ACCUMULATION OF PCB METABOLITES IN SERUM OF SEABASS (*Dicentrarchus labrax*)

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1. INTRODUCTION

Hydroxylated (OH-) polychlorinated biphenyls (PCBs) are derived as metabolites from CYP-mediated Phase I metabolic processes[1]. Metabolic biotransformation is a factor in the bioaccumulation and fate of highly chlorinated PCB congeners that are generally more recalcitrant in fish. OH-PCBs have emerged as important classes of environmental contaminants in wildlife and humans. They are not lipophillic and have not been found to accumulate in fatty tissues. However, it was discovered that some OH-PCBs accumulated in plasma because they bind efficiently to the thyroxine-transport protein[2]. Fish tissue exposure to circulating OH-PCBs is variable, and thus potential OH-PCB-mediated toxicological activity and effects (e.g., endocrine-related) and health risks are different among the fish species[3]. These compounds were found in marine and some terrestrial mammals, and in recent years several authors found OH-PCBs in fish species serum, that range from 1 to 130% of the PCB levels in the serum[4]. This study evaluates the presence of six OH-PCBs (4OH-CB107, 4OH-CB146, 3OH-CB138, 4OH-CB187, 3OH-CB180, and 4OH-CB172) in seabass (*Dicentrarchus labrax*) serum and its relation with the accumulation of PCBs in fish tissues, when exposed to highly contaminated food for a short period of time.

2. MATERIALS AND METHODS

2.1 Laboratory experiment

Seabass was studied for PCB congener's accumulation and elimination, after being fed with food highly contaminated with CBs 18, 44, 49, 52, 101, 105, 118, 138, 180 and 187. Similar concentration values of CB's were used for all studied congeners (1440 to 1750 ng g^{-1}). Specimens of approximately 15 and 250 g in weight were transferred to a 2000 L tank, with natural photoperiod, ambient temperature and aeration was provided to maintain 100% oxygen saturation in water. The water was continuously filtered through an extensive biological filter, and a charcoal filter before being recycled. The fish were exposed to a contaminated diet for 12 days, following a depuration period of 92 days for juveniles and 80 days for adults. The individuals were periodically sampled and the levels of PCBs were quantified in serum, liver and muscle. The levels of OH-PCBs were quantified in serum.

2.2 Analysis

Materials. The following materials were used for sample extraction and aliquot purification: n-hexane (Hx) distilled in the laboratory, hydrochloric acid, 2-propanol, Methyl-terbutyl-ethane (MTBE), ethanol, dichloromethane all p.a. (Merck), KCl, KOH, sodium sulphate – anhydrous (Merck), heated at 440 ºC overnight, Florisil – 60-100 mesh (Merck), activated at 440ºC overnight, and partially deactivated with 1% distilled water, and sulphuric acid p.a. (Merck). PCBs standard was obtained as a certified solution from AccuStandard Inc, (Q-CME-01) containing 40 individual congeners, and internal standards CB30, CB65 and CB204 from Promochem as crystals. OH-PCBs were obtained from Wellington laboratories as individual certified solutions.

Tissue analysis. The liver and muscle were freeze-dried and Soxhlet extracted with *n*-hexane. The method has been described previously in Antunes and Gil, (2004)[5]. Fat content was determined gravimetrically from aliquots of the extracts and the remaining extracts were cleaned with Florisil and sulphuric acid. Gas chromatography

using Electron capture detection was used for PCBs identification and quantification.

Serum analysis, for PCBs and OH-PCBs. Blood samples were collected from sedated fish and serum was separated by centrifugation. Serum was solvent extracted following Hovander et al. (2000)[6]. Serum (about 1.0000g) was transferred to a test tube. Recovery standards (CB30, 65, and 204) were added and mixed in a vortex mixer. For plasma denaturation HCl (6M, 3 mL) was added and vortex mixed, 2-propanol (3 mL) was added and the sample vortex mixed once more. Organic compounds were extracted adding Hx/MTBE (1:1, v/v, 5 mL) and sample gently mixed for 5 minutes. After centrifugation, the organic phase was transferred to a second test tube, containing aqueous KCl solution (1%, w/w, 4 mL). The denaturated plasma was re-extracted with Hx/MTBE (1:1, v/v, 3 mL) and the organic phase combined with the first extract. Potentially co-extracted aqueous compounds in extract were partitioned into KCl-solution by gently mixing. After centrifugation, the organic phase was transferred to a pre-weighted test tube. The aqueous solution was re-extracted with Hx/MTBE (1:1, v/v, 3 mL) which was combined with the extract. The solvent was gently evaporated in nitrogen flow and lipid content determined gravimetrically when constant weight was obtained. The extract, dissolved in Hx (4 mL), was partitioned with a KOH-solution (0.5 M in 50 % ethanol, 2 mL) and gently mixed, after centrifugation the organic phase was transferred to another test tube. The alkaline solution was re-extracted with Hx (3 mL) and the organic phases combined – this aliquot contains the neutral fraction. The alkaline solution was acidified with HCl (2M, 0.5 mL) and phenolic compounds were extracted with Hx/MTBE (9:1, 4 mL) and subsequently extracted once more with 3 mL of the same solution – this aliquot contains the phenolic fraction. Phenolic compounds were derivatized to their corresponding methyl ethers by addition of ethereal diazomethane (0.5 mL, 3 h) prior to any further steps. Ether and excess diazomethane were evaporated under a gentle nitrogen flow.
For lipid removals both fractions were concentrated to 1 mL, sulfuric acid (4 mL) was added and gently mixed. The Hx-phase was transferred to a new test tube, and the sulfuric acid repartitioned with Hx (1 mL).
After adjustment of volume both fractions were analyzed using GC-ECD using the same GC method as for PCB analysis in tissues. It also produced a good separation of phenolic compounds and allowed to confirm satisfactory partitioning between neutral and phenolic phases. Retention times were determined by pure standards injection, and quantification was performed using internal standard method. Detection limit was calculated as 0.1 ng g^{-1}, and quantification limit as 0.4 ng g^{-1}.The internal standard recoveries were between 60 and 105%.

3. RESULTS

The PCB levels in liver increased from 451 to 14398 ng g^{-1} lipids after 40 days in juveniles and from 184 to 14283 ng g^{-1} lipids after 12 days in adults. Levels in muscle increased from 579 to 6033 ng g^{-1} lipids at day 26 and from 201 to 9033 ng g^{-1} lipids at day 26, respectively, in smaller and larger seabass. In serum the maximum levels were coincident with the final of exposure, with maximum of 6945 ng g^{-1} lipids in juveniles and 6474 ng g^{-1} lipids in adults (Figure 1).

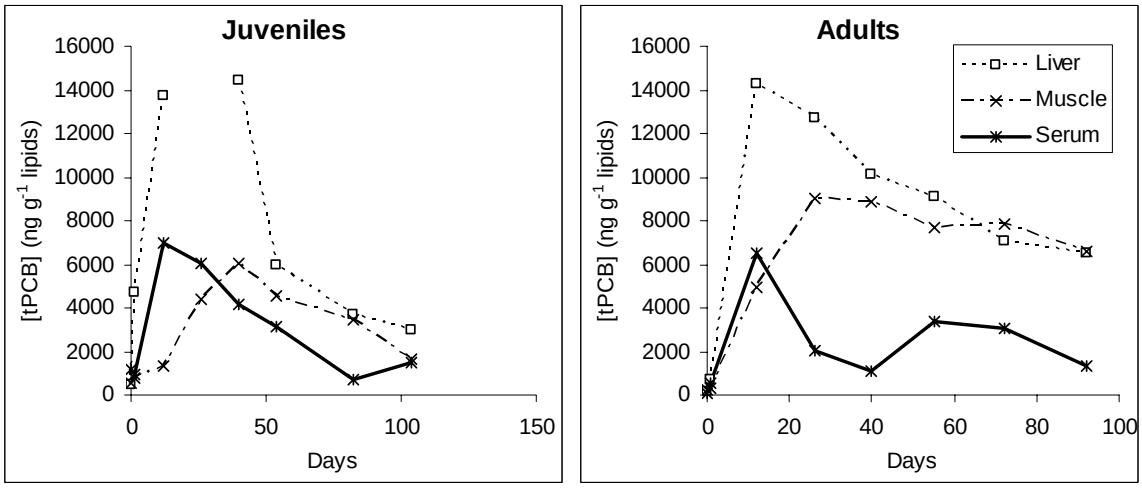

Figure 1. Evolution of PCB levels in liver, muscle and serum over the experiment, for juvenile and adult seabass.

In our study, most of the samples presented values of the investigated OH-PCBs below detection limit. But in the period of higher contamination and consequently higher elimination of compounds it was possible to identify and sometimes quantify these metabolites (Table 1). The values of OH-PCBs reached a maximum of 0.05% of PCBs in juveniles and 0.6% of PCBs in adults.

Table 1: Levels of OH-PCBs in serum (ng g^{-1}.lipids) of seabass during the experiment. Italic denotes values between LOD (0.1 ng g^{-1} lipids) and LOQ (0.4 ng g^{-1} lipids).

	Juveniles								Adults							
days	0	1	12	26	40	54	82	104	0	1	12	26	40	55	72	92
4OH-CB107					*0.3*	*0.1*					5.5	2.3	*0.3*	*0.1*		
4OH-CB146					*0.3*	*0.1*					0.9	*0.2*				
3OH-CB138					*0.2*	*0.1*					*0.3*	*0.3*	*0.2*	*0.1*		
4OH-CB187											0.6	0.4		*0.2*		
3OH-CB180											0.8					
4OH-CB172					*0.2*	*0.1*					*0.3*	*0.2*				
Σ OH-PCBs					1.0	0.4					8.4	3.4	0.5	0.4		

4. DISCUSSION

Results showed that PCB exposure produced a significant uptake in all tissues of seabass. At the end of 12 days, PCB concentrations were two orders of magnitude higher than the initial values, being higher in liver followed by serum and muscle. After the exposure period, levels of PCBs in muscle and liver still increased for a short period, indicating redistribution of the compounds within the organism while serum showed the lowest levels.

Fish have a limited capacity to degrade polyhalogenated contaminants, including PCBs, which tend to be eliminated largely as unchanged compounds to water via gills or to feces[7]. However, the role of metabolic biotransformation on the total elimination rate of polyhalogenated contaminants in fish remains unclear, in the way that most authors consider it negligible[8]. In this experiment all of the metabolites investigated were identified in serum, at very low concentrations. The only metabolite that was found in concentrations above the quantification limit was 4-OH-CB107, derived from CB105 [9]. This result shows that seabass is also able to metabolize PCBs, but due to the lower concentrations found the metabolization rates were not possible to determine. The ratio between OH-PCBs and total of PCBs in serum is lower than the described in other species[3,4]. Due to the short time of exposure in this experiment the compounds will probably not accumulate in the serum, despite the high concentrations of PCBs. In natural environment the OH-PCBs may accumulate progressively.

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