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Application of Bayesian Population Physiologically Based Pharmacokinetic (PBPK) Modeling and Markov Chain Monte Carlo Simulations to Pesticide Kinetics Studies in Protected Marine Mammals: DDT, DDE, and DDD in Harbor Porpoises

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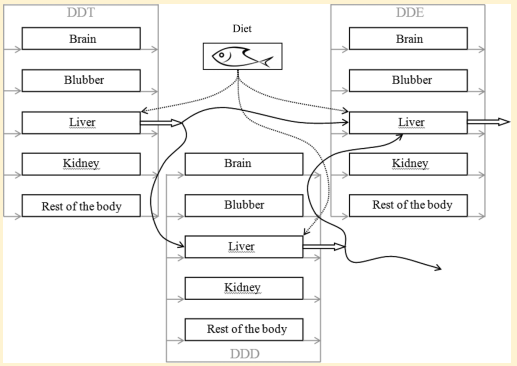
Supporting Information

ABSTRACT: Physiologically based pharmacokinetic (PBPK) modeling in marine mammals is a challenge because of the lack of parameter information and the ban on exposure experiments. To minimize uncertainty and variability, parameter estimation methods are required for the development of reliable PBPK models. The present study is the first to develop PBPK models for the lifetime bioaccumulation of *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD in harbor porpoises. In addition, this study is also the first to apply the Bayesian approach executed with Markov chain Monte Carlo simulations using two data sets of harbor porpoises from the Black and North Seas. Parameters from the literature were used as priors for the first "model update" using the Black Sea data set, the resulting posterior parameters were then used as priors for the second "model update" using the North Sea data set. As such, PBPK models with parameters specific for harbor porpoises could be strengthened with more robust probability distributions. As the science and biomonitoring effort progress in this area, more data sets will become available to further strengthen and update the parameters in the PBPK models for harbor porpoises as a species anywhere in the world. Further, such an approach could very well be extended to other protected marine mammals.



INTRODUCTION

p,p′-Dichlorodiphenyltrichloroethane (DDT) has been used for decades as an insecticide. It was used a lot in the second World War during which it helped to protect soldiers from diseases transferred by insects like malaria and it has later played a major role in agriculture.[1] The use of DDT in agriculture has been banned in several countries starting in the 1970s in the United States and has been included in the Stockholm Convention in 2001. In the 1980s, DDT was replaced by various pyrethroids, but returned eventually as it was proven to be more efficient for mosquito control than pyrethroids. As such, DDT is still used in some countries and continues to be a threat for (marine) wildlife.[2]

The technical mixtures are mainly composed of *p,p′*-DDT and *o,p*-DDT, but also include trace amounts of the *o,p*- and *p,p′*- isomers of the metabolites, e.g., dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD).[1] Metabolically however, DDT can also be transformed into DDE and DDD.[1,3,4] Therefore, the profiles or concentrations of DDT and its metabolites found in wildlife are not solely the result of the intrinsic metabolic capacities of the organisms. DDXs can act as endocrine disruptors.[5] As a result, they can interfere with several systems such as the immune system and eventually be a threat to the overall development and survival of the organism.[6−9]

Harbor porpoises are small cetaceans that inhabit the northern hemisphere. They live in relatively shallow waters, such as the North Sea or Black Sea which are both sinks for pollutants coming from intense ship traffic, harbor activities, and land runoff. Consequently, these animals are exposed to large amounts of pollutants that affect many systems, such as the immune or endocrine system.[10,11] In marine mammals, DDT and its metabolites have often been reported.[11−17] However, since marine mammals are protected from in vivo experiments and since the metabolic capacities of marine mammals regarding the biotransformation of environmental pollutants are poorly understood, there is little or no knowledge

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about the kinetics of DDT and its metabolites in marine mammals. Currently, high numbers of harbor porpoises are present in the European North Sea, making them an important part of the food web. As a result, following up on their current situation in terms of toxicology is definitely worthwhile, especially when the compounds of interest are not totally banned.

In silico toxicology, represented by physiologically based pharmacokinetic (PBPK) modeling in this case, enables us to investigate the different metabolic pathways of DDT, DDE, and DDD, as well as the kinetics of each compound separately inside the body of the organism via computer simulation. For pharmaceuticals, PBPK models are usually linked to pharmacokinetic experiments with laboratory animals or humans. For these compounds, PBPK models are often a necessary part of the approval process that a pharmaceutical product has to undergo before being put on the market. For environmentally relevant chemicals, however, the goal of most PBPK models has been to elucidate the kinetics of a chemical in organisms. In that way, these models can be helpful to understand the potential damage that has been done already if they are linked to effect studies, but can also be helpful for risk assessment in future exposure scenarios. The PBPK models can be linked to experimental work,[18,19] but have also been developed based solely on biomonitoring data.[20−23] In the latter case, variable input concentrations, individual variation, or scattered data lead to a substantial amount of uncertainty to the overall model. This is where the Bayesian statistical approach and Markov chain Monte Carlo (MCMC) simulations are most useful, with "prior knowledge", "randomized parameter sampling from a distribution", and "updated posterior parameter values and distributions" being the key elements in this technique.[24] The "prior knowledge" provides the opportunity to update the model as it refers to knowledge found in the literature, as well as to knowledge resulting from previous model runs. The "randomized parameter sampling" gives a more objective estimate for a specific parameter based on an available data set and its probability distribution where each data point is assumed to have an equal weight of importance. This process can be repeated with each available data set in order to update or optimize posterior parameter values. Because harbor porpoises and other marine mammals are protected species and experimental work is impossible to conduct in these animals, we are left with only scattered data from dead animals due to stranding or accidental deaths with little or no available knowledge on life history and feeding habits of the animals. Under these limitations, the Bayesian approach and MCMC analyses offer the only means to minimize the uncertainty associated with the parameter values and probability distributions in our PBPK models. In that sense, Bayesian population PBPK modeling utilizing MCMC analyses becomes an ideal and noninvasive approach to assess the toxicology and health risk of environmental pollutants to marine mammals. It also serves as an illustration of the utility and power of *in silico* or computational toxicology.

The objectives of the present study were therefore (1) to elucidate the kinetics of *p,p′*-DDT and its metabolites (*p,p′*-DDD and *p,p′*-DDE) in harbor porpoises using PBPK modeling, and (2) to estimate or update species specific parameters with the Bayesian approach and MCMC simulations. To do this, *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE concentrations in male harbor porpoises reported in previously published studies[16,17,25] were used.

MATERIALS AND METHODS

The development of the PBPK model for lifetime exposure to DDT, DDD, and DDE in male harbor porpoises can be separated into two phases: (1) the development of the "Structural PBPK Model", and (2) the estimation/update of some parameters using the Bayesian approach and the MCMC simulations in a "Statistical Model". These "Models" are explained below together with a description of the Black Sea and North Sea data sets which were used to evaluate the models. In this study, the DDT, DDD, and DDE mentioned are the *p,p′*-isomers only. Levels of the *o,p*-isomers are much lower in harbor porpoises than the *p,p′*-isomers and were thus not taken into account. The term "DDXs" is used at some places to represent collectively different forms of DDT, DDE, and DDD.

Black Sea and North Sea Data Sets. DDE, DDD, and DDT concentrations in blubber, brain, kidney, muscle, and liver samples from 20 males and 1 fetus from the Black Sea were used, as well as milk samples (Table 1 for milk; Table 2 for fetus; and Weijs et al.[16] for all other results). These data are referred to as the "Black Sea data set" in the present study. In addition, DDE, DDD, and DDT results in blubber from 9 males and 1 neonate from the North Sea were used (animals from 2000 to 2008 from Weijs et al.[17]), as well as blubber samples from 20 males from the North Sea (unpublished data, but same animals from 1999 to 2004 as discussed in Weijs et al.[25]). All results in the animals from the North Sea were pooled

Table 1. Concentrations of DDT, DDD, DDE, and Sum of DDXs (expressed in ng/g lw) and Lipid Percentages of Milk Samples of Female Harbor Porpoises from the Black Sea in 1998 unless Stated Otherwise[a]

sample ID	% lipid	DDT	DDD	DDE	sum of DDXs	comments
U 31 milk[b]	51.8	12541	33327	51243	101376	female (7 yr), 1997
U 39 milk[c]	36.9	917	2164	3152	6476	female (>1.5 yr), 1997
U 40 milk[b]	36.6	5845	15711	19386	42790	female (>1.5 yr), 1997
U 48 milk	24.7	899	3027	3391	7626	from female U 48[21]
U 67 milk	22.4	467	2294	2710	5649	from female U 67[21]
U 89 milk[c]	26.1	4039	13834	17101	36256	no information available
U 90 milk[c]	36.9	1350	3941	5044	10734	from female U 90[21]
U 94 milk[c]	26.0	4218	10158	12041	27940	from female U 94[21]

[a]Sum of DDXs is the sum of all *o,p*-DDXs and *p,p′*-DDXs. [b]Outlier; excluded from further calculations of the average milk concentration. [c]No information available regarding the situation of death (stranded or by-caught).

Table 2. Levels of DDT, DDD, and DDE Expressed in ng/g lw in Tissues of a Black Sea Harbor Porpoise Fetus[a]

tissue	% lipid	DDT	DDD	DDE	sum of DDXs
brain	6.5	33.7	240.2	365.4	654.3
blubber	85.8	989.6	3170.5	3609.7	8039.9
kidney	5.8	143.9	1097.2	1336.1	2630.0
liver	2.4	ND	1008.4	1354.0	2455.9

[a]Sum of DDXs is the sum of all *o,p*-DDXs and *p,p′*-DDXs. ND = not detected

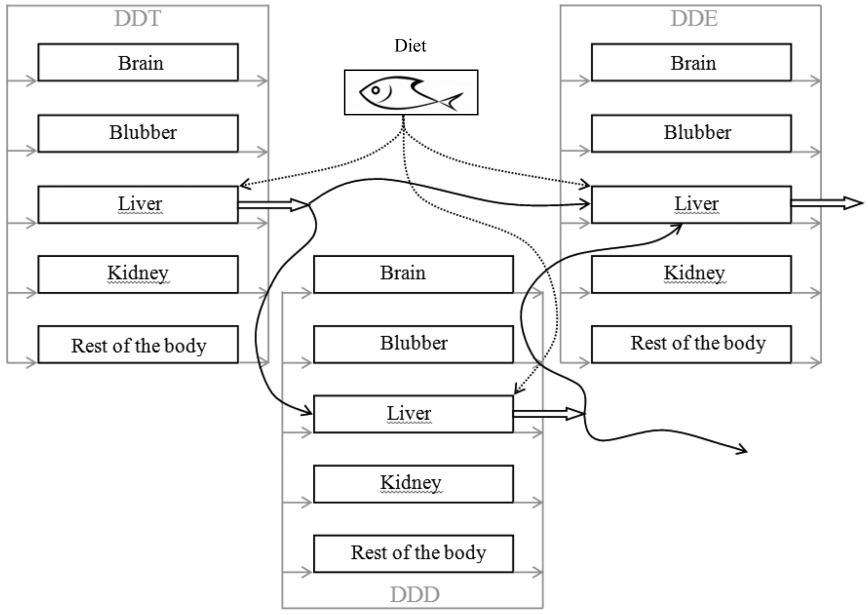

Figure 1. Conceptual diagram of the PBPK model for DDT/DDD/DDE in male harbor porpoises. Solid lines and arrows represent the elimination pathways characterized by their respective elimination half-lives, and dotted lines represent the input pathways. DDXs in the milk for the pups and fish diet for the adults are direct inputs into the liver.

Table 3. Prior Parameter Values, Posteriors, Updated Probability Distributions, and *R*-Values (Convergence Factors) of the Parameters Estimated through Bayesian PBPK Modeling and MCMC Simulations Using the Black Sea Data Set[a]

	prior			posterior		
parameter	value	range	distribution	mean	SD	*R*-value
PF_DDT	331.6	248.7−414.5	normal	331.0	44.2	1.0003
PL_DDT	0.2	0.15−0.25	normal	0.2	0.0	1.0006
PR_DDT	3.5	2.625−4.375	normal	3.5	0.5	1.0000
PK_DDT	0.9	0.675−1.125	normal	0.9	0.1	1.0010
PB_DDT	1.5	1.125−1.875	normal	1.5	0.2	1.0007
PF_DDD	400.0	300.0−500.0	normal	400.8	52.9	1.0006
PL_DDD	7.9	5.925−9.875	normal	7. 9	1.1	1.0009
PR_DDD	15.0	11.25−18.75	normal	15.0	2.0	1.0013
PK_DDD	4.6	3.45−5.75	normal	4.6	0.6	1.0007
PB_DDD	3.0	2.25−3.75	normal	3.0	0.4	1.0012
ElimHL_DDE	15.0	11.25−18.75	normal	11.8	5.7	1.0002
ElimHL_DDD	3.5	2.625−4.375	normal	3.6	1.4	1.0001
ElimHL_DDT	5.0	3.75−6.25	normal	4.8	1.8	1.0016
PercDDD	10.0	7.5−12.5	normal	10.0	1.3	1.0011
PercDDT	50.0	37.5−62.5	normal	50.2	6.6	1.0003

[a]For prior information of parameters, an SD of 15% of the Prior Value was included in the statistical model as well which is not given in the table. Prior Ranges were set at ±25% of the Prior Value. PF − blood/adipose tissue partition coefficient, PL − blood/liver partition coefficient, PR − blood/muscle (rest of the body) partition coefficient, PK − blood/kidney partition coefficient, PB − blood/brain partition coefficient, ElimHL − elimination half-life value, PercDDT − percentage of DDT that metabolically biotransforms into DDD, PercDDD − percentage of DDD that metabolically biotransforms into DDE.

and referred to as the North Sea data set. Animals from the Black Sea and North Sea were victims of accidental by-catch or were found stranded. Extraction and cleanup procedures, details about the GC/MS analysis, and QA/QC results are given in Weijs et al.[16,17,25]

Structural PBPK Model. The structural PBPK model for the kinetics of DDT, DDE, and DDD is similar to the ones developed for lifetime bioaccumulation of PCBs and PBDEs in male harbor porpoises.[21−23] For each of the three compounds, the model consisted of 5 compartments (liver, blubber, kidneys, brain, and muscle (rest of the body)). The three models for DDT, DDE, and DDD are interconnected at the level of the liver compartment, thus the overall model has 15 compartments in total (Figure 1). The entire model includes metabolic biotransformation pathways from the DDT model to the DDD and DDE models, and from the DDD model to the DDE model. While each model is capable of working on its own, the models of DDE and DDD will not give realistic results without being connected to the DDT model. Physiological parameters and equations, such as the growth, the blood flow, cardiac output, and daily fish consumption were taken from the PBPK model for PCB 153 in male harbor porpoises (given in Supporting Information Table S1).[21] Compound specific parameters were either taken from the literature (e.g.,

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Table 4. Prior Parameter Values, Posteriors, Updated Probability Distributions, and *R*-Values (Convergence Factors) of the Parameters That Were Estimated through Bayesian PBPK Modeling and MCMC Simulations Using the North Sea Data Set

	prior			posterior		
	value	range	distribution[a]	mean	SD	*R*-value
TOTDIET_DDE		15−40	uniform	15.2	0.4	1.0039
TOTDIET_DDT		2−10	uniform	2.8	0.7	1.0000
TOTDIET_DDD		5−20	uniform	8.5	2.9	1.0003
CMILK_DDE		50−150	uniform	63.8	12.4	1.0018
CMILK_DDT		0−50	uniform	22.0	13.0	1.0003
CMILK_DDD		40−100	uniform	69.2	15.9	1.0003
PF_DDT	331.0	248.2−413.7	normal	338.3	34.6	1.0002
PL_DDT	0.2	0.15−0.25	normal	0.2	0.0	1.0039
PF_DDE	450.0	225.0−562.5	normal	232.9	9.8	1.0003
PL_DDE	7.0	3.5−14.0	normal	7.1	1.0	1.0014
PF_DDD	400.8	347.9−543.7	normal	406.8	41.9	1.0004
PL_DDD	7.9	5.9−9.9	normal	7.9	0.8	1.0020

[a]Prior Value, SD (Table 3 for PFs and PLs of DDD and DDT, but not given in this table), and Range (Prior Value ±25% of Prior Value) are specified for normal distributions, whereas only a Range is specified for uniform distributions. TOTDIET − concentration of DDE, DDT, or DDD in the fish diet of the harbor porpoises, CMILK − concentration of DDE, DDT, or DDD in the milk diet of the harbor porpoises, for descriptions of PF and PL see Table 3.

assimilation efficiencies or average net absorptions from Thomas et al.[14] which were 98% for DDT, 99% for DDD, and 97% for DDE) or were estimated using the Bayesian/MCMC approach (Tables 3 and 4). The structural model was developed initially with Berkeley Madonna software; it was later transformed with AcslX/Libero software (AEgis Technologies, Orlando, FL).

Statistical Model. Since there were 49 parameters in the PBPK model for DDT, DDE, and DDD, only a selection of those parameters that were shown to be sensitive in the global sensitivity analyses (GSA) were included in the statistical model. The GSA (Table S2 and Figures S1, S2, and S3) followed the scheme of McNally et al.[26] with the Morris test to deselect the least sensitive parameters followed by the eFAST (extended Fourier amplitude sensitivity) test to quantitatively test the influence of the most sensitive parameters on the model output throughout the entire lifetime of the animals. The Bayesian approach with MCMC simulations allows updating the parameters in a model with every data set that becomes available. In the present study, two data sets were used: one from male harbor porpoises from the Black Sea, the other from male harbor porpoises from the North Sea. Since the Black Sea data set was much more extensive compared to the North Sea data set, the statistical model was run using the Black Sea data first, followed by a second run using the North Sea data. The initial values (priors) of the parameters with their probability distribution characterized by a mean and standard deviation are therefore different for the two model runs (Tables 3 and 4). To allow the MCMC simulation to converge and to calculate convergence factors (*R*-values), three chains of 15,000 iterations each were used for the statistical model with the Black Sea data and for the statistical model with the North Sea data.

RESULTS

Sensitivity Analyses. Morris sensitivity tests were performed with the entire set of parameters (49 parameters; Table S2), whereas the eFAST tests were performed with only a subset of parameters (19 parameters) selected based on the availability of parameter information in the literature and the results of the Morris test. Percentages of DDT transforming into DDD and DDE, percentages of DDD transforming into DDE, and tissue/blood partition coefficients of DDT and DDD were selected because there was no information about these parameter values in the literature (for DDE[1]). Therefore, these parameters needed to be estimated regardless of their sensitivity in the model. Partition coefficients between liver, kidney, and blood for the DDD model (PL_DDD and PK_DDD) were not included in the eFAST test exclusively due to practical constraints since the eFAST test in AcslX/Libero does not work with more than 20 parameters. However, they were included in the statistical model and were estimated using the Bayesian approach and MCMC simulations. Concentrations in the fish or milk diet (TOTDIET and CMILK parameters) were selected, because these parameters are generally unknown for most harbor porpoise populations, but were known for the Black Sea data set. Hepatic elimination rates of DDT, DDE, and DDD were initially calculated using elimination half-life values from Verner et al.,[27] so these values were included in the eFAST tests because of their sensitivity in the models.

Selection of Parameters in the Statistical Models for MCMC Analyses. *Black Sea.* For the Black Sea data set, the fish diet was assumed to consist of European anchovy and whiting.[28] The levels of total DDXs in these two fish species from the Black Sea were taken from Tanabe et al.[29] Concentrations of DDT, DDD, and DDE in the fish diet were recalculated according to the composition of DDT (15%), DDD (31%), and DDE (52%) in the total DDTs[29] resulting in input concentrations of 33.9, 117.4, and 70.0 ng/g ww, respectively. The concentrations of DDT, DDD, and DDE in the milk of Black Sea harbor porpoises were measured in milk samples of female porpoises and the average values (Table 1) were used in the model. Partition coefficients for DDE in various tissues of pregnant rats were available in the literature,[1] but partition coefficients for DDD and DDT were not. Likewise, percentages of DDT metabolically transformed into DDD or DDE and of DDD transformed into DDE were scarce in the literature. These parameters were therefore included in the statistical model and estimated using the Bayesian approach. The prior values for these parameters were initially taken from our earlier study on harbor porpoises,[21] but were updated in subsequent range finding model runs (executed with

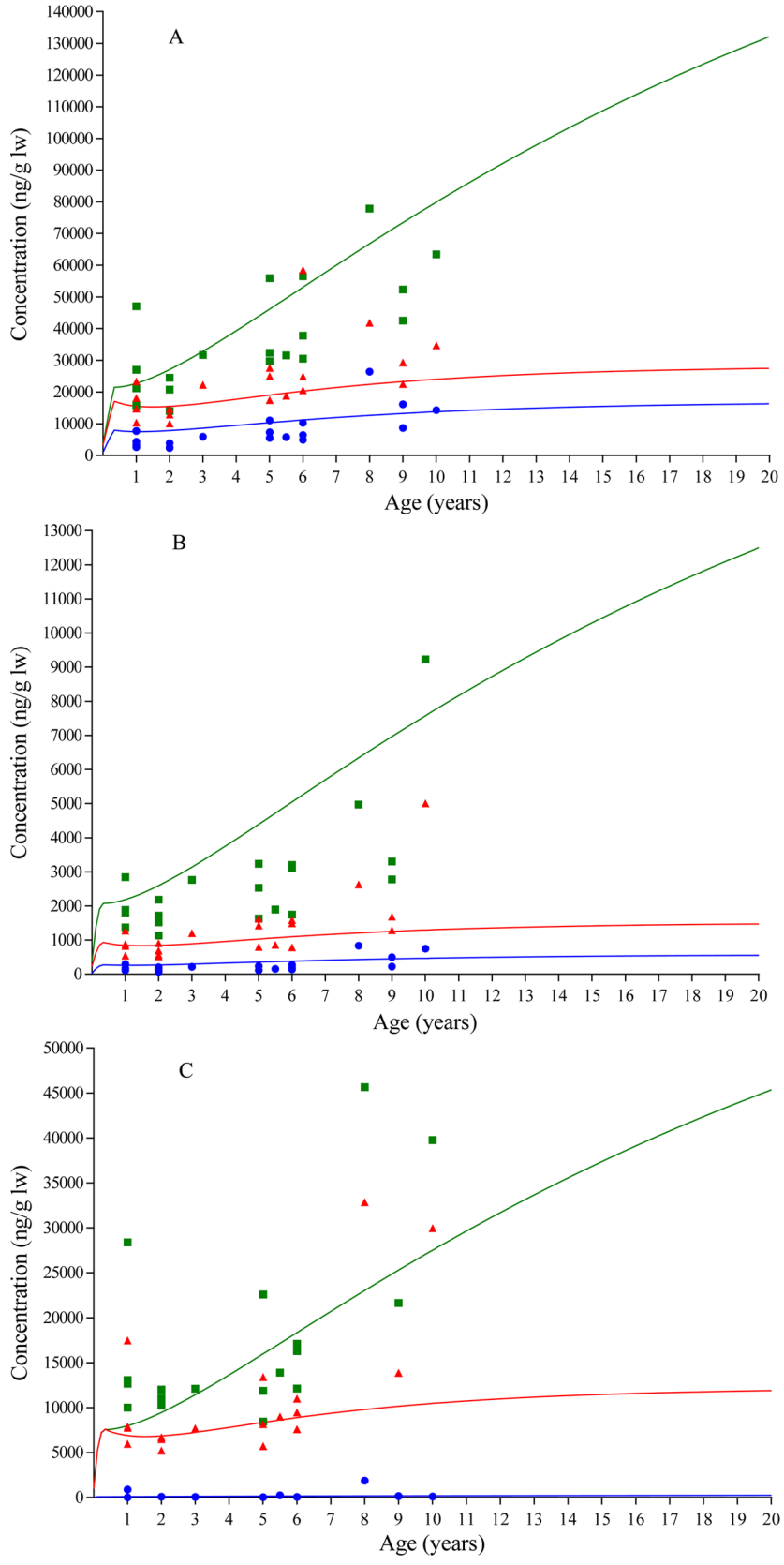

Figure 2. continued

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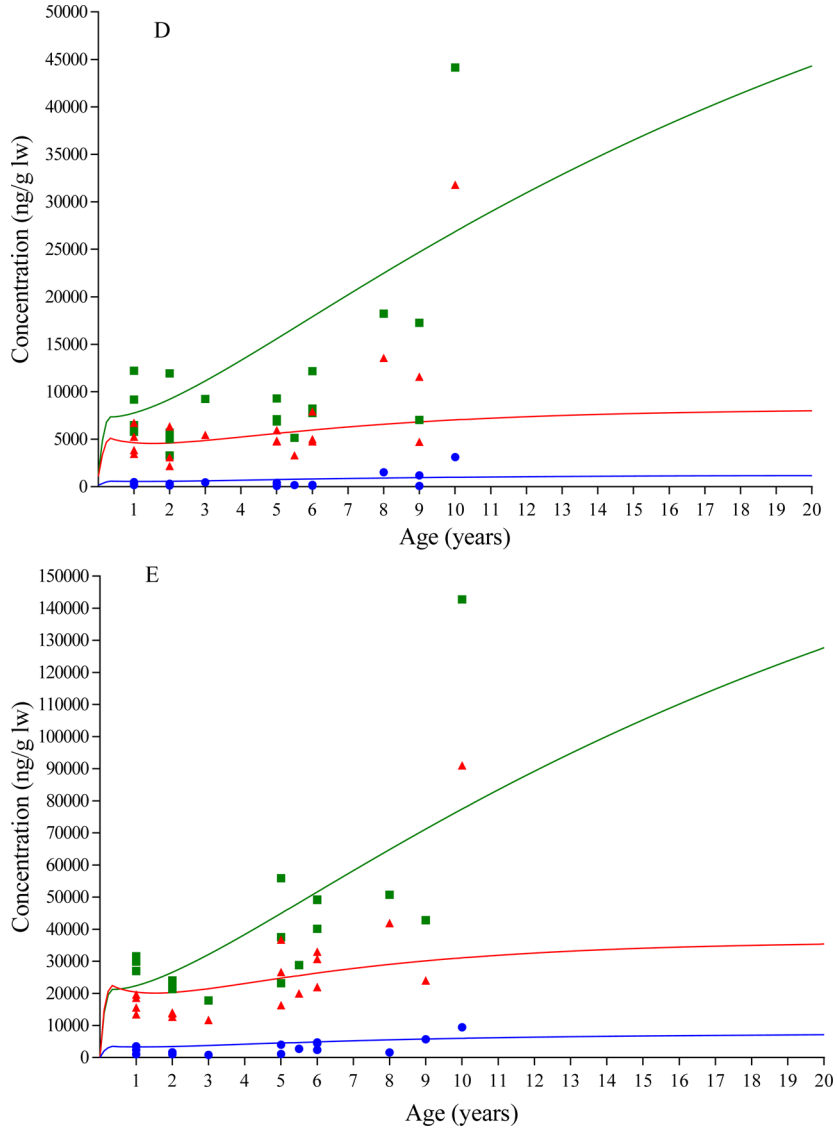

Figure 2. Concentrations of *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD in (A) blubber, (B) brain, (C) liver, (D) kidney, and (E) rest of the body (muscle) of Black Sea harbor porpoises. Concentrations are expressed in ng/g lipid weight (lw), by the age of the animals in years. __= model predictions with posterior mean values of the parameters of Table 3, ■/▲/● = Black Sea data set.[16] Green, red, and blue curves/data points represent *p,p′*-DDE, *p,p′*- DDD, and *p,p′*-DDT, respectively.

the Bayesian method and MCMC simulations, 5000 iterations). The posterior values of those range finding model runs were then included in the statistical model characterized as a normal distribution with their randomly chosen standard deviation (SD) of 15% and a range of ±25%. Elimination half-life values for DDE of 15 years and for DDT of 5 years in humans were taken from Verner et al.[27] Both parameters were also included in the statistical model for further optimization using the Bayesian approach which was also used to estimate the elimination half-life value of DDD. In total, there were 15 parameters included in the statistical model (Table 3). As noted in Table 3, the posterior means were very similar to the prior values. This is not unexpected since the prior ranges of 10 out of 15 parameters were already updated once with the Bayesian approach/MCMC under the assumption of uniform distributions because of lack of information. The posterior means for the elimination half-lives did not differ much from the prior values either, but had relatively greater SDs compared to all other parameters.

Figure 2 shows all model predictions (curves) and data points (squares) in all five compartments of male harbor porpoises from the Black Sea. In all compartments or tissues, DDE levels were highest followed by DDD and DDT. Although the trend was less clear for DDT, all levels, as well as the differences in levels of DDE, DDD, and DDT appear to increase with age.

North Sea. In total, 12 parameters were included in the statistical model (Table 4) which were: TOTALDIET_DDT, TOTALDIET_DDD, TOTALDIET_DDE (concentrations of DDT, DDD, and DDE in the fish diet), CMILK_DDT, CMILK_DDD, CMILK_DDE (concentrations of DDT, DDD, and DDE in the milk diet), PF_DDT, PF_DDD, PF_DDE (blubber/blood partition coefficients for DDT, DDD, and DDE), and PL_DDT, PL_DDD, and PL_DDE (liver/blood partition coefficients for DDT, DDD, and DDE). These parameters were selected based on the following rationale:

(1) The PF and PL parameters were important because they were very "sensitive" to the changes in parameter value

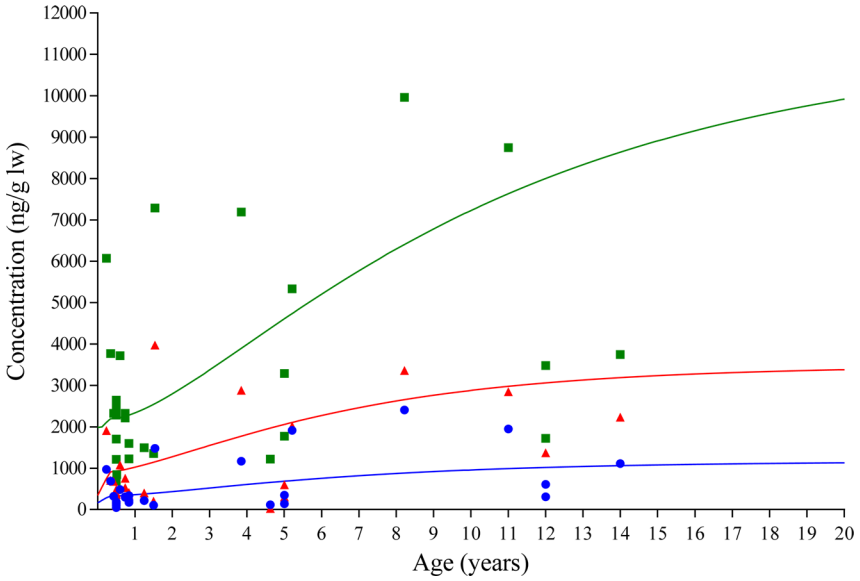

Figure 3. Concentrations of *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD in blubber of North Sea harbor porpoises. Concentrations are expressed in ng/g lw, by the age of the animals in years. __= model predictions with posterior mean values of the parameters of Table 4, ■/▲/● = North Sea data set.[17] Green, red, and blue curves/data points represent *p,p′*-DDE, *p,p′*- DDD, and *p,p′*-DDT, respectively.

according to the results of Morris sensitivity analyses (Table S2). Also, liver and blubber samples of marine mammals are more easily selected for POP analyses compared to muscle samples. Therefore, updating liver or blubber related parameters was given priority.

(2) The TOTDIET and CMILK parameters were selected because it was impossible to find any information in the literature. Therefore, the best option was to make assumptions of the priors (parameter values) and their respective probability distributions and let MCMC analyses optimize the parameter values based on the newly available data set.

For the partition coefficients of DDT and DDD, the prior values (Table 4) were the posterior values (Table 3) from the model runs with the Black Sea data, whereas the range was, similar to the previous run with the Black Sea data set, set at ±25% of the prior parameter value. Partition coefficients for DDE taken from the literature[2] got a SD of 15% and a range of ±25%. Concentrations in the fish diet (TOTDIET parameters) were assigned a range taking into account the proportion of DDT, DDD, and DDE in North Sea fish species.[30] Concentrations in the milk diet (CMILK parameters) were higher in the Black Sea milk samples than in the Black Sea fish input parameters. Therefore, ranges for CMILK in the North Sea data set were also assumed to be higher than the input parameters. The DDD, DDE, and DDT proportions were also taken into account as was done for the TOTDIET parameters of the North Sea model.

Figure 3 gives all model predictions (curves) and data points (squares) in blubber of male harbor porpoises from the North Sea. Similar to the Black Sea data set, DDE levels were highest followed by DDD and DDT. Although not that clear for DDT, all levels of DDE, DDD, and DDT increased with age.

■ DISCUSSION

The present study is the first to describe the kinetics of *p,p′*-DDT and its metabolites *p,p′*-DDE and *p,p′*-DDD in multiple tissues of a marine mammal species using Bayesian Population PBPK modeling with MCMC analyses. The importance of this work lies in the fact that DDXs are currently still produced and used as they are more useful for malaria control than alternative substances (pyrethroids). The present models are therefore not only suitable for explaining past exposures but can also be used in a proactive way for future exposure scenarios.

The presence of DDT, DDD, and DDE in the environment and biota can be partly explained by the composition of the technical mixture and partly by the metabolic biotransformation of the organism. A realistic model should therefore reflect both issues. From the literature it is known that DDT can be converted metabolically into DDE and DDD in mammals, including humans,[1] and in birds.[31] In these studies, the pathways suggested are from DDT to DDD and from DDT to DDE. In sheep, however, DDE was suggested to appear as a metabolite of both DDT and DDD[32] and this finding was confirmed later in rats.[3,33] In the past, DDXs have been found in marine mammal species, but the origin of DDD and DDE could not be determined. Given the above literature evidence, we believe that two major sources for DDD and DDE in harbor porpoises are through direct food intake and from biotransformation of DDT. Accordingly, the DDT model has only one input factor, the presence of DDT in the fish/milk diet of the porpoises while the DDD model has two input factors, the presence of DDD in the fish/milk diet of the porpoises as well as a percentage of the metabolism of the DDT model. Similarly, the DDE model has three input factors, the presence of DDE in the fish/milk diet of the porpoises, a percentage of the metabolic end products of the DDT model and a percentage of the metabolism of the DDD model. Because of this, the three models are metabolically connected through the liver compartment.

Parameter estimation through the Bayesian approach/MCMC analyses are such that all succeeding available data sets, as well as all the biological and toxicological information associated with the PBPK models, are taken into consideration. Theoretically, during the optimization process, numerous possible values for a given parameter may be suitable for the simulation outcome to be consistent with the available data.

However, the Bayesian approach/MCMC analyses narrow down these numerous possible values for the given parameter because all data sets and other biological/toxicological information including the interdependence of all parameters are taken into consideration. The resulting final "posteriors" for the updated parameters and their respective probability distributions are therefore the most robust estimates based on the presently available information. It is conceivable, as the science in this domain continues to advance, that more and more data will be available for further updates/refinements of the parameters by the Bayesian Approach/MCMC analyses.

DDX PBPK Model for Harbor Porpoises from the Black Sea. The Black Sea data set is useful to work with from a modeling perspective. All individuals died in the same region, had limited migration, and were at different ages. Furthermore, several tissues, and the subsequent analytical data, are available for each individual; this, plus the dietary information from Tanabe et al.[28,29] constitutes a more suitable database than any other harbor porpoise data set to date. For that reason, the Black Sea data set was chosen as the first step in the whole modeling exercise. In the Black Sea data set, only 3 out of 20 animals were found stranded. Victims of by-catch are often considered healthy in contrast to stranded animals. However, the three stranded animals did not visibly stand out compared to the concentrations of the 17 victims of by-catch so all data were pooled. In the model, prior information of the parameters was taken from the literature (e.g., for the elimination half-lives of DDE and DDD) and estimated in short and simple model runs using uniform parameter distributions if necessary (e.g., for some of the partition coefficients). Later, the parameter values were all estimated together in the entire model as the short and simple model runs included only maximum 3 parameters at the same time thereby ignoring potential covariations between parameters.

The posterior parameter values estimated through the Bayesian approach and MCMC simulations (Table 3) were for most parameters close to the prior parameter values. A potential explanation for this could be that the prior values were, unknowingly, already good enough to represent the Black Sea data set. On the other hand, there is also the possibility that the Black Sea data set was not informative enough for some of the parameters to be updated. In both cases, adding more data sets will provide a bigger challenge for the parameters to be updated or optimized.

Compared to the PCB 153 model[21] or other PCB models,[22,23] the growth dilution effect in the present PBPK models for DDT, DDE, DDD did not seem to play a large role because of the high concentrations in the fish diet for all three compounds. For PCB 153 and other PCB congeners, the growth dilution effect represented a time period in the entire lifetime of the harbor porpoises where the animals were exposed to relatively lower concentrations of PCBs. The same cannot be found for the PBPK models for DDT, DDD, and DDE. Concentrations of DDT were consistently lower than those of DDE and DDD, whereas levels of DDE were highest, a common pattern in marine mammals.[11,12] In the present study, the DDE levels in blubber were higher than those found in male ringed seals from Svalbard (300 ng/g lw),[15] in blubber of harbor seals ($\sum$DDXs = 1780 ng/g lw),[13] and in blubber of striped dolphins ($\sum$DDXs = 61 100 ng/g lw).[13] Das et al.[11] suggested that the levels of PCBs, DDT, DDE, and PBDEs in porpoises from European coasts potentially interfere with the thyroid functions in these animals. Since the levels of DDE found in the younger Black Sea harbor porpoises (the present study) were much higher than those reported by Das et al.,[11] it is possible that even the lowest concentrations in the Black Sea animals are already toxic to the animals. Such risk undoubtedly increases with age as the Black Sea harbor porpoises accumulate higher levels of these pollutants through feeding.

Further Update of DDX PBPK Models Using North Sea Data Set. The sample size of the North Sea data set was larger than the Black Sea data set (29 animals versus 20 animals), but smaller in several other aspects. Blubber was the only tissue in the North Sea data set, animals were from a longer time span (1999−2008), and information about the concentrations of DDXs in the animals' diet was not available in the literature. However, the model with the North Sea data set could benefit from the posterior parameter information deduced via the model with the Black Sea data set. For historical samples of marine mammals where the diet was not simultaneously analyzed, the pollutant input parameters will always need to be estimated from little or no background information. An alternative for those samples is the possible use of the existing age-dependent bioaccumulation models for the respective chemical[21−23] even if input parameters are unknown (reverse dosimetry modeling[20]). Information regarding the health status was not available for all 29 North Sea animals: 12 were reported as victims of accidental by-catch whereas 4 were found on the beach. Similar as for the Black Sea data set, concentrations in those 4 animals did not differ from the others upon visual inspection. All data were therefore pooled. For all parameters other than the input parameters, the posterior values of the model with the North Sea data set were, where possible, based on and estimated with the posterior values of the Black Sea data. Consequently, the model with the North Sea data set covers the Black Sea data as well. There was a big difference between the prior blubber/blood partition coefficient value of 450 for DDE and the posterior value of 233 even though the prior value worked fine for the model with the Black Sea data set. This low partition coefficient could be due to the North Sea data set where data are more scattered than in the Black Sea data set. The Bayesian approach does not distinguish between these data and does not recognize outliers. By excluding outliers in the North Sea data set, the value of this parameter will change. However, outliers or exceptions are undoubtedly part of any data set in wild populations and will perhaps be more "normal" in data sets with larger sample sizes or when more data sets are included in the model. Therefore, the decision was made to keep the parameter value of 233 and test it in the future whenever more, bigger, and/or better data sets become available.

With respect to the North Sea data, it is important to note that there were no data of DDXs in tissues other than blubber. Thus, parameters for liver, brain, kidney, and "rest of the body" could not be updated in this iteration. The accuracy of these other parameters could therefore benefit from further modeling with more elaborate data sets. Similar to the Black Sea model predictions, the DDE concentrations were highest, followed by DDD and DDT. For all three compounds, concentrations were increasing over the entire lifetime of the porpoises with no growth dilution effect. Levels of DDE from the present study were about 5 times higher compared to DDE levels reported in Das et al.,[11] but approximately 13 times lower than the levels in the Black Sea data set.

Future Perspectives. In this work, we demonstrated the utility of refined and updated parameter values of PBPK models

for DDXs using Bayesian Population PBPK modeling and MCMC analyses. As indicated earlier, such an approach may be repeated when new data become available. Assuming that all future data sets are sufficiently informative, the posterior distribution ranges will become narrower than the prior ones and all resulting updates will encompass all earlier data sets. Having narrower posterior distribution ranges for the parameter values with every update has two advantages for the entire model.

First, the model uncertainty will be reduced until, in theory, only the model variability is left. As the model variability reflects the differences between individuals, this is an intrinsic source of variation in the model that cannot be reduced by the Bayesian approach. However, since marine mammals in general are not organisms typically used in experiments, there is a great deal of variability among wild individuals which we do not know. Consequently, the variability in the model parameters will present a challenge for all future investigators of marine mammals.

Second, by using knowledge from previous studies to get posterior parameter distributions, the updated PBPK model and parameters not only reflect the situation in the current data set, but also inclusive of the data sets from previous studies. Such an integration of computational technology and chemical, physiological, and toxicological information reflects the essence of systems biology. Altogether, the Bayesian Population PBPK modeling and MCMC analyses approach applied here may lead to a useful, reliable, species-specific model or tool with parameter ranges that mimic the variability between individuals in reality. This is a very powerful and efficient way to assess pollution and risk in these protected wild marine mammal species.

■ ASSOCIATED CONTENT

Supporting Information

Tables, results of the sensitivity tests, and model codes. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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