FIRST ATTEMPT TO EVALUATE PCDDs, PCDFs AND PCB ENANTIOMERIC RATIOS IN STRIPED DOLPHINS (*Stenella coeruleoalba*) FROM THE MEDITERRANEAN SEA USING SKIN BIOPSIES.

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Introduction

Several studies[1,2] conducted in the Mediterranean basin have revealed that top predators, and particularly cetaceans, accumulate high concentrations of Organochlorine Contaminants (OCs). However it should be pointed that most of these studies are based on tissues obtained from dead specimens such as blubber or liver. Limited information exists on the ecotoxicological status of free ranging specimens of high interest, in particular of top predators.

In this study, we used skin biopsy as a non-lethal approach to investigate the presence of OCs, in particular polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorobiphenyls (PCBs) in striped dolphins (*Stenella coeruleoalba*). Skin biopsy has certain advantages in ecotoxicological studies: it enables a large number of samples to be obtained across a wide geographic range; it could be suitable for residue analysis and, biomarker analysis such as Cytochrome P4501A (CYP1A1) induction[3,4].

Recently, increasing attention has also been paid on PCBs which display axial chirality in their non-planar conformations. Nineteen out of 78 chiral PCBs exist as stable atropisomers at ambient temperature and have been introduced into the environment as racemates. But it has been shown that their intake or metabolism by organisms may be enantioselective and that PCB atropisomers may have different activities, toxicities, or metabolic pathways[5]. This makes their determination highly important and interesting in different research topics, especially in the environmental field. Moreover, few studies deal with their determination in marine mammals such as stripped dolphins, being this study the first one performed in skin biopsy.

Among the objectives of this work, are to provide for the first time baseline data on PCDD and PCDF levels, as well as PCB enantiomeric ratios for an endangered population of South-West Mediterranean striped dolphins using skin biopsy as a non destructive sampling procedure.

Materials and Methods

Sampling

In 2002 during the summer season, subcutaneous blubber samples were obtained from a total of 10 specimens of Striped Dolphin (*Stenella coeruleoalba*) from the Mediterranean Sea. Samples of skin biopsies were obtained using biopsy tips mounted on a 2-m pole. Biopsies were immediately

frozen in liquid nitrogen and stored at –80ºC until analysis. Due to the small size of each sample (0.20-0.02g) and the low levels expected for PCDD/Fs, biopsies were pooled for residue analysis.

Analytical procedure

Sample preparation

Sample treatment involved a procedure described elsewhere[6]. Basically, the extraction of PCDDs/Fs, PCBs and DDTs involved a Matrix Solid Phase Dispersion (MSPD) of the sample and clean-up using acid and basic modified silica gel multilayer columns. Final fractionation among the studied compounds was achieved by using SPE carbon tubes.

Instrumental Analysis

Ortho substituted PCBs and DDTs

PCB congeners (#28, 52, 95, 101, 132, 138, 149, 153, 170, 180, 183, 194, 105, 114, 118, 123, 156, 157, 167, 189), DDT and its metabolites (DDE and TDE) were analysed by GC-μECD using an Agilent 6890 Series II as described by Gomara et al. (2002)[7].

Non*-ortho *substituted PCBs (PCB 77, 126 and 169) and PCDDs/Fs

Identification and quantification of non-*ortho* PCBs, PCDDs and PCDFs, as described by Abad et al. (1997)[8] was performed by GC-HRMS/EI(+)-SIM on a Carlo Erba GC 8000 series coupled to an Autospec Ultima Mass Spectrometer from Micromass.

Enantiomeric Ratio Determination of Chiral PCBs

The enantiomeric ratios of 9 chiral PCBs were determined using a Varian MDGC system equipped with a DB5 as achiral column and a Chirasil-Dex as main column. The transfer of the PCB congener to the chiral column was done by means of a Deans valve.

Results and Discussion

This study represents the first report on PCDDs and PCDFs in Striped Dolphin skin biopsies from the Mediterranean Sea. All 2,3,7,8-substituted PCDDs and PCDFs, except OCDF, were detected in the samples studied. Total PCDD/F levels were 73.91 pg/g on a wet weight basis (ww). Regarding the contribution of PCDDs and PCDFs to total PCDD/F levels, it was found the same percentage contribution from both PCDDs and PCDFs. The most abundant congener was OCDD, representing a 20% of the total levels, followed by 1,2,3,4,6,7,8-HpCDD which represented the 12% of total PCDD/Fs. The remaining congeners in most cases had a contribution under 5% as show in Fig. 1 where is presented the characteristic 2,3,7,8-substituted pattern. Total PCDD/F levels found in this study are higher than those reported for other groups of marine mammals using the same sampling procedure[9]. Sea lion skin biopsies from a polluted area in Argentina exhibited 10.92 pg/g (wet weight) of total PCDD/Fs. In both cases, sea lion and striped dolphin data are consistent with previous studies[10,11] which indicate that dioxins occur at relatively low levels in marine mammals, possible due to rapid catabolism or elimination. Non-*ortho* PCBs (#77, #126 and #169) were found at higher levels than PCDD/Fs, with a value of 372.81 pg/g ww, being PCB#77 the congener exhibiting the highest levels. *Ortho*-substituted PCB levels (as the sum of the individual congeners determined) were 6.22 ppm (μg/g ww), being congeners #153, #138, #180 the most abundant contributing with 72% to total levels. Regarding DDTs (as the sum of DDT and its two main metabolites, DDE and TDE) it was found a concentration of 5.08 μg/g ww, being DDE the main contributor to total levels, with a 91%. PCB and DDT levels found in this study are lower than those reported in the literature for subcutaneous blubber from Mediterranean striped dolphins[2].

Total TEQs, based on TEF values for mammals proposed by the WHO[12], were 47.3 pg/g on a fresh weight basis being the highest contributor mono-*ortho* PCBs (60.2 %), followed by PCDDs (15.9%), non-*ortho* PCBs (13.4%) and PCDFs (10.5%).

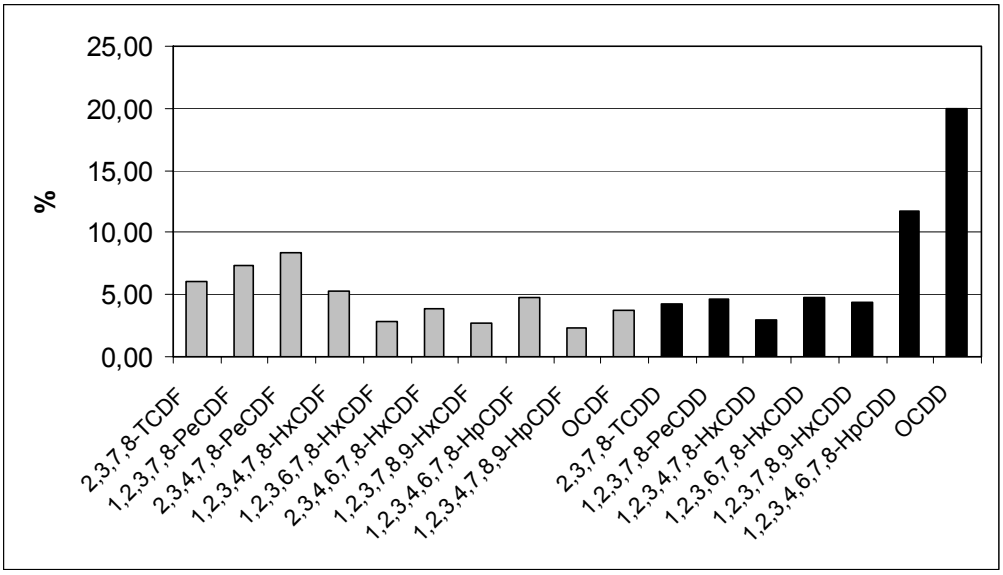

Figure 1. Percentage contribution of 2,3,7,8-substituted PCDDs and PCDFs in skin biopsy from striped dolphins (*Stenella coeruleoalba*).

Regarding the 9 chiral PCBs investigated, the study revealed an enantiomeric excess (ee) of the second enantiomer eluted higher than 10% in 5 of them (PCB 91, 135, 136, 149 and 176) as shown in Table 1. A lower enantiomeric excess was found for congeners 95, 132 and 174. Other studies[13] have reported ee in blubber samples from the same species, showing ee higher than 10% for PCB 132, 135, 149, and between 5-10% for PCB 136 and 174.

Table 1. Enantiomeric ratio (ER, ratio of the first eluted enantiomer to the second) and enantiomeric excess (expressed in percentage) determined in the skin biopsy by MDGC.

	PCB 84	PCB 91	PCB 95	PCB 132	PCB 135	PCB 136	PCB 149	PCB 174	PCB 176
ER	nd	0,72	0,93	0,88	0,77	0,73	0,81	0,95	0,73
ee (%)	-	15,9	3,5	5,9	12,9	15,3	10,5	2,56	15,2

This preliminary study shows that dolphin's skin biopsies are a useful tool to evaluate the presence of highly toxic contaminants (PCDDs, PCDFs and PCBs) in top predators from the marine trophic web. In conclusion the use of skin biopsies appears to be a powerful tools to assess the ecotoxicological status of species of interest.

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