Biomagnification Factors of 20 Selected PCBs after oral exposure in Zebrafish (*Danio rerio*)

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Introduction

The polychlorinated biphenyls (PCBs) are widely spread in the environment and a large number of congeners have been found in biota. The uptake of PCBs for aquatic species is the sum of magnification from the food and concentration from the water phase. For fish the uptake from the food is considered as the major pathway for such lipophilic compounds as the PCBs (1). The biomagnification potential of a specific compound is the ratio of uptake from the food and the clearance. The substitution pattern of the individual PCBs determines their environmental behaviour due to different physico-chemical features. For example, the toxicity of the PCBs, measured as the potency to induce different cytochrome P450 isoenzymes, as well as the metabolism and excretion of the compounds primarily relies on the substituents in the *meta* and *para* positions (2,3). In order to access structure-specific features of the PCBs, in this case related to the biomagnification, the present study has applied a set of PCBs, which is selected to represent the diversity in substitution pattern of the compound group. We have recently determined biomagnification factors of the PCBs after oral exposure to three-spined sticklebacks (*Gasterosteus aculeatus*) (4) and present here a similar study for zebrafish (*Danio rerio*). The present study also includes the impact on reproduction, histological changes and the ability of the PCB mixture to induce hepatic ethoxyresorufin *O*-deethylase (EROD) activity, which is presented elsewhere (5).

Material and Methods

By using factorial design in combination with principal component analysis, 20 PCBs have been selected to represent the 154 tetra to hepta chlorinated biphenyls (6). The 20 PCBs were dissolved in isooctane and mixed with freeze-dried chironomids to prepare three different dose levels. The PCBs, viz. PCB#41, #51, #58, #60, #68, #78, #91, #99, #104, #112, #115, #126, #143, #153, #169, #173, #184, #188, #190, and #193, were purchased from Accustandard, New Haven, CT, USA. After preparing the food in concentrations of 0.008, 0.08, and 0.4 μg of each congener per gram food, the solvent was removed in a rotary evaporator at 50°C. The food of the controls was prepared with isooctane only. The zebrafish were fed with the chironomids at about 2% of their

body weight per day over the experimental period. The weight of the fish at the start of the experiment was 150-200 mg.

Sampling of the fish for biomagnification, biochemical and histological studies was carried out after 4 and 13 weeks of exposure, except the reproduction studies, which were initiated after 9 weeks. At each sampling occasion 10 females were randomly selected, pooled and homogenised with Na_2SO_4, after excluding livers and gonads, which were dissected for histological and biochemical analyses. After extraction of the lipids by acetone:hexane (5:2) and hexane: diethylether (9:1), the amount of lipids was gravimetrically determined. The lipids were then removed by dialysis with cyclohexane, followed by a florisil-gel open column and a 40% H_2SO_4 prepared silica column. The internal standards PCB#50 and #189 were added before the extraction procedure and the recovery standard #199 after the silica column. The analyses of the PCBs were performed on a HRGC/LRMS (Fisons GC 8000/Fisons MD 800) using selected ion recording. A non-polar capillary column (J&W DB-5, Folsom, CA, USA) was used. The PCBs were quantified by calculating the relative response factor of each native compound against the internal standard in the standard calibration solution, which contained one congener of each homologue group. The biomagnification factors (BMFs) were calculated by dividing the PCB concentration in the fish by the concentration in the chironomids, both on lipid basis.

Results and Discussion

Zebrafish were exposed orally to a mixture of 20 selected PCBs in three dose groups for 4 and 13 weeks, respectively. The present paper consider the two lowest concentrations tested, viz. 0.008 and 0.08 μg of each congener/gram food. Generally, a dose dependent increase over time was seen resulting in the total PCB concentrations on lipid basis; 1.4 μg/g and 2.6 μg/g on lipid basis for the low dose after 4 weeks (ld4) and 13 weeks (ld13), respectively, and 9.7 μg/g and 25.9 μg/g for the intermediate dose groups (id4 and id13), respectively. The control fish contained only minor amounts of PCB#153, generally less than 10%. To determine the uptake of the PCBs over time and to verify if a steady state was reached during the experimental period, the relative change from ld4 to ld13 was calculated. All congeners, except PCB#51, #78, #104, and #143, showed increasing BMFs and PCB#115, #126, #173, #190, and #193, increased more than 100% from week 4 to 13. Hence, a steady state was not reached or could not be confirmed and the BMFs presented are to be considered as time specific. Displayed in Figure 1 are the calculated BMFs for all congeners, except the coeluting isomers PCB#41 and #68. The BMFs increase over the time for both levels and are constantly higher in the low dose group for all congeners. A weak increase of the BMFs can be seen as the number of chlorine atoms increases, but other structural characteristics of the PCBs seem to be stronger determinants of the BMFs. PCB#153, #190, and #193 magnified most and PCB#51, #78, #104, #143, and #184 were found in very low concentrations or below the detection limit. Notably, the non-*ortho* substituted PCB#126 and #169 have moderate BMFs if compared in each group of homologues. This is in accordance with earlier findings and may be related to a lower bioavailability due to adsorption to the food (4,7). In Figure 2, the molecular structures of the congeners of BMFs <0.5 and BMFs >1.5 are shown. The congeners of low biomagnification have, with the exception of PCB#184, adjacent unsubstituted *meta* and *para* positions, which is known to facilitate metabolism of the PCBs. Accordingly, the congeners of BMFs >1.5 have no open positions for metabolism, with the exception of PCB#173. The substituents in the *meta* and *para* positions seem to be the most

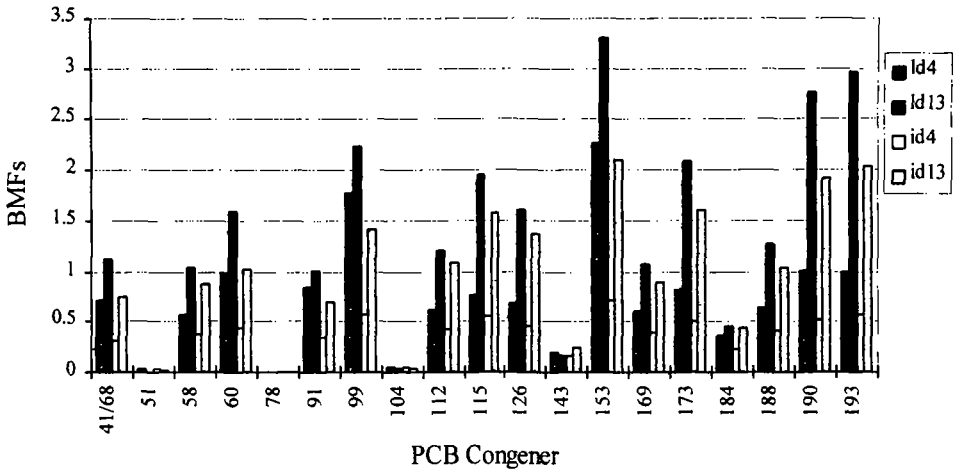

Figure 1. The BMFs of the PCBs for the low- (ld4, ld 13) and the intermediate dose (id4, id13).

important structural feature of the PCBs, determining the BMFs in zebrafish. Congeners having a different number of chlorine atoms as well as different number of substituents in the *ortho* position are represented across the two groups presented in Figure 2.

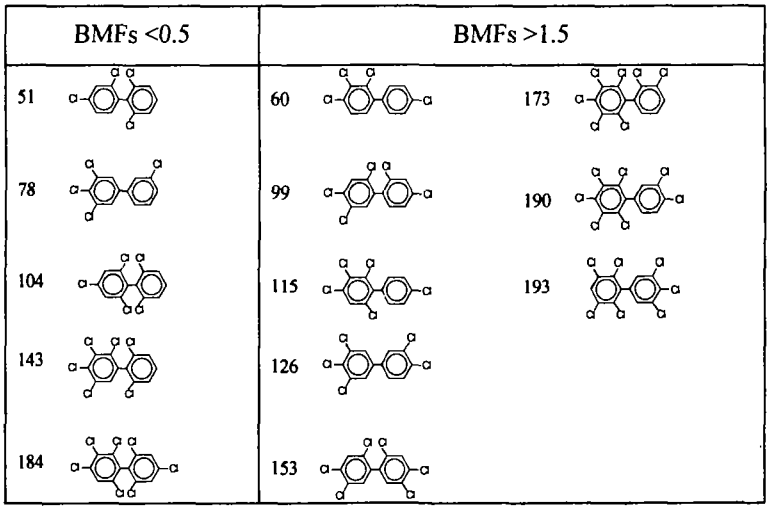

Figure 2. The molecular structure of the PCBs with BMFs <0.5 and >1.5 in ld13.

The results are compared with BMFs obtained in a similar project using three-spined sticklebacks fed with the same food (4). In Figure 3 the BMFs of the three-spined sticklebacks versus the zebrafish are plotted for the low dose at 12 and 13 weeks of exposure, respectively. The BMFs of

the zebrafish are notably lower and with the exception of PCB#173, #184, and #188, the correlation is fairly good (r^2=0.83). The levels found of especially PCB#173 and #184 in zebrafish are unexpected and disagree with known structure-specific metabolism.

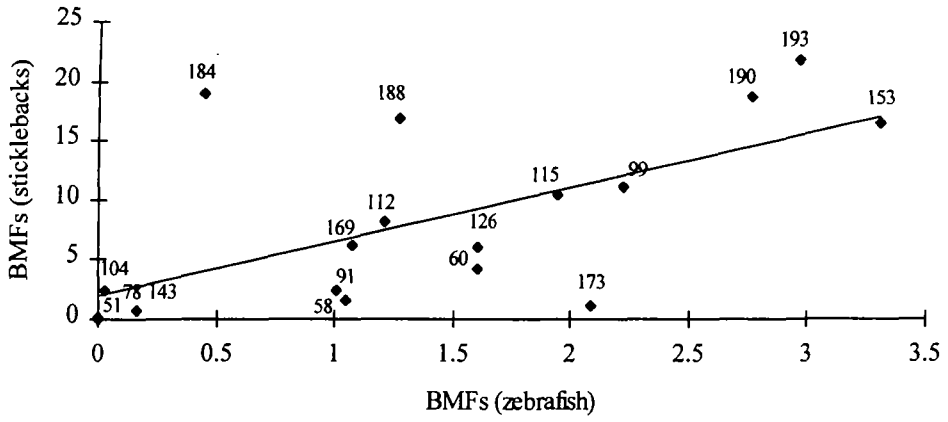

Figure 3. The BMFs of the three-spined sticklebacks (4) versus the BMFs of the zebrafish.

In conclusion, a structure-specific biomagnification pattern was shown in zebrafish, but the calculated BMFs were considerably lower than the BMFs for three-spined sticklebacks exposed to the same PCBs. The BMFs of the zebrafish were comparable to those found for sticklebacks exposed to higher concentrations. This might be explained by, e.g. toxic stress, differences in metabolic system or growth. For both dose groups, the hepatic EROD activity was induced after 13 weeks of exposure. In addition, the reproduction studies performed after 9 weeks showed a dose dependent reduction in number of deposited eggs/female, which is in accordance with the histological changes observed in the oocytes (5). The substitution pattern crucial for a low biomagnification is correlated to adjacent unsubstituted *meta* and *para* positions. This indicates a metabolic activity in the zebrafish with a favoured substitution pattern governing excretion in fish of these compounds.

Acknowledgement

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