Development of physiologically based pharmacokinetic models for the bioaccumulation of persistent organic pollutants in marine mammals

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Factors, such as a greater demand for products and more sophisticated industrial techniques, have caused a growing number of chemicals in the environment. Due to a lack of efficient metabolic breakdown or elimination processes in organisms and in the environment, these chemicals can be passed on in the aquatic and terrestrial food webs leading to higher levels in the predator compared to its prey. Marine mammals are apex predators in marine ecosystems and as such, they accumulate considerable amounts of chemicals through their diet. Evidence in the literature has shown that these amounts can have a major negative impact on their immune, endocrine and reproductive system or even on their survival in general. Marine mammals do not seem to have the appropriate enzyme systems to be able to deal with chemicals. They experience the negative effects of pollution themselves and at the same time, pass the chemicals on to their offspring. As a consequence, levels of contaminants in these animals decrease only slowly even though the industrial production of several chemicals has been restricted or banned in the past.

In order to prevent repetitions of past situations, it is important to gain knowledge about the absorption, distribution and elimination of known or 'old' chemicals in marine mammals. Understanding the kinetics and effects of old chemicals can be useful to assess the impact of new chemicals with comparable properties compared to the old ones before these new chemicals are being manufactured. From this point of view, studying known, old chemicals is undoubtedly useful for the risk assessment of new compounds.

In this thesis, the kinetics of known or traditional chemicals such as PCBs and PBDEs, was investigated in harbour porpoises and long-finned pilot whales by using physiologically based pharmacokinetic (PBPK) models. These computerized models combine physiological information of the organism of interest and chemical properties of the chemical of interest to reflect the kinetics of that compound in the body of the organism. Similar to exposure experiments in which all factors are controlled to minimize the large degree of variability, models for wild populations are also more reliable if the datasets used for evaluation of the models are somewhat 'robust' or 'uniform'. For practical reasons, trends are easier to visualize and parameters are easier to estimate if the number of interfering, external factors are reduced to a minimum. For theoretical reasons, scattered data can lead to parameter estimates that are not reflecting the intrinsic, physiological capabilities of the species.

Consequently, it was important to investigate first which factors were influencing the levels of pollutants in the blood since blood is the only circulation medium in the models. Blood samples of harbour seals and porpoises of different health condition, origin (captivity versus wild), gender and age were analysed. Results showed that only emaciated animals had deviating concentrations and profiles of PCBs and PBDEs in their blood compared to animals that were not emaciated. Of course, starvation can occur in wild populations, but is definitely not common for all wild animals. The conclusion here was thus that datasets of blood could be used in the bioaccumulation models. However, blood is never sampled at the same time as tissues in marine mammals. In multi-compartmental models as the ones developed in this work, data of more tissues was preferred to evaluate the model predictions for as much compartments as possible simultaneously.

Because the Black Sea harbour porpoise dataset was both restricted in time (animals were from 1997-1998) and space (animals from the Black Sea do not leave the Black Sea area), these results were preferably used to evaluate the very first preliminary harbour porpoise model predictions. These models were developed to explain the bioaccumulation of several PCB congeners (PCB 153, PCB 180, PCB 101, PCB 149, PCB 118, PCB 99, PCB 170) and PBDE congeners (PBDE 47, PBDE 99, PBDE 100 and PBDE 153) in male harbour porpoises. Model outputs showed that levels of all PCBs and PBDEs reached high levels at the end of lactation period (e.g. first year of life) after which the growth dilution effect and a change in diet caused a decline in concentrations followed by an increase in concentrations for the rest of the lives of the Black Sea harbour porpoises. The models

were then applied to assess temporal trends by using the dataset of harbour porpoises from the North Sea. During this modelling exercise, levels of PCBs and PBDEs were found to decrease from 1990 until 2008, although not at the same rate for all PCB and PBDE congeners. For some PCB congeners, the PBPK models were also used to test the metabolic biotransformation capacity for PCB 118, PCB 149 and PCB 101. Results suggested a fairly weak metabolic breakdown of PCB 118 and an enhanced capacity for metabolic breakdown of PCB 101 with higher age. In contrast, results were inconclusive about the metabolic capacities for PCB 149. So far, all attempts to estimate parameters were performed manually and the sensitivity of the parameters on the model output was tested by a 'one-at-a-time' or local sensitivity analysis. However, this type of sensitivity analysis ignores potential correlations between the parameters. Hence, more statistically sound parameter estimation methods and global sensitivity tests that take into account potential interactions between the parameters were needed in order to improve the robustness of the models.

Applying new methods for parameter estimation and sensitivity analyses was, thus, the next step. So, in the most recent PBPK model for bioaccumulation of pesticides (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD) in harbour porpoises, parameters were estimated using Bayes' theorem executed with Markov chain Monte Carlo (MCMC) simulations. In addition, the influence of changes in parameter values on the model output was tested using global sensitivity analyses. Compared to all previous PBPK models, this model for bioaccumulation of pesticides differed not only in the statistical techniques, but also in its complexity. Whereas all previous models showed the kinetics of a single compound, the pesticide model showed the kinetics of *p,p'*-DDT and its two metabolites *p,p'*-DDE and *p,p'*-DDD at the same time ensuring a high connectivity between the kinetics of these three compounds. Similar to the previous harbour porpoise models, the structural model was first evaluated using a dataset of harbour porpoises from the Black Sea after which the parameter range estimates were further optimized using the dataset of harbour porpoises from the North Sea.

The same techniques (Bayesian PBPK modelling and MCMC simulations) were also used for a PBPK model for the lifetime bioaccumulation of PCB 153 in long-finned pilot whales. For this species, two datasets were available from two mass stranding events. Long-finned pilot whales have tight family group bonds so whenever an individual ends up on the beach, all other members of the group follow. For the animals, these mass strandings are traumatic, but they are a great opportunity for monitoring and modelling as a dataset cannot possibly be more 'homogeneous'. In contrast to the dataset of the Black Sea harbour porpoises, only blubber samples were available for the long-finned pilot whales. The PBPK models for the bioaccumulation of PCB 153 in pilot whales are therefore smaller than the models in harbour porpoises. Nevertheless, parameters were estimated with the most suitable technique for this type of models, making the pilot whale model already a useful framework for evaluating similar or more elaborate datasets in the future.

This work provides new ideas and innovative approaches to study biomonitoring data. The bioaccumulation models developed here can already be used as a framework to compare to new datasets, but can also be further optimized and expanded in the future. These results are, therefore, not only a useful addition to existing knowledge, but provide also new perspectives to assess pollution and its effects in marine mammals. Such an integrated approach is required to set up guidelines for conservation of a species. As a result, the models developed in this work are undoubtedly useful tools for risk assessment purposes.