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Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants

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Abstract

Various studies on Mediterranean cetaceans have revealed bioaccumulation of contaminants such as organochlorines (OCs) and heavy metals. The susceptibility of these animals to organic pollutants and the relationship between bioaccumulation and population decline (as in the case of *Delphinus delphis*) are unexplored fields. In this study, we used a non-destructive approach (skin biopsy) to explore OC bioaccumulation processes and mixed-function oxidase activity (BPMO) in four species of cetaceans: striped dolphin (*Stenella coeruleoalba*), bottlenose dolphin (*Tursiops truncatus*), common dolphin (*D. delphis*) and fin whale (*Balaenoptera physalus*). Significant differences in BPMO induction and OC levels were found between odontocetes and mysticetes, the former having mixed-function oxidase activities four times higher than the latter, binding with levels of OCs one order of magnitude higher in odontocetes. A significant correlation ($P < 0.05$) between BPMO activities and OC levels was found in *B. physalus*. In an ongoing project, fibroblast cultures have been used as an alternative in vitro method of evaluating interspecies susceptibility to contaminants such as OCs and polycyclic aromatic hydrocarbons (PAHs). These results suggest that cetacean skin biopsies are a powerful non-invasive tool for assessing ecotoxicological risk to Mediterranean marine mammals species.

Keywords: Marine mammals; Mediterranean Sea; Non-destructive biomarkers; Skin biopsy; BPMO activity; Cell culture

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In this paper we focus on the 'history' of the ecotoxicology of cetaceans from the 'perception' to the 'prediction' of toxicological risk to these marine mammals. In the 1970s the first scientific interest in the ecotoxicology of marine mammals was born. Toxic compounds, such as polyhalogenated aromatic hydrocarbons (PHAHs) and toxic metals were found to be biomagnified in the marine food-chain (Geraci & St. Aubin, 1980; Marsili & Focardi, 1996). Top predators such as marine mammals, and particularly odontocetes and pinnipeds, tend to accumulate high concentrations of these contaminants and are subject to high toxicological risk. In the late 1980s the biomarker approach is proposed to evaluate the ecotoxicological risk of these marine organisms (Brouwer, Reijnders & Koeman, 1989; Goksøyr, Tarlebø, Solbakken & Klungsøyr, 1985; De Swart et al., 1994); however there was no consideration to the fact that several species are threatened. During this period, most toxicological studies, including biomarker studies were based on marine mammals killed by hunting, tacitly approving this activity (Fossi & Marsili, 1997). The ecotoxicological risk to some species is also related to their 'biochemical vulnerability' to xenobiotic lipophilic contaminants. Tanabe and Tatsukawa (1992) report that "...these animals have a low capacity for degradation of organochlorines due to a specific mode of their cytochrome P450 enzyme system". Finally, in the early 1990s non-destructive biomarkers are proposed for hazard assessment, protection and conservation of endangered species of marine mammals (Fossi, 1994; Fossi & Marsili, 1997; Fossi, Marsili, Leonzio, Notarbartolo di Sciara, Zanardelli & Focardi, 1992; Marsili et al., 1998).

In this context, we explore the potential use of skin biopsy for the studying of Mediterranean cetaceans: from the 'perception' to the 'prediction' of their ecotoxicological risk. First of all, skin biopsy was used to explore organochlorine (OC) bioaccumulation processes and mixed-function oxidase (MFO) activity (BPMO) in four free-ranging species of Mediterranean cetaceans. Finally, as 'prediction' models, fibroblast cultures were employed as an alternative in vitro method for evaluating interspecies susceptibility (MFO responses, DNA damage) to Mediterranean contaminates such as OCs and PAHs (Marsili et al., 2000).

Subcutaneous tissue consisting of skin and blubber (about 1×2 cm) was obtained from *Stenella coeruleoalba, Tursiops truncatus, Delphinus delphis* and *Balaenoptera physalus*. Sampling was performed in the western Ligurian Sea (1992 until 1998), between Corsica and the French–Italian coast, and Ionic Sea using biopsy darts launched with a crossbow. The 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. To avoid the possibility of infection, the bolt tip was sterilised with alcohol before shooting. Biopsy specimens were taken in the dorsal area near a dorsal fin and on the upper part of the caudal peduncle. All material was immediately placed in liquid nitrogen. The small size of the biopsy samples (between 0.200 and 0.002 g) did not permit isolation of the microsomal fractions. BPMO activity was detected in whole tissue. 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 nitrogen and pulverizing in a Potter apparatus with ultrasound. BPMO activity was assessed

using the incubation mixture proposed by Fossi et al. (1992). Incubating each sample (plus the blanks) in a shaking bath for 2 h at 37°C. The activity was expressed in arbitrary units of fluorescence (A.U.F./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. Sample purification was carried out adding concentrated sulphuric acid to the extracts; after elimination of 'black' residues, the extracts were reconcentrated and purified by Florisil column chromatography. The analytical method used was high-resolution capillary gas chromatography with a Perkin-Elmer Series 8700 GC and a 63Ni ECD. Capillary gas chromatography revealed the presence of *op′*- and *pp′*-isomers of DDT and its derivatives, DDD and DDE, and about 30 polychlorinated biphenyl (PCB) congeners.

Comparing *S. coeruleoalba*, *T. truncatus*, *D. delphis* and *B. physalus*, significant differences in BPMO and OC levels were found between odontocetes and mysticetes, the former having MFO activities approximately four times higher than the latter, binding with levels of OCs one order of magnitude higher in odontocetes (Fig. 1A). Moreover, statistical correlation ($P < 0.05$) was found between BPMO activity and OC levels in *Balaenoptera* skin biopsies (Marsili et al., 1998) (Fig. 1B). High induction of BPMO may therefore be an early warning sign of exposure to endocrine disruptors such as OCs and potential alert of transgenerational effects, related to exposure of future generations via the placenta and milk. It is therefore a powerful 'prognostic' indicator of the health of cetacean populations.

How can we use the non-destructive biomarker approach to study interspecies susceptibility to contaminants in Mediterranean cetaceans? Are some cetacean species more sensitive than other to the toxicological effects of xenobiotic compounds or organic chemicals? The motivation behind these theoretical questions comes from the observation that Mediterranean species, such as the *D. delphis* (common until this century), have almost completely disappeared from the Mediterranean sea. In order to explore the role of detoxification enzymes, such as MFO system, in the different interspecies susceptibility to Mediterranean xenobiotic contaminants, we are running a project using fibroblast cell cultures of different species, as 'in vitro surrogate' (Fig. 1C). Samples of different species of Mediterranean cetaceans have already been collected in several parts of the Mediterranean. Skin biopsy samples are stored in a cell medium and taken to the lab within 24–36 h. Fibroblasts of the different species (*S. coeruleoalba, T. truncatus, D. delphis* and *B. physalus*) will be cultured, and when the number of cells is sufficient for the experimental treatment, interspecies differences in susceptibility to the main Mediterranean contaminants (PCBs, DDTs, PAHs) will be investigated using several in vitro biomarkers techniques. In the interpretation of the results we have take into consideration that in vitro studies cannot replace in vivo studies. Nevertheless, the difficulties associated with the species studied made the utilization of fibroblast cell culture the most powerful non-destructive method to study the 'biochemical bases' of the susceptibility of the different species.

In conclusion, skin biopsies of cetaceans can be used to assess ecotoxicological risk to Mediterranean species. Diagnostic and prognostic tools, based on skin

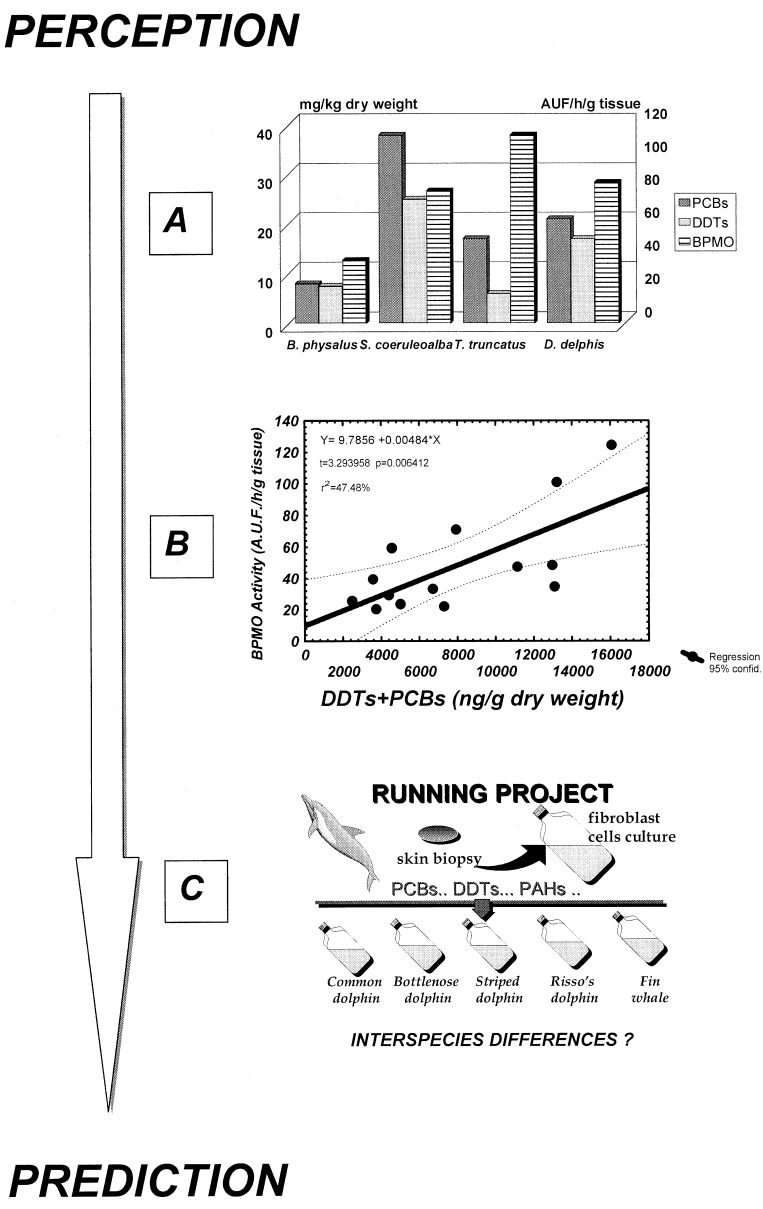

Fig. 1. From the 'perception' to the 'prediction' of ecotoxicological risk of Mediterranean cetaceans. (A) Organochlorine (OC) levels and mixed-function oxidase (BPMO) activities in skin biopsy samples of *Stenella coeruleoalba* ($n=18$), *Tursiops truncatus* ($n=1$), *Delphinus delphis* ($n=3$) and *Balenoptera physalus* ($n=14$) (means); (B) statistical correlation ($P<0.05$) between BPMO activity and OC levels in *B. physalus* skin biopsies; (C) experimental design of fibroblast cell cultures project (see explanation in text).

biopsies, can be used to help protect and conserve endangered species of marine mammals.

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