acutus*). Aquat. Toxicol. 96, 216–224.

Wolkers, J., Burkow, I.C., Lydersen, C., Dahle, S., Monshouwer, M., Witkamp, R.F., 1998. Congener specific PCB and polychlorinated camphene (toxaphene) levels in Svalbard ringed seals (*Phoca hispida*) in relation to sex, age, condition and cytochrome P450 enzyme activity. Sci. Total Environ. 216, 1–11.

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncatus*) resident to Sarasota Bay, FL. Sci. Total Environ. 408, 2163–2172.