COMPUTATIONAL ECOTOXICOLOGY; MODELLING THE BIOMAGNIFICATION OF ORGANOCHLORINES AND BROMINATED FLAME RETARDANTS IN A BALTIC SEA FOOD WEB

Lundstedt-Enkel, Katrin[1,2] Lek, Per M.[3] Lundstedt, Torbjörn [3,4] and Örberg, Jan[1]

[1] Environmental Toxicology, Department of Physiology and Developmental Biology, Uppsala University, Norbyvägen 18A, SE-752 36, Sweden. email, katrin.lundstedt-enkel@ebc.uu.se
[2] Contaminant Research Group, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden.
[3] AcurePharma AB, Ulleråkersvägen 38, SE-756 43 Uppsala, Sweden.
[4] Division of Organic Pharmaceutical Chemistry, BMC, Uppsala University, P.O. Box 574, SE-751 23 Uppsala, Sweden

Introduction

Concentrations of contaminants in organisms in a food-web can increase with increasing trophic level and concentrations, especially in predators, can reach harmful levels. This increase in contaminant concentration from prey to predator is called biomagnification (for an overview see[1]). A biomagnification factor (BMF) is defined as the concentration of a contaminant in an animal divided with the concentration of that contaminant in its food and contaminants with BMF > 1 is said to biomagnify.

Here our research with developing a quantitative model, that we term QSBMR - Quantitative Structure Biomagnification Relationships, is presented. This model describes the relationship between the BMFs for several organochlorines (OCs) and brominated flame retardants (BFRs), *e.g.*, polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCD), and the contaminants' descriptors (*e.g.* physico-chemical properties and structural descriptors). We evaluated the model, in part by using multivariate design (MVD)[2, 3] by selecting a training set (50% of the contaminants), calculate a model and then predict the BMF for the rest of the contaminants that were not included in the training set.

Our ultimate aim is to use biomagnification models as an aid in risk assessments for *in silico* predictions of the biomagnification of new, not yet investigated compounds. The models would also facilitate a deeper understanding of mechanisms behind *e.g.* biomagnification and toxicity and increase the possibilities to develop environmentally friendly flame-retardants. I will present this project, published in several papers[4-7]

Materials and Methods

In order to perform the QSBMR modelling several preparatory studies had to be completed. The first was to determine concentrations for the OCs and BFRs in herring (*Clupea harengus*) muscle and guillemot (*Uria aalge*) egg from the Baltic Sea [4, 5, 8]. The second was to determine biomagnification factors (BMFs) with a new statistical method (randomly sampled ratios method) that denotes the BMFs with a measure of variation [7] and the third was to generate a wide variety of descriptors for the OCs and BFRs[6]. We hypothesized before starting this work that there were several descriptors in combination that determine the important events such as uptake, distribution, biotransformation and excretion of contaminants in organisms, events that together might lead to the biomagnification of a contaminant. For data mining purposes we therefore wanted to include many descriptors in the QSBMR. We have used several different approaches to generate a wide range of diverse descriptors: *a*, by using the software TSAR[9] and logP; *b*, finding $\log K_{ow}$ values from the literature, and *c*, by creating binary fingerprint variables that described the position of the halogens for the respective PCB and PBDE molecules. Partial least squares (PLS) regression[10] was used to model the relationship between the contaminants' BMF and

their descriptors. For validation, a training set of 7 contaminants was selected by multivariate design (MVD)[2] and a model was established. This model was then used to predict the BMFs of the test set (7 contaminants not included in the model). The resulting R^2 for the regression Observed BMF *versus* Predicted BMF was high (0.65). The good models showed that descriptors important for the biomagnification of OCs and BFRs had been used.

Results and Discussion

The calculated biomagnification factors (BMFs) showed a large span, *e.g.* PCB 101 and *p,p*'DDT that had BMFs below one, to PCB118 with a BMF of 42.5 (22.3-80.8) (as mean, mean±SD). The large variation (±SD) is also clear and shows the importance of using large numbers of sampled animals when calculating BMFs as well as the advantage of the novel method, the Randomly Samples Ratio method[7]. Noteworthy is that all analysed BFRs biomagnify, *e.g.* HBCD with the highest BMF (for the BFRs) of 9.1 (5.1-16.1).

The model including all contaminants (R^2X= 0.73, R^2Y= 0.87 and Q^2= 0.63, RMSEE= 5.73, three components) show high predictive ability (the R^2Y) meaning that descriptors important for the biomagnification are included in the modelling. The picture is the same when modelling the PCBs separately (R^2X= 0.83, R^2Y= 0.87 and Q^2= 0.58, RMSEE= 6.88, two components) (Figure 1) as with the BFRs separately (R^2X= 0.68, R^2Y= 0.88 and Q^2= 0.41, RMSEE= 1.04, two components).

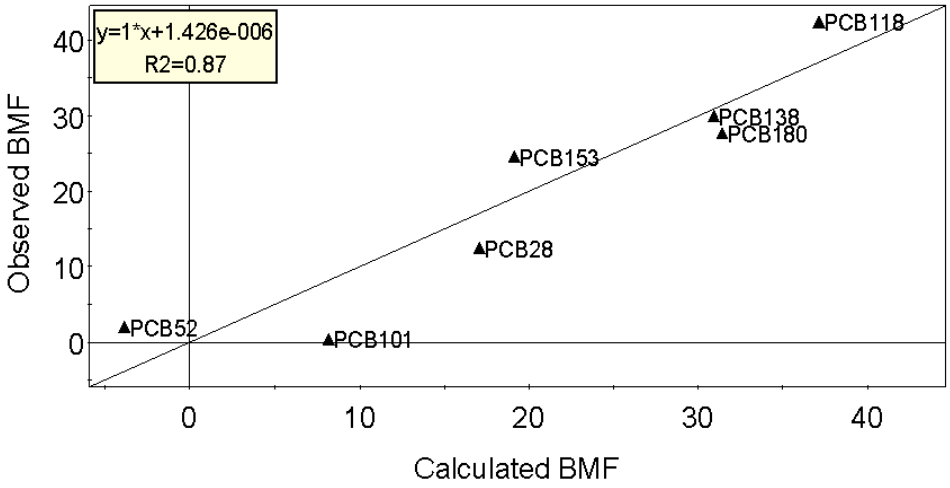

Figure 1. OBS/PRED plot showing observed biomagnification factors (BMF) *vs* calculated BMF based on a PLS model (R^2X= 0.83, R^2Y= 0.87 and Q^2= 0.58, RMSEE= 6.88, two components) of PCBs biomagnification factors (BMF) *versus* their descriptors.

To be a candidate for biomagnification a substance has to be bioavailable and not easily eliminated. The models presented here include important descriptors that in combination determine properties that lead to the biomagnification of a contaminant. The resulting QSBMR revealed that more than 20 descriptors *in combination* were important for the biomagnification of OCs and BFRs between herring muscle and guillemot egg. Descriptors that were either *positively correlated* with BMF *e.g.* EV (Ellipsoidal Volume), DMX (Dipole Moment X Component (Whole Molecule)), Halogens in *meta-para* position and the Number of halogens, or *negatively correlated* with BMF *e.g.* VHF (Heat of formation), VTE (Total Energy), HA (Number of H-bond acceptors), DMZ (Dipole Moment Z Component (Whole Molecule)) LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) [6]. Most interestingly, the $\log K_{ow}$ of the contaminants did not show any significant correlation to the BMF (Pearson, p=0.62, R^2= 0.01).

We discuss that the PCBs and PBDEs included in the Swedish monitoring program span a limited chemical variation, compared to what is possible for these compound classes. Therefore, a complimentary set of compounds is suggested to be included in future studies (Figure 2). These models will be useful for *in silico* predictions of the biomagnification of new, not yet investigated, compounds as an aid in risk assessments. The models will facilitate a deeper understanding of mechanisms behind *e.g.* biomagnification and toxicity and increase the possibilities to develop environmentally friendly chemicals such as flame retardants.

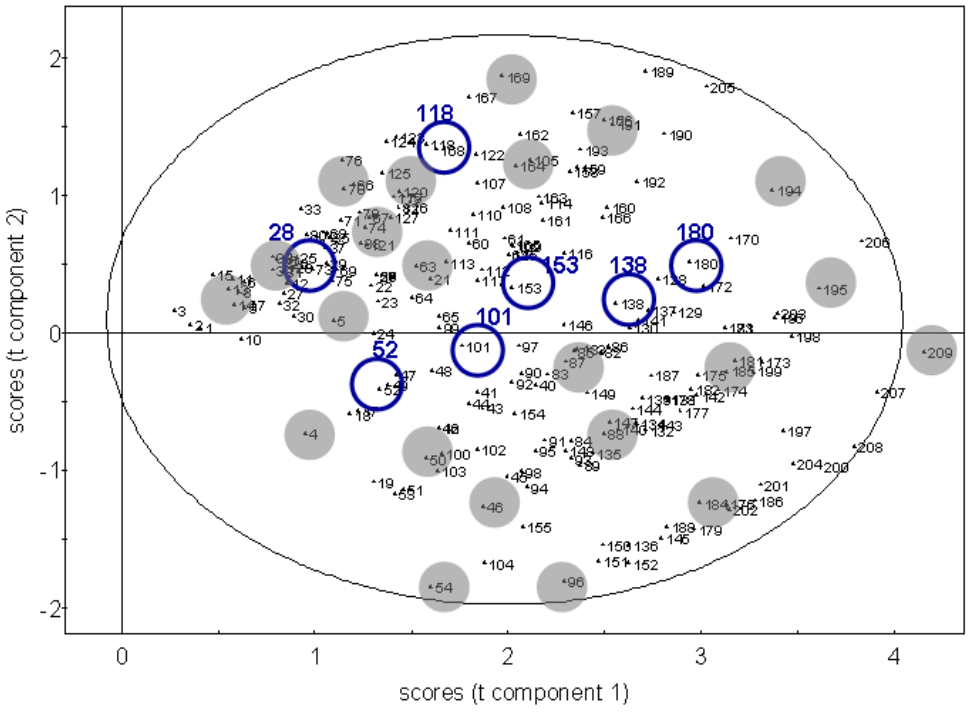

Figure 2. Score plot from a principal component analysis with PCB/PBDEs' binary fingerprint variables as X. Numbers encircled with an open ring are the PCB congeners that today are included in the Swedish monitoring programme[8]. The congeners marked with grey dots are complimenting ones, selected by us in order to span the variation.

Acknowledgements
We wish to thank: The Foundation for Strategic Environmental Research, MISTRA; The Swedish Environmental Protection Agency (EPA); The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas); The staff at the Contaminant research group at the Swedish Museum of Natural History that run the monitoring programme.

References

1. Gray, J. S., Biomagnification in marine systems: the perspective of an ecologist. *Mar. Pollut. Bull.* **2002,** *45*, (1-12), 46-52.
2. Wold, S.; Sjöström, M.; Carlson, R.; Lundstedt, T.; Hellberg, S.; Skagerberg, B.; Wikström, C.; Öhman, J., Multivariate design. *Anal. Chim. Acta* **1986,** *191*, 17.
3. Carlson, R.; Lundstedt, T.; Albano, C., Screening of suitable solvents for organic Synthesis. Strategies for solvent Selection. *Acta Chem. Scand. B* **1984,** *39*, 79-91.
4. Lundstedt-Enkel, K.; Asplund, L.; Nylund, K.; Bignert, A.; Tysklind, M.; Olsson, M.; Orberg, J., Multivariate data analysis of organochlorines and brominated flame retardants in Baltic Sea guillemot (*Uria aalge*) egg and muscle. *Chemosphere* **2006,** *65*, (9), 1591-1599.
5. Lundstedt-Enkel, K.; Johansson, A. K.; Tysklind, M.; Asplund, L.; Nylund, K.; Olsson, M.; Örberg, J., Multivariate data analyses of chlorinated and brominated contaminants and biological characteristics in adult guillemot (*Uria aalge*) from the Baltic Sea. *Environ. Sci. Technol.* **2005,** *39*, (22), 8630-8637.
6. Lundstedt-Enkel, K.; Lek, P. M.; Lundstedt, T.; Örberg, J., QSBMR - Quantitative structure biomagnification relationships: Physicochemical and structural descriptors important for the biomagnification of organochlorines and brominated flame retardants. *J. Chemometrics* **2006,** *20*, (8-10), 392-401.
7. Lundstedt-Enkel, K.; Tysklind, M.; Trygg, J.; Schuller, P.; Asplund, L.; Eriksson, U.; Häggberg, L.; Odsjö, T.; Hjelmberg, M.; Olsson, M.; Örberg, J., A statistical resampling method to calculate biomagnification factors exemplified with organochlorine data from herring (*Clupea harengus*) muscle and guillemot (*Uria aalge*) egg from the Baltic sea. *Environ. Sci. Technol.* **2005,** *39*, (21), 8395-402.
8. Bignert, A.; Nyberg, E.; Danielsson, S.; Asplund, L.; Eriksson, U.; Berger, U.; Wilander, A. Comments concerning the national Swedish contaminant monitoring programme in marine biota, 2007. http://www.nrm.se/download/18.5a9ee81411905b544f680001317/Marina+programmet+2007.pdf (accessed April 2008),
9. TSAR *Accelrys Software Inc. (Previous Oxford Molecular Ltd.)*.
10. Wold, S., Personal memories of the early PLS development. *Chemom. Intell. Lab. Syst.* **2001,** *58*, (2), 83-84.