ORGANOCHLORINES WITH ENDOCRINE DISRUPTING CAPACITY IN MARINE MAMMALS: THE CASE STUDY OF MEDITERRANEAN CETACEANS

M.Cristina Fossi and Letizia Marsili,

Department of Environmental Sciences, Siena University, Via Mattioli 4, 53100 Siena, Italy.

Introduction

In the last few decades various studies have shown that marine mammals are sensitive to the toxicological effects of certain xenobiotic compounds, including the large class of Endocrine Disrupting Chemicals (EDCs). Fish-eating aquatic mammals may be extremely vulnerable to EDCs because of (a) their position in the food chain, (b) dependence on an aquatic/marine food web, (c) they live in areas influenced by industry and agriculture and (d) their specific reproductive physiology[1]. Since several EDCs and particularly organochlorines, tend to bioaccumulate and biomagnify in the aquatic food chain[2,3], various aquatic mammals, particularly top predators such as pinnipeds, odontocete cetaceans[4] and polar bears, are potentially "at risk" due to EDC contamination.

EDCs are a structurally diverse group of compounds that may damage the health of humans, wildlife, fisheries and their progeny, by interaction with the endocrine system[5-8]. They include chemicals used heavily in the past in industry and agriculture, such as polychlorinated biphenyls and organochlorine pesticides, and chemicals under current use, such as plasticizers and surfactants. Many known EDCs are estrogenic, affecting reproductive function. Because of the lipophilic persistent nature of most xenobiotic estrogens and their metabolites, many bioaccumulate and biomagnify[2,3]; organochlorines are an example.

There are four types of organochlorine endocrine disruptors commonly found in aquatic mammals[9-11]: environmental estrogens, environmental androgens, anti-estrogens and anti-androgens. These endocrine disruptors act by mimicking steroid sex-hormones, both estrogens and androgens, by binding to hormone receptors or influencing cell pathways (environmental estrogens and androgens), or by blocking and altering hormonal binding to hormone receptors (anti-estrogens, anti-androgens).

Materials and Methods

Sampling

Subcutaneous blubber samples were obtained from Mediterranean fin whales (*Balaenoptera physalus*) using biopsy darts launched with a crossbow. A biopsy dart, a regular aluminium crossbow bolt with a modified stainless steel collecting tip and floater, was fired into the whale with a Barnett Wildcat II crossbow with a 150-pound test bow. The procedure consisted of approaching the whale at low-to-moderate speed as it surfaced, and shooting the dart at a range of 10 - 30 m. The striped dolphins (*Stenella coeruleoalba*) were sampled from the prow of the boat, while they were riding the bow wave, using biopsy tips mounted on a 2-m pole. In the Ionian Sea, samples were obtained from common dolphins (*Delphinus delphis*) and bottlenose dolphins (*Tursiops truncatus*). The principal problem in sampling these two species was their diffidence towards humans the man and the boat. We therefore could not use the modified pole and opted for a Barnett Trident crossbow with a 15-pound test bow. The biopsy samples were immediately stored in liquid nitrogen.

Laboratory analysis

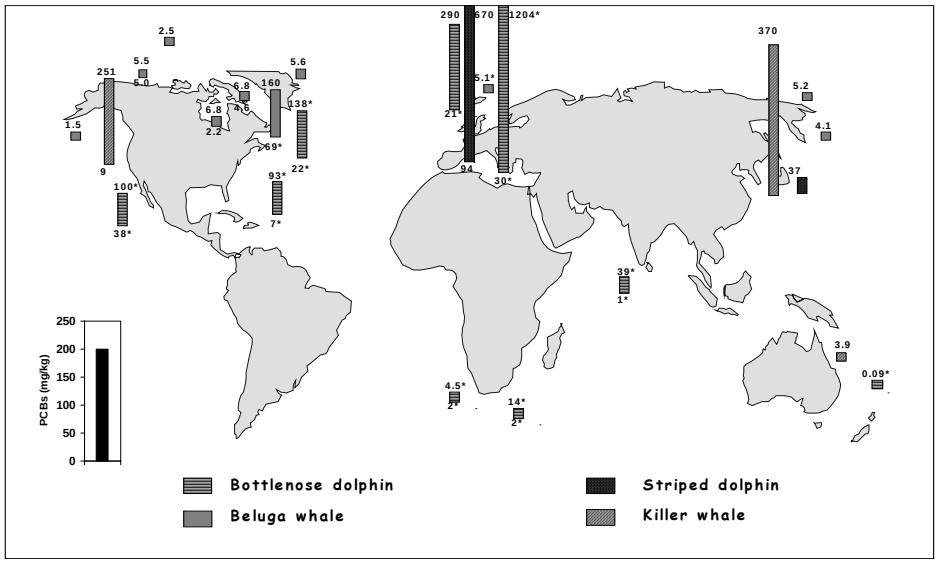
The small size of the biopsy samples (between 0.20 g and 0.02 g) did not permit isolation of the microsomal fractions for MFO assay. Benzo(a)pyrene monooxygenase (BPMO) activity was detected in whole tissue following the procedure proposed by Fossi et al.[14]. Since the connective tissue was very tough, the epidermis was homogenized in 1.15% KCl buffer at pH 7.5 by thermal shock and separated by freezing in liquid N_2 and pulverizing in a Potter apparatus with ultrasound. BPMO activity was assessed using the incubation mixture proposed by Kurelek et al. (1977), incubating each sample (plus the blanks) in a shaking bath for 1 h at 37°C. The activity was expressed in arbitrary units of fluorescence (AUF/h/g tissue).

The samples of subcutaneous blubber (about 0.3 g) were freeze-dried and extracted with n-hexane in a Soxhlet apparatus for analysis of chlorinated hydrocarbons, using the method proposed by Marsili[10]. The analytical method used was High Resolution Capillary Gas Chromatography with a Perkin-Elmer Series 8700 GC and a 63Ni ECD. A mixture of specific isomers was used to calibrate the system, evaluate recovery and confirm the results which were expressed in µg/g d.w. Capillary gas-chromatography revealed op'- and pp'- isomers of DDT and its derivatives DDD and DDE, and about 30 PCB congeners.

The case study of Mediterranean Cetaceans

Man-made EDCs range across all continents and oceans; some geographic areas are potentially more threatened than others: one of these is the Mediterranean Sea. This basin has limited exchange of water with the Atlantic Ocean, and is surrounded by some of the most heavily populated and industrialised countries in the world. Levels of some xenobiotics are therefore much higher here than in other seas and oceans[12,13]. In this peculiar environment top predators (such as large pelagic fish and marine mammals) tend to accumulate large quantities of polyhalogenated aromatic hydrocarbons (PHAHs) and toxic metals[10,14]. Levels of PHAHs in a top predator of the Mediterranean, the striped dolphin, are 1-2 orders of magnitude higher than in Atlantic and Pacific individuals of the same species[15] (Figure 1).

Figure1: Minimum and maximum mean levels of total PCBs in blubber (mg/kg fresh weight) in cetaceans from various part of the world. Number with * are in mg/kg lipid weight.

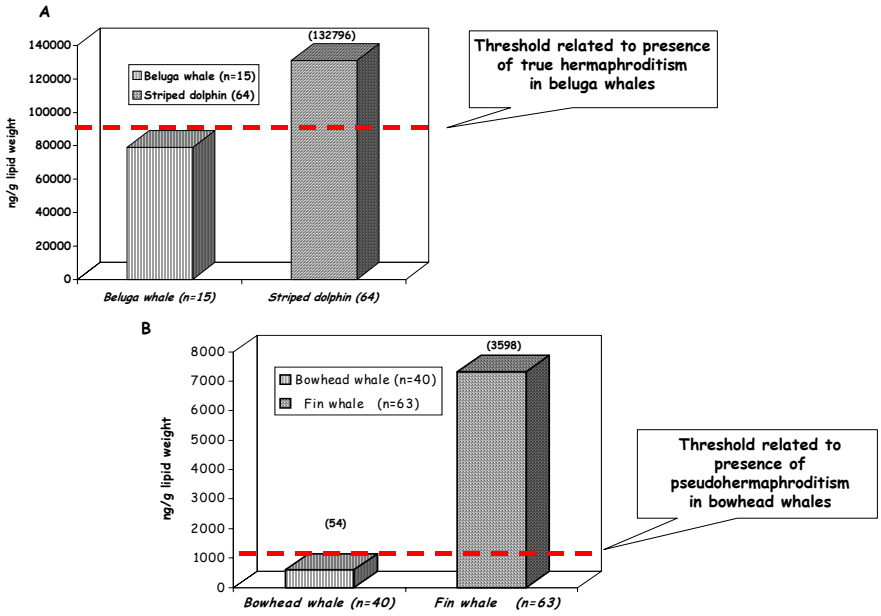


Non-destructive studies of Mediterranean cetaceans have revealed the hazard to which odontocete species are exposed in relation to organochlorines in blubber[16]. Significant differences in total levels of organochlorine EDCs (OC-EDCs) were found between odontocetes and mysticetes. Highest mean levels were found in striped dolphins [(OC-EDCs = 40.0 µg/g fresh weight (f.w.)], followed by bottlenose dolphins (OC-EDCs = 24.3 µg/g f.w.) and common dolphins (OC-EDCs = 15.0 µg/g f.w.). Differences in organochlorine bioaccumulation and consequent potential risk due to endocrine disruptors, are primarily related to different positions in the marine food chain. Of the Mediterranean cetaceans investigated, the species with the highest levels of OCs and the highest biomarker response seems to be the striped dolphin.

Interspecies differences in susceptibility to EDCs must be considered because high levels of contaminants and high biomarker responses do not necessarily mean high risk for the species.
Some cetaceans, such as the common dolphin, have almost completely disappeared from the Mediterranean Sea. Using skin biopsy as an ecotoxicological tool to study intraspecific susceptibility to contaminants, a high statistical correlation was found between Benzo (a)pyrene monoxigenase (BPMO) activity and Total DDTs, pp'DDE, op'DDT, Total PCBs and 153 congener (Pearson correlations significant at $p<0.05$) in male specimens of common dolphin. This suggests that EDCs may be one of the major ecotoxicological stress for common dolphin populations in the Mediterranean Sea. Similar correlation (Spearman Rank Correlations significant at $p<0.05$) was obtained in fin whales, sampled in the Ligurian Sea from 1992 to 1995[17], between BPMO activity and organochlorine levels in skin biopsy specimens from males but not females or males and females together. Future studies are needed to explore the role of detoxification enzymes and estrogen receptors (ER) in interspecies susceptibility to EDC contaminants using fibroblast cell cultures of different species[18].

Some general considerations on potential hazard to these Mediterranean species can be drawn from comparison of OC levels detected in Mediterranean cetaceans[14-17] and that of other cetacean species with known reproductive impairment. Several examples suggest that exposure to OC insecticides and PCBs has affected endocrine function and reproduction in marine mammals. For example, transformation of epididymal and testicular tissue has been observed in north Pacific minke whales (*Balaenoptera acutorostrata*)[19]. Tumours and reproductive problems are documented in beluga whales of the St. Lawrence estuary, now among the most contaminated animals on earth[20,21]. De Guise *et al.*[22] reported a true hermaphrodite beluga whale. Here it is worth noting that levels of PCBs found in Mediterranean odontocetes sampled in the period 1992-1999 (striped dolphin, bottlenose dolphin and common dolphin, mean value = 54587 ng/g l.w.; 44924 ng/g l.w.; 25032 ng/g l.w. respectively)[23] are similar to those detected in hermaphrodite beluga whales of the St. Lawrence estuary (mean value = 78900 ng/g l.w.)[24]; moreover the levels of PCBs detected in Mediterranean fin whales in the same period (mean value = 7331 ng/g l.w.)[23] are approximately 10 times higher than those found in bowhead whales (*Balaena mysticetus*) with pseudohermaphroditism and other reproductive dysfunctions (mean value = 610 ng/g l.w.)[25,26]. This observation suggests the potential risk to which these species are exposed in the Mediterranean Sea (Figure2).

Figure 2: PCB levels in blubber of Mediterranean striped dolphin (A) and of Mediterannean fin whale (B). The broken line represent the threshold related to presence of true hermaphroditism in beluga whale (A) and the threshold related to presence of pseudohermaphroditism in bowhead whale (B).



In conclusion future research on stranded Mediterranean cetaceans will help to clarify potential effects of these chemicals on gonad integrity and the development of a series of non-lethal techniques to evaluate residue levels and biomarker responses is recommended for hazard assessment and conservation of endangered species of marine mammals exposed to OCs with ED capacity, instead of lethal approaches. The present results validate the use of skin biopsies as a suitable non-lethal biological material for the general assessment of exposure of Mediterranean cetaceans to OCs, enabling evaluation of OC levels with ED capacity and BPMO induction as an early warning sign of exposure to organochlorines.

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