RELATIONSHIPS BETWEEN PHYSICOCHEMICAL AND STRUCTURAL DESCRIPTORS AND BIOMAGNIFICATION OF ORGANOCHLORINES AND BROMINATED FLAME RETARDANTS

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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 Gray 2002). 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, 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) (Carlsson *et al.* 1984) to select a training set (50% of the contaminants), calculate a model and then predict the BMF for the contaminants that were not included in the training set.

The ultimate aim is to use these models for *in silico* predictions of the biomagnification of new, not yet investigated, compounds as an aid in risk assessments. The models would facilitate a deeper understanding of mechanisms behind *e.g.* biomagnification and toxicity and *e.g.* increase the possibilities to develop environmentally friendly flame-retardants.

Materials and Methods

In order to perform the QSBMR modeling 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 (Lundstedt-Enkel *et al*. 2005a, 2005b; Bignert *et al.* 2005; Lundstedt-Enkel *et al.* 2006). 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 (Lundstedt-Enkel et al. 2005a). The obtained BMFs are summarized in Table 1. The third part was to generate a wide variety of descriptors for the OCs and BFRs. 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); *b*, finding $\log K_{ow}$ values from the literature, and *c*, creating binary fingerprint variables that described the position of the halogens for the respective PCB and PBDE molecules. Partial least squares (PLS) regression (Wold 2001) was used to model the relationship between the contaminants' BMF and the descriptors.

For validation, a training set of 7 contaminants was selected by multivariate design (MVD) 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 are shown in Table 1 where it can be seen that there is a large span of BMFs from *p,p'*DDE that biomagnifies almost 60 times to CB101 that do not biomagnify, with lower concentration of CB101 in guillemot than in herring. The large variation shown (as –SD and +SD) is also clear and show the importance of using large numbers of sampled animals when calculating BMFs. Noteworthy is that all BFRs biomagnify, *e.g.* HBCD approximately 9 times.

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) as with only the BFRs (R^2X= 0.68, R^2Y= 0.88 and Q^2= 0.41, RMSEE=1.04, two components) (Figure 1).

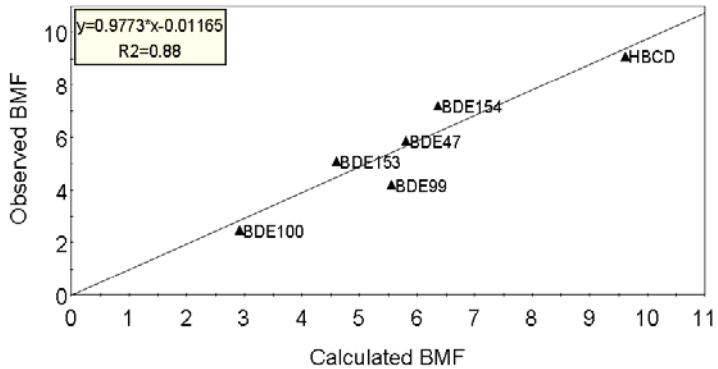

Figure 1. OBS/PRED plot showing observed biomagnification factor (BMF) *vs* calculated BMF based on a PLS model (R^2X= 0.68, R^2Y= 0.88 and Q^2= 0.41, RMSEE=1.04, two components) between brominated flame retardants' BMF and their descriptors.

Table 1. Biomagnification factors (BMF) calculated with the RSR method for contaminants in herring (*Clupea harengus*) muscle (*n*= 72) and guillemot (*Uria aalge*) egg (*n*= 30), collected in the Baltic Sea.

	BMF [1]	-SD [2]	+SD [3]	BM [4]
*p,p'*DDE	57.1	28.8	113.1	yes
CB118	42.5	22.3	80.8	yes
ΣDDT	36	19.4	66.9	yes
HCB	33.8	19.3	59.2	yes
CB138	30	15.1	59.4	yes
CB180	27.8	12.3	62.6	yes
CB153	24.7	12.1	50.4	yes
ΣPCB [5]	24.6	12.5	48.4	yes
βHCH	18.6	12.7	27.3	yes
CB28	12.5	7.2	21.8	yes
HBCD	9.1	5.1	16.1	yes
BDE154 [6]	7.2	4.5	11.6	yes
BDE47	5.9	2.6	13.4	yes
ΣPBDE [7]	5.5	2.6	11.7	yes
BDE153	5.1	2.3	11.6	yes
BDE99	4.2	1.9	9.2	yes
BDE100 [6]	2.5	1.3	4.8	yes
γHCH [8]	2.1	1.82	2.4	-
CB52 [8]	2	0.94	4.1	no
αHCH [8]	1.4	1.1	1.8	no
*p,p'*DDT [8]	0.93	0.48	1.82	no
CB101 [8]	0.46	0.25	0.84	no
*p,p'*DDD [8]	0.42	0.22	0.79	no

[1] The animal material for OCs was collected the years 1996, 1997 and 1999 and for BFRs the years 2000, 2001 and 2002.
[2] BMF=BMF_{RSR}; calculated with the "randomly sampled ratios" (RSR) method using 50000 iterations resulting in geometric mean (GM) and GMSD.
[3] -SD; lower limit (calculated as BMF_{RSR} / GMSD) and +SD; upper limit (as BMF_{RSR} * GMSD).
[4] BM= Biomagnification; yes= BM of contaminant with BMF_{RSR} significantly ($p<0.05$) higher than 1, no= no BM, BMF_{RSR} not significantly higher than 1.
[5] ΣPCB = sum of ICES 7 marker PCBs: IUPAC no. CB28, CB52, CB101, CB118, CB138, CB153 and CB180
[6] n= 20 for BDE 154 and BDE100.
[7] ΣPBDE = sum of BDE congeners no. BDE47, BDE99, BDE100, BDE153 and BDE154.
[8] The level of quantification was used as the concentration in guillemot.

To be a candidate for biomagnification a substance has to be bioavailable and not easily eliminated. The models 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.

We found 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 (Lundstedt-Enkel *et al.* 2007).

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 flame-retardants.

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