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ScienceDirect
Chemosphere 67 (2007) S58–S64
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Depuration of PCBs and DDTs in mullet under captivity clean conditions

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Accepted 26 May 2006
Available online 17 January 2007

Abstract

Contaminated mullet (*Mugil cephalus*) from Douro estuary was allowed to depurate in clean water and fed with uncontaminated food. Levels of PCBs and DDTs in muscle and liver, and ethoxyresorufin O-deethylase (EROD) activity were measured at day 0, 21, 120 and 270. In specimens captured in the estuary total PCB and total DDT concentrations were 311 and 65 ng g^{-1} in muscle and 686 and 115 ng g^{-1} in liver, respectively. At day 21, after an initial 10–15 days period of starvation, organochlorines levels increased in both analyzed tissues. Thereafter levels of all PCB congeners and DDT compounds decreased in muscle, and at the end of the 270 days were 49 ng g^{-1} and 13 ng g^{-1}, respectively. These decreases were correlated to the lipids consumption. In liver no relationship between those variables was observed, suggesting different elimination processes and eventual exchange of contaminants between muscle and liver. EROD activities decreased in the first days of depuration experiment, but showed no relations with analysed organochlorines.

Keywords: PCBs; DDTs; Depuration; *Mugil cephalus*; Douro estuary

1. Introduction

Thousands of tons of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) were produced in the 20th century and partially released to the environment. Either due to diffuse sources or direct discharges to the terrestrial and atmospheric compartments, the aquatic environment is the ultimate sink for these contaminants (Stegeman and Hahn, 1994). Fish captured in the coastal zone often contains enhanced residues of these compounds in their tissues, as result of environmental contamination. Accumulation in the tissues does not necessarily imply injurious effects to the organisms. The biological response is usually evaluated through the presence of chemical biomarkers (van der Oost et al., 2003), namely ethoxyresoruf in O-deethylase (EROD) one of the hepatic cytochrome P-450 dependent monooxidase (Ferreira et al., 2004).

The elimination of organochlorines in fish is barely known despite the number of studies related to the uptake kinetics by fish. Organochlorines can be eliminated by excretion (Moermond et al., 2004) and lower chlorinated PCB congeners biotransformed (van der Oost et al., 2003). However, most of the understanding comes from laboratory experiments with species exposed to contaminants (Goerke and Weber, 2001), and few works report results from naturally contaminated fish. In a long-term elimination study, PCB-contaminated eels captured in a natural environment were transferred to a relatively clean lake (de Boer et al., 1994), and half-lives of tetra- and penta-CBs ranged from 340 to 1450 days, but most of hexa-, hepta- and octa-CBs showed no measurable elimination.

This work presents the levels of PCBs and DDTs in muscle and liver of contaminated mullet (*Mugil cephalus*)

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0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.05.091

from the Douro estuary when exposed to clean sea water and uncontaminated food, and examines whether elimination of organochlorines is a realist mechanism during the life-time of species that migrate from estuarine contaminated systems to coastal waters.

2. Methods and materials

2.1. Sampling

Twenty two mullets (*M. cephalus*) were captured in the Douro estuary in May 2001. Five individuals were sacrificed within 24 h after capture, body liver and gonads were dissected, weighted and the hepato-somatic (HSI: ratio between the weights of the liver and the fish), and gonado-somatic indices (GSI: ratio between the weights of the gonads and the fish) calculated. The remaining liver and small pieces of muscle were frozen in liquid nitrogen and stored at −80 °C until they were assayed for EROD activity and the concentration of PCB congeners and DDT compounds. The other specimens were allowed to depurate in a 3000 l tank with a flow rate of 5 l/minute of brackish water (salinity 20‰). Water was continuously filtered through an extensive biological filter coupled to a charcoal filter before being recycled. The tank was aerated to maintain 100% oxygen saturation in the water. Fishes were maintained in natural photoperiod and temperature, and fed with uncontaminated hake fillet. During the first 10 days the added food was not consumed, probably due to the stress of captivity. At the day 15 it was observed that all individuals were eating normally. Five individuals were sampled at days 21, 120 and 270 following the same procedure.

2.2. Analytical procedure

Samples for analysis of PCBs and DDTs were prepared individually. The method has been described previously in Antunes and Gil (2002, 2004) 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 in a HP 5890 series II gas chromatograph, equipped with an electron capture detector, and a DB-5 (J&W Scientific) capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness). PCBs and DDTs were quantified using a six point calibration curve. A standard solution containing 18 PCB congeners (IUPAC Nos. 18, 26, 52, 49, 44, 101, 151, 149, 118, 153, 105, 138, 187, 183, 128, 180, 170, 194), *p*,*p*′-DDT and metabolites (*p*,*p*′-DDD and *p*,*p*′-DDE) was used as external standard. Procedural blanks were analyzed each 10–16 samples to monitor possible laboratory contamination and blank subtractions were made before quantification. 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 (1998). One way analysis of variance or student's *t*-test was used to compare concentrations. A 5% significance level was used for the statistical tests.

3. Results and discussion

3.1. PCBs and DDTs in mullets from the Douro estuary

Mullets captured in the Douro estuary contained relatively high values of PCBs and DDTs in their muscle and liver tissues. The mean concentrations and standard error ($n = 5$) of tPCB (calculated as the sum of individual analysed CB levels) in muscle and liver were 311 ± 58 and 686 ± 135 ng g^{-1}, respectively, and of tDDT (sum of concentrations of *p*,*p*′-DDE, *p*,*p*′-DDD and *p*,*p*′-DDT) were 65 ± 35 and 115 ± 5.7 ng g^{-1}. In spite of the differences of residue levels among the five analysed individuals, values are one order of magnitude higher than the concentrations reported by Antunes et al. (2001) for golden mullet (*Liza aurata*) captured in Ria de Aveiro (80 ng g^{-1} of tPCB and 22 ng g^{-1} of tDDT), a coastal lagoon with permanent connection to the sea, located about 70 Km south of the Douro estuary. Mullets can run long distances in short periods of time eventually moving out of the estuarine systems, or remain inside particularly around urban sewages discharge (Chubb et al., 1981). Levels of organochlorines in the specimens captured in the Douro indicate local contamination. Values are one to two orders of magnitude higher than concentrations of PCB (2.5 ng g^{-1} fresh weight, sum of the IUPAC congeners Nos 28, 52, 101, 118, 138, 153 and 180) and DDTs (7.2 ng g^{-1}) in sea mullet from the Ebro Delta, a zone influenced by agro-industrial activities, and comparable to levels of PCBs in samples of mackerel and anchovy from an area exposed to pollution (Bayarri et al., 2001).

The contribution of each analysed CBs to the total PCB was the same in muscle and liver in mullets from Douro, and the CB180 (hepta-), CB153 and CB138 (hexa-chlorinated) were the predominant congeners (Fig. 1). These compounds contain chlorines at *para* positions in both biphenyl rings, and usually are the prevailing compounds reported in biological samples (Bayarri et al., 2001). The CB138 and CB153 are dominant components in *Platichthys flesus* from Douro (Ferreira et al., 2004), in several species from Ria de Aveiro (Antunes et al., 2001) and in sea bass from Seine estuary (Loizeau et al., 2001). The metabolite *p*,*p*′-DDE accounted to more than 69% of the total DDT in muscle and liver tissues of mullet from Douro.

3.2. Laboratory experiment

3.2.1. Mullet conditions

The physiological conditions of mullet during the experiment are presented in Table 1. The specimens kept in