POPs IN TOP PREDATORS FROM THE MEDITERRANEAN SEA.

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Introduction

Persistent Organic Compounds (POPs) such as PCDDs, PCDFs and PCBs are man-made chemicals that are persistent and highly lipophilic and thus biomagnify in food chains. There is growing evidence that these compounds are extremely harmful to marine and freshwater ecosystems, especially when they bioaccumulate through aquatic foodwebs[1].

Cetacean populations living in the Mediterranean Sea are at considerable risk from levels of persistent chemicals in the marine environment, which have risen rapidly in the second half of this century. Cetaceans are marine mammals at the top of the food chain, making them among the most exposed of all marine animals. Moreover, they have been found to be very much less able to metabolize organochlorine than birds and land mammals[2]. Although there is as yet no evidence that pollutants are causing the death of marine mammals, organochlorines such as PCBs, PCDDs and PCDFs are known to cause immune and reproductive dysfunction[3]. In a number of regions contamination in cetaceans has now reached concentrations at which there are known sub-lethal effects sufficient to harm populations of other species.

Several studies assessing the ecotoxicological impacts of toxic and bioaccumulative compounds, in particular non-ortho PCBs have been published related to the high mortality occurred in 1990 among dolphins from the Mediterranean Sea[4, 5], but to our knowledge, no data have been published investigating the contribution of the highly toxic PCDDs and PCDFs to the overall toxicity. Therefore, the isomer specific analysis of these substances by HRGC-HRMS in liver samples of a variety of cetacean species found stranded along the Italian coasts was performed. The goal of this study was to determine the levels of PCDDs and PCDFs and to establish a comparison among different areas. The toxicological significance of PCDD and PCDF levels compared to PCB levels is discussed.

Materials and Methods

Study area.

Some useful information is missing and difficult to avoid due to logistical difficulties in obtaining samples at some locations.

Sampling

Liver samples were obtained from five different cetacean species: Striped dolphin (*Stenella coeruleoalba*), Bottlenose dolphin (*Tursiops truncatus*), Risso's dolphin (*Grampus griseus*), Pilot whale (*Balaenoptera physalus*), and Long-finned pilot whale (*Globicephala melaena*). All the animals studied were found dead along the Italian shore of the Tyrrhenian, Adriatic and Ligurian Seas in the period 1987-1992. Liver samples were frozen and stored at -20° C until analysis.

Analytical determination

Extraction and clean up

Samples for residue analysis were lyophilized and amounts of approximately 0.5 grams of lyophilized tissue were used for analysis.

Extraction and clean up were performed as previously described in detail elsewhere[6]. Basically this consisted of low-pressure chromatography on neutral and base-modified silica gel and activated carbon dispersed on glass fibers. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDDs and PCDFs, respectively. The lipid content was determined gravimetrically.

Quantification

Resolution and quantification of PCDDs and PCDFs were performed by HRGC-HRMS using a VG AutoSpec Ultima (VG Analytical, Manchester, UK) coupled to a Fisons Series 8000 (8060) gas chromatograph. A fused silica capillary DB-5 column (60m, 0.25 mm id., 0.25μm film thickness, J&W Scientific, USA) and a DB-DIOXIN column were used. The carrier gas was Helium at a column head pressure of 175 Kpa. A minimum resolution of 10,000 was used when operating with the HRMS instrument. Methods blanks were routinely analyzed, and no contributions were detected. Total values reported were calculated assuming that all values less than the limit of detection (LOD) are equal to the half the LOD.

Results and Discussion

A specific 2,3,7,8-substituted PCDDs and PCDFs pattern was found in all samples studied and no contributions were found from other congeners. Total PCDD and PCDF levels ranged from 13 to 112 pg/g on a wet weight basis. Regarding the contribution of PCDDs and PCDFs to total levels, in general a higher percentage contribution was found from PCDFs, which in some cases contributed up to 77%. When sampling areas are compared it can also be seen that individuals from the Adriatic Sea exhibited the lowest PCDD and PCDF levels while total levels were higher in those individuals from the Ligurian Sea which is known to be heavily contaminated with organochlorines from the nearby Rhone estuary[7]. The widespread occurrence of OCDD, OCDF and TCDF found in the cetaceans from the Mediterranean Sea indicates the local influence of combustion sources already reported in previous studies conducted in the region. High levels of OCDD and OCDF have been reported in crabs and sediments from different areas in the Mediterranean Sea and this prevalence has been attributed to combustion sources[8]. The congener 2,3,4,7,8-PnCDF, which is also present in combustion sources[9], was also found in all samples studied, thus supporting the hypothesis that combustion sources play a significant role in pollution in the Mediterranean Sea.

Most studies dealing with PCDDs and PCDFs in marine mammals have been carried out with seals and data on PCDD and PCDF levels in dolphins and whales are scarce. It is interesting to note that 1,2,3,7,8-PnCDD, 2,3,4,7,8-PnCDF and 1,2,3,7,8-PnCDF were found in all samples analyzed in the present study, which agree with general findings in European samples[10]. There are some studies in dolphins from the U.S. Atlantic Coast including Atlantic Bottlenose obtained during the 1987/88 mass mortality occurred along the central and southern Atlantic coast of the United States[11]. It was reported that 2,3,7,8-TCCD has not been previously detected (< 15 pg/g lipid) in any of the animals studied. PCBs, however, were quantified at concentrations as high as 195 μg/g lipid, suggesting that PCBs may be a causative factor in the mortality event but not PCDDs and PCDFs. Muir et al.[12] studied levels of PCDDs and PCDFs in Beluga whales (*Delphinapterus leucas*) from the St. Lawrence river estuary. No PCDDs or PCDFs were detected

in beluga liver samples (MDL<1 pg/g TCDD) and low pg/g levels of PCDFs were detected in the beluga blubber samples while PCDDs were undetectable. The major PCDF congeners found were 1,2,4,6,8,9-HxCDF, 1,2,4,7,8-PnCDF and 1,2,4,8,9-PnCDF. These findings suggest that some cetaceans have an unusual ability to metabolize and excrete PCDDs, but not PCDFs. However, this is probably not a universal rule for all cetaceans, since Buckland[13] showed Hector's dolphin (Cephalorhynchus hectori) in New Zealand to contain only 2,3,7,8-substituted TCDD and TCDF congeners, the usual finding for birds, fish and land mammals.

Calculated TEQs for PCDDs, PCDFs and biochemically active planar PCBs.

2,3,7,8-TCDD equivalents (TEQs) were estimated for PCDDs, PCDFs and PCBs based on the new mammalian Toxic Equivalency Factors (TEFs) reported by the World Health Organization[14]. Considering total TEQs due to biochemically active PCBs, PCDDs and PCDFs, total levels ranged from 5 to a maximum of 1458 pg/g (wet weight), the highest value being from the Pilot whale. However TEQs reported in the present study are well below the high values reported in similar marine mammals studies, including those reporting only TEQs for PCBs[4].

In a research of organochlorines in Ringed seals and Grey seals, ecotoxic significance of PCDDs and PCDFs was evaluated and in all the species studied it was found that the toxic load of PCDDs and PCDFs as TCDD-equivalents is much lower than that of PCBs, concluding that PCBs, especially toxic coplanar congeners, are at present the most hazardous known organochlorines in the Baltic and Finnish environment[15]. The toxic threat of coplanar PCBs to marine mammals has been describe by Tanabe and Kannan[16]. They have suggested that although the toxic and biological responses to coplanar PCBs are much lower than those of PCDDs and PCDFs, their impact may be more significant because they are found at much higher concentrations, especially going from land to ocean mammals.

Kannan et al.[4] have reported isomer-specific concentrations of PCBs including di-, mono- and non ortho-coplanar PCB congeners in Striped dolphins affected by the morbillivirus epizootic in the Mediterranean Sea in 1990. They concluded that the mono-ortho PCB congener contribution to TEQ in these marine mammals was consistently higher than that of non-ortho PCB congeners. When the contribution from PCDDs and PCDFs to total TEQs is compared with that of PCBs in studies carried out in the same individuals as in the present study [5,17] it can be seen that it is lower than that coming from PCBs. In most cases the largest percentage contribution to total TEQ came from mono-ortho PCBs. This situation was clear in all the Striped dolphins studied with percentages ranging from 75 to 99%. Also the Long-finned pilot whale had a percentage contribution of 61%, the Risso's dolphin and the Bottlenose dolphins had percentages ranging from 42 to 89%. The case of the Pilot whale was unusual since practically all the total TEQ comes practically from mono-ortho PCBs with a value of 98%, the contribution of PCDDs, PCDFs and non-ortho PCBs being almost negligible. There were a few cases in which non-ortho PCBs made the largest contribution to total TEQs. Similar studies in other marine mammals have reported that PCDDs and PCDFs are not important contributors to TEQs. The most recent report[18] using new TEFs recommended by the WHO examined the ability of Ganges River dolphins (Platanica gangetica). The total TEQs of non- and mono-ortho substituted congeners in Ganges dolphin blubber was 175 pg/g wet weight. Authors found the greater contribution to TEQs to be due to mono-ortho congeners.

Therefore the relative contribution of PCDDs, PCDFs, non- and mono-ortho PCBs found in different cetaceans may reflect geographic differences in sources of PCBs, age and sex differences, and differences in metabolic capability among odontocetes.

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