Determination and Modelling of Biomagnification Factors for Polychlorinated Naphthalenes (PCNs) in Salmon (*Salmon salar*)

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Introduction

Polychlorinated naphthalenes (PCNs) have been identified as a group of persistent substances and the environmental concentrations of these compounds have gained increasing interest during the last years. PCNs are found in biological and abiotic samples from basically all compartments of the environment (1). In many cases the isomeric patterns and congener profiles identified in most abiotic samples, resemble technical PCN mixtures. However, in biota a structure-dependent retention of specific PCNs is found (2,3). In this project the possibilities to calculate quantitative structure-activity relationships (QSARs) for biomagnification of PCNs in salmon were investigated.

Material and Methods

Exposure. Fry of salmon *(Salmon salar)* were exposed to PCNs via contaminated food. The fish were divided into five different groups living in separate aquariums and exposed to different doses. The food was contaminated with a mixture of three Halowaxes (Halowax 1001, 1014, and 1051) (Koppers Co., USA) in equal amounts (based on weight) in four different doses, viz. 0.1 μg, 1 μg, 2 μg, and 10 μg PCN/g food, respectively. One control group was fed uncontaminated food. During the study, fish samples were collected at three different occasions, after 8 weeks (sampling A), 13 weeks (sampling B) and 17 weeks (sampling C). After collection of samples the fish were killed, weighed and stored at -20°C until cleanup and analysis.

Cleanup and analysis. Five fish from each dose group and sampling occasion were pooled, homogenised with anhydrous sodium sulphate (5 times the wet weight of the sample) and packed in glass columns with little sodium sulphate on the top. Prior to lipid extraction ^{13}C-labelled chlorinated dibenzofurans were added to the column as an internal standard. The extraction was performed with 80-90 mL of acetone/n-hexane (5:2) and n-hexane/ diethylether (9:1). The lipid contents were determined gravimetrically. The lipid samples were dissolved in a mixture of n-hexane and methylene chloride (50:50) and added to a Florisil column for clean up (8 g Florisil gel with 2 g Na_2SO_4 on the top). The samples were eluted with 80 mL n-hexane/

methylene chloride (50:50) and then left to vaporise after addition of 25 μL tetradecane. Two ^{13}C-labelled dibenzofurans were added as recovery standards to each sample and the samples analysed using GC/MS (Fisons MD800) using a DB5 fused silica capillary column (60m x 0.32 mm, film thickness 0.25 μm). Injections of 2 μL were made using the splitless mode with helium as carrier gas. Identification of the different congeners was done using the retention order for PCNs in Halowaxes on a DB5 capillary column determined by Takasuga et al. (4). The numbering of the individual congeners was done according to Wiedmann and Ballschmiter (5). Quantification standard, internal and recovery standards was used to quantify the samples and estimate recoveries for the method applied. The concentrations of PCNs in the food were determined according to the same method as the fish samples. Biomagnification factors, BMFs, were calculated for all single eluting PCN congeners in fish for the dose 2 μg total-PCN/g food. BMF was defined as conc. in fish (ng/g lipids)/conc. in food (ng/g lipids).

Data interpretation and calculation QSARs. Principal component analysis (PCA) and partial least square (PLS) modelling were used for the interpretation of the data and to calculate QSARs. PCA, which is a multivariate projection method, combines a large number of variables into a few underlying descriptive dimensions, which summarise the systematic variation and give an overview of the dominant patterns in the data. PLS operates in much the same way as PCA but simultaneously model two data matrices in order to calculate the relation between the two sets of data. In this study the relation between physico-chemical parameters reported by Tysklind et al. (6) and BMFs was investigated. Both PCA and PLS were performed with the SIMCA-S 6.01 software package (Umetri AB, Umeå, Sweden).

Results and Discussion

The levels of PCNs in the salmon increased in a dose dependent manner. In the exposed fish, the penta-, hexa-, and heptachlorinated PCNs were dominating, as compared to the Halowax mixture (and the food), which was dominated by tri- and octachlorinated congeners. To evaluate the PCN patterns in the food and exposed fish, a PCA model was calculated. Prior to the calculations the data set was normalised to the total PCN concentration and thus the data resembles the relative differences in PCN composition in food and in the fish after 8, 13 and 17 weeks of exposure, respectively. This two-dimensional model explained as much as 91% of the variance in the data. The score plot (Figure 1A) shows the relation between the food and fish samples. The first principal component (t1) separates the food sample from the three fish samples, and the second PC (t2) separates the different fish samples from each other (8 week exposure (A4) in the upper left corner of the plot, followed by the 13 (B4) and 17 (C4) week exposure samples). Figure 1B shows the corresponding loading plot, which reveals the PCN congeners of importance for the separation in score-plot. The triCNs as well as octaCN are dominating in the food and thus all these congeners show up to the right in the loading plot. Moving from the food to the fish sample taken after 8 weeks (A4) the influence from the tetra- and pentaCNs increase. Further, the separation of sample A4 (8 weeks) to C4 (17 weeks of exposure), can be seen in the shift of congeners within the penta, hexa and hepta homologue groups. Characteristic for sample C4 is a group congeners which retain in the fish, e.g. 1,2,3,7-TeCN(#42), 1,2,3,4,6,7-/1,2,3,5,6,7-HeCN(#66/67), 1,2,3,5,7,8-HxCN(#69),and 1,2,4,5,6,8-/1,2,4,5,7,8-HxCN(#71/72), and thus expected high biomagnification factors.

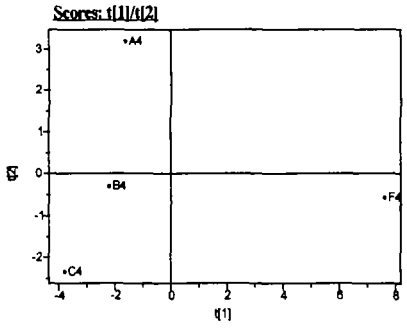

Figure 1A. Score plot of PC1 versus PC2 based on the normalised PCN concentrations in food and fish exposed to dose 2μg PCN/g food.

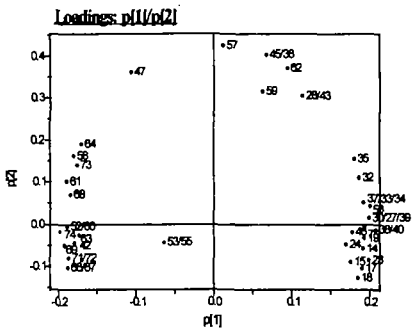

Figure 1B. Loading plot of loading vector 1 versus 2. For the numbering of individual congeners, see Wiedmann and Ballschmiter (5)

QSARs for BMFs. QSARs were established for modelling of BMFs for 26 (on the DB5 GC column) single-eluting tri- to hepta PCN congeners for the 2 μg PCN/g food dose group. In the PLS model we used 29 physico-chemical variables for the structural description (6). A one-dimensional PLS model explained 69 % (R^2) of the variation in BMF, with a cross-validated explained variance (Q^2) of 64%. Figure 2 shows the relation between the physico-chemical variables (X) and BMFs as the independent variable (Y) expressed in the score vectors of X and Y, respectively. Important physico-chemical variables in the model were related to size and electronic/substitution related properties. The most important variables were the absolute hardness, ionisation potential, heat of formation and molecular weight. Within each homologue group, large variations in BMF were observed which also can be seen in Figure 2, e.g. the TeCNs 1,2,4,5 (#32), 1,3,5,7 (#42), and 1,4,6,7 (#47).

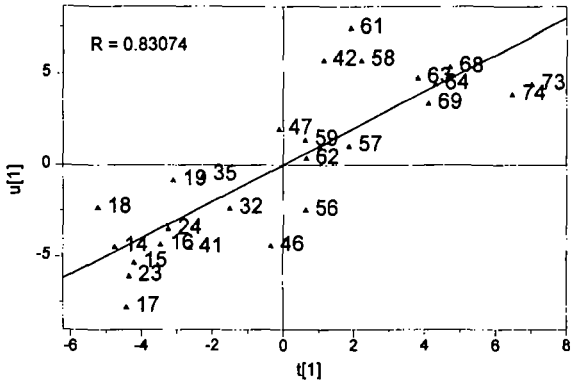

Figure 2. The PLS score vectors t1 vs. u1, showing the correlation between the physico-chemical data (t1) and BMF-factors (u1).

To increase the resolution of the QSAR models, specific QSARs for tri-, tetra-, and penta-CNs were calculated. These models explained 96%, 90%, and 87% of the variation in BMF, respectively. In summary, the chemical factors determining the differences in BMFs can be divided into a lipophilic size influenced factor which explains roughly 2/3 the BMF and a second substitution dependent factor explaining the remaining 1/3 of the variation. The relative importance of the two factors depends on the degree of chlorination and for e.g. the tetraCNs and pentaCNs, which show large chemical variation, the substitution dependent factor is dominating. The QSAR models were used for prediction of other not included (co-eluting or not present) PCN congeners. Table 1 shows the 16 congeners with the highest measured or predicted BMF. Among these, several have been found in high concentrations in biota (2).

Table 1. Observed and predicted BMFs for the 16 PCN congeners showing the highest biomagnification potential.

Congener (no)	BMF	Congener (no)	BMF
1,3,5,7-TeCN (#42)	1.4[a]	1,2,3,5,6,7-HxCN (#67)	1.5[b]
1,3,6,7-TeCN (#44)	0.73[b]	1,2,3,5,6,8-HxCN (#68)	1.0[b]
2,3,6,7-TeCN (#48)	0.73[b]	1,2,3,5,7,8-HxCN (#69)	1.2[a]
1,2,4,5,7-PeCN (#58)	1.4[a]	1,2,3,6,7,8-HxCN (#70)	0.91[b]
1,2,4,6,8-PeCN (#61)	2.5[a]	1,2,4,5,6,8-HxCN (#71)	0.97[b]
1,2,3,4,5,7-HxCN (#64)	0.96[b]	1,2,4,5,7,8-HxCN (#72)	0.78[b]
1,2,3,4,5,8-HxCN (#65)	1.0[a]	1,2,3,4,5,6,7-HpCN (#73)	0.90[a]
1,2,3,4,6,7-HxCN (#66)	1.2[b]	1,2,3,4,5,6,8-HpCN (#74)	0.75[a]

[a] observed, [b] predicted

Notable is that several of these highly bioaccumulative congeners are substituted in the 2,3,6,7-positions and thus can be expected to exhibit dioxin-like properties. Toxicological studies have also shown that lateral substituted PCNs induce ethoxyresorufin-*O*-deethylase (EROD) activity (7), which indicate dioxin-like toxicity. Some of the congeners which were identified as having high biomagnification factors, e.g. PCN#48 and #70 (see Table 1), are not present in Halowax mixtures, however they are found in fly ash from different incineration plants (8,9). Hence, the toxicological relevance of the PCNs should be further investigated.

Acknowledgements

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References

1.Crookes M J, Howe P D, Environmental hazard assessment: Halogenated naphthalenes. Report TSD/13, **1993**, Department of the Environment, London, Great Britain.
2. Falandysz J, Rappe C; *Environ. Sci.Technol.* **1996**, 30, 3362.
3. Harner T, Bidleman T F; *Atmos.Environ.* **1997**, 31(23), 4006.
4. Takasuga T, Inoue E O, Ireland P; *Organohal. Comp.* **1994**, 19, 177.
5. Wiedmann T, Ballschmiter K; *Fresen. Anal. Chem.* **1993**, 346, 800.
6. Tysklind M, Elg K, Andersson P, van Bavel B, Haglund P; *Organohal. Comp.* **1996**, 28, 545.
7. Hanberg A, Waern F, Asplund L, Haglund E, Safe S; *Chemosphere* **1990**, 20(7-9), 1161.
8. Adab, E, Caixach J, Rivera J; *Organohal. Comp.* **1997**, 32, 403.
9. Imagawa T, Yamashita N;*Organohal. Comp.* **1994**, 19, 215.