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Short communication

Development of new-tools to investigate toxicological hazard due to endocrine disruptor organochlorines and emerging contaminants in Mediterranean cetaceans

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Abstract

The possibility that certain Mediterranean cetaceans are subject to toxicological hazard due to organochlorines and emerging contaminants, such as polybrominated diphenyl ethers (PBDEs) with endocrine disrupting capacity, was investigated using non-lethal methods. The need for new biomarkers for EDCs and for a "cell model" to explore the different susceptibilities to several classes of ECDs, including emerging contaminants, led us to culture fibroblasts of different cetacean species ("dolphins in test tubes"). We then explored interspecies and gender susceptibility to OC-EDCs and PBDEs using qualitative and semi-quantitative evaluation of target proteins, such as CYP1A and CYP2B in cultured cetacean fibroblasts (*Stenella coeruleoalba*, *Tursiops truncatus* and *Balaenoptera physalus*), by western blot and immunofluorescence techniques.

Keywords: Mediterranean cetaceans; Organochlorine contaminants; Brominated flame retardants; Endocrine disrupting chemicals; Fibroblasts cell culture; Immunofluorescence technique; Western blot

Mediterranean top predators, and particularly cetacean odontocetes, accumulate high concentrations of organochlorine contaminants (OCs) and this exposure may increase their risk of disease. Some organochlorine compounds are also known as endocrine

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disrupting chemicals (EDCs) (Fossi et al., 2003). General considerations on the potential hazard to these Mediterranean species can be drawn from comparison of the levels of OC-EDCs commonly detected in Mediterranean cetaceans and that of other cetacean species with known reproductive impairment. In fact several examples suggest that exposure to levels of OC insecticides and PCBs, commonly detected in Mediterranean odontocetes, has affected endocrine function and reproduction in other marine mammal species (Fossi and Marsili, 2003).

Moreover there is growing concern about accumulation and effects of emerging contaminants such as polybrominated diphenyl ethers (PBDEs) (a major family of flame retardants) in the food chain. PBDEe are lipophilic, persistent and toxic to fauna and humans (Alaee et al., 2003). The highest levels of PBDEs have been found in top marine predators, including Mediterranean odontocetes.

All these considerations orientated a decade of our ecotoxicological research in Mediterranean cetaceans towards field application of a powerful indicator of exposure to lipophilic contaminants, namely CYP1A1 induction (benzo(*a*)pyrene monooxygenase activity) in skin biopsies, and quantification of OCs in blubber, to assess different exposure of species, populations and genders to OCs in the Mediterranean Sea (Fossi et al., 1992, 2003; Marsili et al., 1998; Fossi and Marsili, 2003). Several questions remain still unanswered in ecotoxicological studies of Mediterranean cetaceans. The need for new biomarkers for EDCs and for a "cell model" to explore the different susceptibilities to several classes of ECDs, including emerging contaminants, led us to culture fibroblasts of different cetacean species as a non-lethal new investigation tool ("dolphins in test tubes"). In this study we evaluated three methodologies to detect cultured fibroblast responses to OC-EDCs and PBDEs: immunofluorescence technique,western blot and real time PCR. Here we present the preliminary results of the first two. We explored interspecies and gender susceptibility to OC-EDCs and PBDEs using qualitative and semi-quantitative evaluation of the target protein CYP2B in cultured cetaceans fibroblasts (*Stenella coeruleoalba*, *Tursiops truncatus* and *Balaenoptera physalus*). Particular attention was paid to the role of detoxification enzymes (CYP2B) and the related biochemical susceptibility of the different species to different classes of chemicals. The role of CYP2B in *in vitro* metabolism of two tetrachlorobiphenyl congeners were previously studied in beluga and pilot whale (White et al., 2000).

Sampling: Skin and blubber samples were obtained in striped dolphin, bottlenose dolphin and fin whale from the western Ligurian Sea, between Corsica and the French-Italian coast, using an aluminium pole armed with biopsy tips or biopsy darts launched with a crossbow (Fossi et al., 2003). All material was immediately placed in liquid nitrogen or stored in cell medium.

Sex identification: Cetacean gender was determined genetically according to Berube and Palsboll (1996).

Fibroblasts cell culture: The development of a non-lethal sampling method for obtaining viable tissue samples for cell cultures from skin biopsies of free-ranging cetaceans was described in Marsili et al. (2000). Successful cell cultures were obtained from striped dolphin, bottlenose dolphin and fin whale. The first fibroblasts were observed after 7–21 days. Cultures reached 90% confluence in 15–20 days, when they were trypsinized, washed and placed in Falcon 50 and 125 flasks, after two and three trypsinizations, respectively.

Experimental design: Fibroblast cultures (third generation) from striped dolphin ($n = 15$), bottlenose dolphin ($n = 2$) and fin whale ($n = 3$) were subjected to two different