DEPURATION OF PCBS AND DDTS IN MULLET UNDER CAPTIVITY CONDITIONS

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Introduction

Fish captured in the coastal zone and estuaries often contains enhanced residues of organochlorine compounds in their tissues, in response to environmental contamination. Residues in fish tissues may be eliminated by different pathways, but most of what is known comes from laboratory studies with species that are exposed to contaminants[1]. In recent years, the importance of ethoxyresorufin O-deethylase (EROD), one of the hepatic cytochrome P-450 dependent monooxidase, has become widely known, and it is increasingly accepted as an indicator of exposure to common organic pollutants.
The mullet (*Mugil cephalus*) from the Douro estuary may present relatively high content of PCBs and DDTs. The objective of this study was to examine the levels of PCBs and DDTs in muscle and liver when individuals are exposed to clean sea water and uncontaminated food, and to evaluate whether this is a feasible option for depuration.

Methods and Materials

Sampling
Twenty two mullets were captured in Douro estuary in May 2001. Five individuals were sacrificed within 24 hours after capture, body liver and gonads were dissected, weighted and the hepato-somatic (HSI), and gonado-somatic indices (GSI) calculated. The remaining liver and small pieces of muscle were frozen in liquid nitrogen and stored at -80ºC until they were assayed for EROD activity and the concentration of PCB congeners and DDT compounds. The remaining fish were allowed to depurate in a 3000 L tank at the salinity of 20 within a flow rate of 5 L/minute. Water was continuously filtered through an extensive biological filter, and a charcoal filter before being recycled. Aeration was provided in the tanks to maintain 100% oxygen saturation in the water. Fish were maintained in natural photoperiod and temperature, and fed with uncontaminated hake fillet. Five individuals were sampled after 21, 120 and 270 days following the same procedure.

Analytical procedure
Samples for analysis of PCBs and DDTs were prepared individually or composite of three individuals for the small liver samples. The method has been described previously[2] and is summarized below. Freeze dried tissues were extracted with hexane using Soxhlet apparatus. Fat content was determined gravimetrically from aliquots of the extracts and the remaining extracts were cleaned with Florisil before the analysis by gas chromatography using a DB-5 column with electron capture detection. PCBs and DDTs were quantified using a standard solution containing 18 PCB congeners, p,p'-DDE and metabolites. Procedural blanks were analyzed each 10 to 16 samples to monitor possible laboratory contamination. Recovery of the Florisil column was evaluated with a standard solution and more than 85% of each compound was obtained. The ethoxyresorufin O-deethylase (EROD) activity was evaluated by the fluorimetric method described by Pacheco and Santos[3]. One way analysis of variance was used to compare concentrations. A 5% significance level was used for the statistical tests.

Results and Discussion

Levels of PCBs and DDTs in muscle and liver of mullet from Douro estuary
Sea mullet (*Mugil cephalus*) from Douro estuary contain relatively high concentrations of PCBs and DDTs both in muscle and liver. At capture day, the mean concentrations of tPCB (calculated as the sum of individual CB levels) in muscle and liver of mullet were 311 and 686 ng g^{-1} and of tDDT (calculated as the sum of concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDE) 65 and 115 ng g^{-1}, respectively. These values are one order of magnitude higher than the maximum values observed by Antunes et al.[4] in golden mullet from Ria de Aveiro (80 ng g^{-1} for tPCB and 22 ng g^{-1} for tDDT), a large coastal lagoon in the NW Portugal with a permanent connection to the sea. The highest muscle concentrations, on a wet weight basis (PCBs: 201 ng g^{-1} ww ; DDTs: 60 ng g^{-1} ww) were also much higher than PCBs (sum of 7 congeners IUPAC Nos 28, 52, 101, 118, 138, 153 and 180) and DDTs reported by Pastor et al.[5] in sea mullet from the Ebro Delta, a zone influenced by agro-industrial activities, respectively, 2.5 and 7.2 ng g^{-1} ww.
The concentrations of PCBs in muscle of wild mullet (311 ng g^{-1}) were lower than the corresponding levels in liver (686 ng g^{-1}), however, the contribution of each component to tPCB was very similar in both tissues (Fig. 1) and characterized by the predominance of the CB 180 (hepta-) and CBs 153, 138 (hexachlorobiphenys). These dominant congeners contain chlorines on the *para* positions on both biphenyl rings and are the prevailing congeners usually reported to be present in biological samples[6]. CBs 138, 153 are also reported as dominant components in *Platichthys flesus* from the same environment[7], in some fish species from Ria de Aveiro[4] and in sea bass from Seine estuary[8].

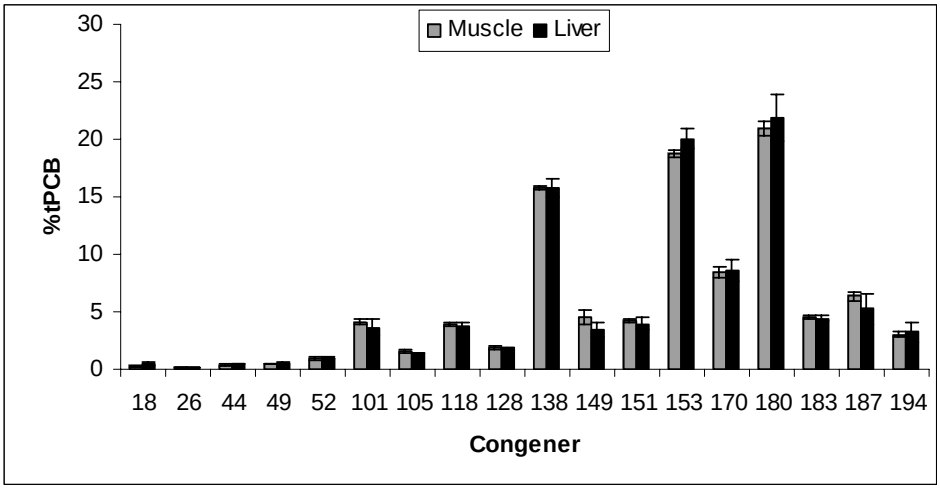

Figure 1: PCB pattern in muscle and liver of mullet from Douro estuary (mean ± SE).

From the DDT compounds, DDE was present in the highest concentration in the analyzed tissues, representing more than 69% of tDDT.

Elimination experiment

The elimination experiment was carried out in captivity under clean experimental conditions during 270 days. In Table 1 are given some individual physiological data.

Table 1: Physiological conditions of mullet (*Mugil cephalus*) during the experiment. Mean values are given ± SE. Different letters denotes significant differences between sampling days.

Sampling day	Whole fish		Liver		Gonad	Muscle
	Length (cm)	Weight (g)	HSI	Lipids (%)	GSI	Lipids (%)
0	36.1±0.3	459±20.3	1.85±0.04^{b}	23±1.3	0.28±0.05	8.0±1.6$^{a,.b}$
21	35.7±0.8	412±16.5	1.31±0.05$^{a,.b}$	23±2.3	0.13±0.01	11±0.6^{b}
120	33.9±0.4	325±14.6	0.94±0.05^{a}	18±0.8	0.28±0.07	3.6±0.4^{a}
270	35.6±0.3	316±6.0	1.26±0.05$^{a,.b}$	12±1.0	0.12±0.01	3.0±0.2^{a}

HIS, hepato-somatic index; GSI, gonado-somatic index.

HSI decreased after 120 d. Liver and muscle lipid content also tend to decrease after 120 days although differences were not always significant. Gonad weight was, however, almost constant indicating that fish did not attained sexual maturation.

The concentrations of tPCB and tDDT in mullet tissues along the experiment including those of wild fish are given in Table 2. The results indicate that, in general, there was absence of elimination

after 21 days. This was also observed by the same authors (unpublished data) in other two 21 days experiments carried out in different seasons. Elimination only occurred in muscle after 120 days. Mean initial concentrations were 311 ng g^{-1} for tPCB and 65 ng g^{-1} for tDDT and at the end of the 270-d study were, respectively, 49 and 13 ng g^{-1}. The concentrations in liver varied, in general, less than in muscle, ranged mean tPCB from 686 to 503 ng $g^{-1}$and tDDT from 115 to 153 ng g^{-1}.

Table 2: Concentrations of organochlorines (ng g^{-1} dw) in muscle and liver of mullet over the 270-d study. Each value represents the mean ± SE of five fishes. Statistical differences from day 21 ($p<0.05$) are identified by an asterisk.

Compound	Depuration time (days)				Depuration time (days)			
	Muscle				Liver			
	0	21	120	270	0	21	120	270
∑PCB	311±58*	821±270	88±2*	49±6.7*	686±135	870±350	259±39*	503±64
p,p'-DDE	40±7.8	55±3.0	29±3.6*	11±0.7*	81±2.65	79±14.7	79±8.4	123±8.5
p,p'-DDD	8.3±2.3	14±0.9	5.0±0.6*	1.2±0.08*	30±1.4	25±1.6	12±0.85*	18±1.1*
p,p'-DDT	17±5.6	23±1.8	7.4±1.0*	0.5±0.08*	3.4±0.9	4,0±1.1	20±1.5*	14±1.2*
∑DDT	65±35	106±27	41±12*	13±1.8*	115±5.7	108±27	111±23	153±22

Lipid content in muscle showed a similar trend as the contaminants during the study, resulting in small variations in lipid base concentrations. This is confirmed by the significant ($p<0.001$) relationships (r^2 ranged from 0.61 to 0.94 for PCB congeners and from 0.81 to 0.93 for DDT compounds) indicating that lipid content is a major factor in elimination kinetics of PCBs and DDTs in muscle of mullet. In liver, the evolution of lipids and contaminants with time is quite different and no relationships were obtained.

Levels of EROD in liver of mullet

Several authors have been used EROD induction as a biomarker for assessment PCB and PAH pollution[9]. Mullet livers of the animals collected in Douro estuary, presented high induction of EROD (328 ± 53 n mol/min. mg protein) which reflect the exposure to pollution. This is in accordance with the relatively high concentrations of PCBs and DDTs which are found in muscle and liver of the wild fish. After 21 days of depuration, levels showed a substantial decrease of EROD activity and the mean value during the experiment was 38 ± 1.4 n mol/min. mg protein. However, this decrease did not correspond to a decrease on concentrations of PCBs and DDTs in liver (Table 2). This lack of correspondence was observed along all the experiment. This indicates that the high values of EROD activity of mullet from Douro estuary cannot be attributed solely to the accumulation of these organochlorine compounds but to a mixture of non-persistent contaminants present in the estuary eventually eliminated after 21 days under captivity conditions.

Dependence on component

The decreasing time of elimination was not related to decreasing chlorine content of PCB congeners. This is illustrated in Figure 2. Concentrations of most compounds increased in the first sample interval in muscle then decreased thereafter, so the variation of each individual congener concentration will be discussed on the basis of concentrations at day 21. Tri- and tetrachlorobiphenyls were eliminated in muscle 33-60% after 120 d, while the other congeners decreased more than 64%. At day 270, the concentrations of all the analyzed congeners were lower than 29% of the concentrations at day 21. In liver significant differences were observed only after 120 days for CB 101 (penta-) and for the hexa- and hepta- CBs. After 270 d concentrations of most congeners increased which may be attributed to a mobilization to the liver.

In a bioconcentration/elimination laboratory experiment Goerke and Weber[1] observed that the lower level of chlorination and free *m,p* position of CBs favoured elimination in *Platichthys flesus*. In our study the reductions in muscle were lower for tri- and tetra- CBs. All the other congeners were eliminated fastest and there were no substantial dependence on the component. Most compounds showed, however, after 120 d mean concentrations in muscle below 50 % than those at day 21. So, a differentiation in congener half-lives may be probably observed in shorter experiment times. The lower reduction of tri- and tetra CBs may be related to its low levels in mullet tissues.

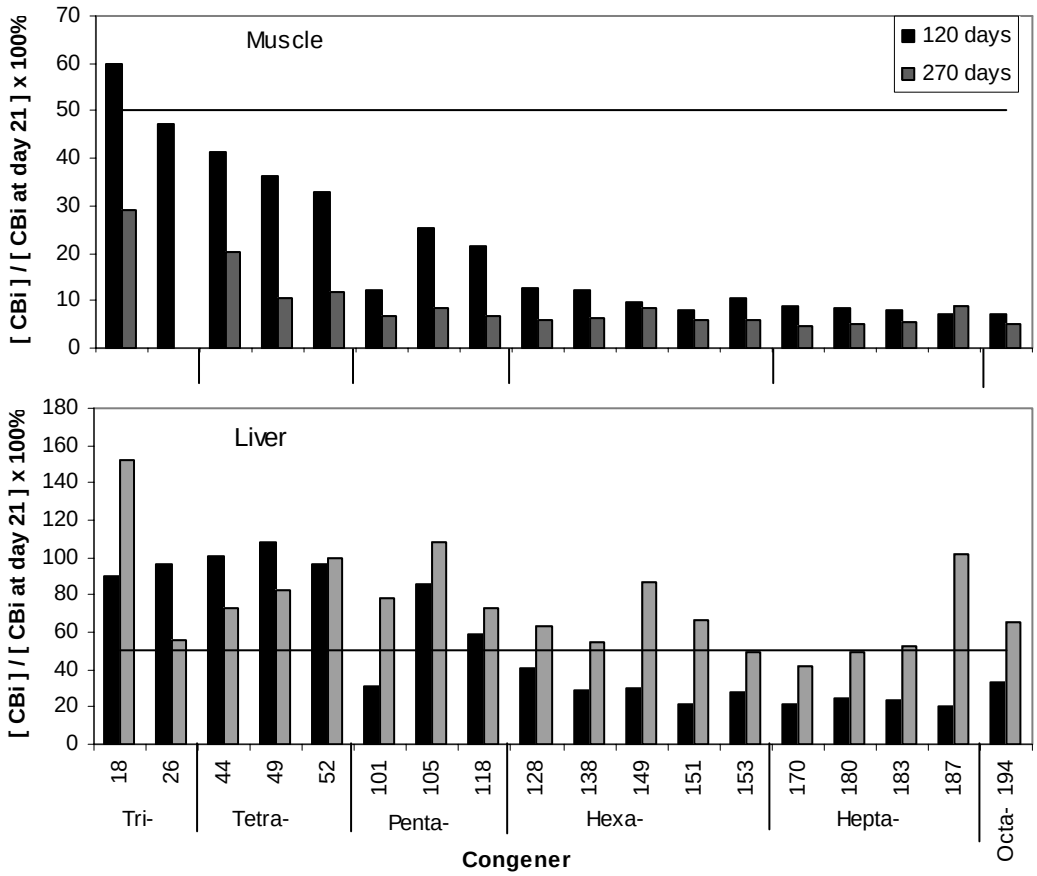

Figure 2: Percentages of the concentration of individual CBs in relation to concentrations at day 21, after 120 and 270 days of depuration.

In mullet muscle DDD and DDT were eliminated faster than DDE. At day 120, DDD and DDT were eliminated more than 60% of the initial values and more than 91% at day 270, while concentrations of DDE decreased only 48% and 80%, respectively. As it is known that the conversion of p,p'-DDT to p,p'-DDE occurs in fish, the slower decrease of DDE in muscle may be a result of metabolism. In liver an erratic variability of these compounds was recorded.
In conclusion, in clean sea water the levels of organochlorines decreased due to the decrease of lipids in muscle. The PCB pattern changed mainly due to the lower reduction of tri- and tetrachlorinated CBs, probably related to the low levels recorded in muscle.

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