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Assessment of toxicological status of a SW Mediterranean segment population of striped dolphin (*Stenella coeruleoalba*) using skin biopsy

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Abstract

Various studies have revealed high concentrations of contaminants such as organochlorines (OCs) and heavy metals in Mediterranean cetaceans. A geographical trend of contamination (PCBs and DDTs) has been found for striped dolphin (*Stenella coeruleoalba*). In this study we used a non-lethal approach (skin biopsy) to investigate bioaccumulation of OCs, including polychlorobiphenyls (PCBs), DDTs, polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), trace elements (Hg, Cd, Pb) and CYP1A activity (BPMO) in nine striped dolphins sampled in the Aeolian area (Sicily – Italy) in summer 2002. The arithmetic mean value of BPMO activity in this group was 43.46 AUF/g tissue/h. This value is approximately 3 times and 5 times lower, respectively, than the value found in the Ionian and in the Ligurian groups. Skin biopsies of striped dolphins emerged as a suitable material for assessing the toxicological status of the various Mediterranean groups.

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In the Mediterranean environment, top predators, particularly cetacean odontocetes, accumulate high concentrations of organochlorines (OCs) and toxic metals, incurring high toxicological risk (Marsili, Casini, Marini, Regoli, & Focardi, 1997). A geographical contamination trend has been found in this basin for striped dolphin (*Stenella coeruleoalba*): PCB and DDT levels decreased from the north (Ligurian Sea) to south-east (Ionian Sea) (Marsili, 2000). Limited information exists on the toxicological status of south-west Mediterranean groups of this top predator.

In this research, we used a non-lethal approach (skin biopsy) to investigate bioaccumulation of OCs and trace elements (Hg, Cd, Pb) as well as CYP1A induction (BPMO) in nine specimens of *Stenella coeruleoalba* biopsied with a pole during a survey of the Aeolian area in summer 2002. This study is also the first attempt to investigate the presence of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) and co-planar PCBs in skin biopsies of cetacean species. One aim was to compare the resulting data with that of striped dolphin from other Mediterranean areas, obtained with the same methodological approach (Fossi et al., 2000).

Some OC compounds, now with world wide distribution, are known as endocrine disrupting chemicals (EDCs). Four types of organochlorine endocrine disruptors (Hilscherova, Machala, Kannan, Blankenship, & Giesy, 2000) are commonly found in Mediterranean cetaceans (Fossi et al., 2003): environmental estrogens, environmental androgens, anti-estrogens and anti-androgens. Endocrine disruptors act by mimicking sex steroid hormones, both estrogens and androgens, by binding to hormone receptors or influencing cell pathways (environmental estrogens and androgens), or by blocking and altering hormone receptor binding (anti-estrogens, anti-androgens). The relative estrogenic power of these chemicals, identified by in vitro and in vivo screening methods (Safe, 1995) is rather weak (10^{-3} or less) compared with the reference power of 17-estradiol or DES. However, the high levels of organochlorine compounds detected in mediterranean marine mammals, particularly in odontocetes, and consequently, the high levels of organochlorines with ED capacity, cannot be ignored.

The hypothesis that Mediterranean cetaceans, and particularly striped dolphin, are potentially exposed to the toxicological risk of organochlorines with endocrine disrupting capacity has been investigated using CYP1A induction in skin (Fossi et al., 2003). Induction of CYP1A is associated with exposure to polynuclear aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons. Both classes of compounds are known to initiate the down-stream cascade of events that results in transcription of CYP1A by binding to the aryl hydrocarbon receptor (AHR). The activity of this enzyme can be measured in liver microsomes by ethoxyresorufin-O-deethylase (EROD) and BPMO activities (Lewis, 1996) and in marine mammal skin, mainly by BPMO activity (Fossi et al., 2003).

Nine biopsy specimens of striped dolphins were collected using a biopsy pole in the Mediterranean Sea (southern Italy – Aeolian area) in summer 2002. A survey, assisted by ICRAM, was carried out in this area where no data on density and/or distribution were available. Sampled specimens could belong to a striped dolphin

population inhabiting southern Italy. Their reaction varied from a slight start to no reaction at all. The biopsy samples were immediately stored in liquid nitrogen.

The small size of the skin samples (between 0.20 and 0.02 g) did not permit isolation of microsomal fractions for CYP1A assay. Benzo(a)pyrene monooxygenase (BPMO) activity was detected in whole tissue following the procedure proposed by Fossi et al. (1992). BPMO activity was assessed using the incubation mixture proposed by Kurelec, Britvic, Rijavec, Muller, and Zahn (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/g tissue/h).

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 (2000). 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 ng/g d.w. Capillary gas-chromatography revealed *op'*- and *pp'*-isomers of DDT and its derivatives DDD and DDE, and about 30 PCB congeners.

Eight samples were pooled for PCDD/Fs and co-planar PCBs analyses. The analytical method involved a solid phase matrix dispersion (SPMD) procedure. Fractionation among the studied compounds and other possible interferences was achieved by using Supelclean™ Supelco ENVI™-Carb tubes. Resolution and quantification of PCDDs, PCDFs and co-planar PCBs were performed by HRGC-HRMS on a GC 8000 series gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a CTC A 200S autosampler and coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK), using a positive electron ionization (EI+) source and operating in the SIM mode at 10000 resolving power (10% valley definition). Chromatographic separation was achieved with a DB-5 (J&W Scientific, CA, USA) fused-silica capillary column (60 m × 0.25 mm ID, 0.25 μm film thickness) with helium as carrier gas in the splitless injection mode. Quantification was carried out by the isotopic dilution method (USEPA 1613, 1994). Methods blanks were routinely analysed, and low contributions were detected.

For heavy metal analysis, tissues were lyophilised, homogenised, weighed and then digested with HNO₃ in high-pressure vessels. Metal concentrations in samples were determined by atomic absorption spectrometry with graphite furnace (Pb, Cd, Cr) and flow injection mercury system (Hg) detection. The data were processed using Statistica 5.0 (Microsoft). Differences between groups of data were detected by *t*-test for independent samples (significance level: $p < 0.05$).

In Table 1 the values of CYP1A (BPMO) activity, organochlorine and trace elements levels detected in skin biopsy samples of specimens of *Stenella coeruleoalba* collected the Aeolian area are reported. The arithmetic mean value of BPMO activity in this group was 43.46 AUF/g tissue/h.

All 2,3,7,8-substituted PCDDs and PCDFs except OCDF were detected in the samples analysed. Total PCDD/F levels were 73.91 pg/g on a wet weight basis (w.w.). 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

Table 1

Levels of organochlorine contaminants, trace elements and CYP1A (BPMO) activity in nine specimens of striped dolphins sampled in the Aeolian area (S.W. Mediterranean Sea – Italy)

	BPMO (AUF/g/h)	HCb (ng/g d.w.)	DDTs (ng/g d.w.)	PCBs (ng/g d.w.)	Hg (µg/g d.w.)	Cd (µg/g d.w.)	Pb (µg/g d.w.)
Arithmetic mean	43.46	72.89	10043	13233	6.15	0.040	0.610
Median	32.05	39.06	10444	13991	5.36	0.040	0.400
Standard deviation	35.68	95.36	5309.8	6349.2	1.58	0.021	0.483
Standard error	11.89	31.78	1769.9	2116.4	0.53	0.007	0.161
Minimum	1.500	6.940	2187.5	3009.4	3.92	0.026	0.186
Maximum	117.3	305.6	18173	22714	8.38	0.090	1.490

abundant congener was OCDD which contributed 20% to total levels, followed by 1,2,3,4,6,7,8-HpCDD with a 12% percentage contribution. The remaining congeners in most cases, had a contribution under 5% as can be seen in Fig. 1(a). Coplanar PCBs (77, 126 and 169) were found at higher levels than PCDD/Fs, with a mean value of 372 ppt (pg/g wet weight), with PCB 77 being the congener with the highest levels. Calculated TEQs, based on TEF values for mammals proposed by WHO (Van den Berg et al., 1998), were 18 ppt (pg/g on a wet weight basis), considering PCDDs, PCDFs and co-planar PCBs. The lowest contribution to total TEQs came from PCDFs, while the contribution of PCDDs and co-planar PCBs was almost the same.

Literature data on heavy metal skin content in *S. coeruleolaba* are very spare. However Hg and Cd levels were found to be lower than those reported in specimens from French Mediterranean coasts and from other western Mediterranean areas (Augier, Bencoel, Chamlian, Park, & Ronneau, 1993; Monaci, Borrel, Leonzio, Marsili, & Calzada, 1998). Lead levels in skin biopsies were reported for the first time.

It is interesting to show the levels of CYP1A activities (BPMO) found in the Aeolian population, appear respectively three times and five times lower than the levels previously found (Marsili, 2000) in the Ligurian (199.7 AUF/g tissue/h) and Ionian (125.5 AUF/g tissue/h) striped dolphin groups (Fig. 1(b)). Moreover, organochlorine levels appear significantly higher in the Ligurian samples (Marsili, 2000) with respect to the Aeolian group.

These results suggest that: (a) the non-lethal approach (skin biopsy) is suitable for investigating bioaccumulation of OCs, including PCDDs and PCDFs, and trace elements, as well as CYP1A induction (BPMO) in free-ranging Mediterranean striped dolphin; (b) the Aeolian group seems the less exposed of the groups analysed, to potential effects of major OCs Mediterranean contaminants; (c) CYP1A (BPMO) induction may be an early sign of exposure to AH receptor agonist, some of which may also be endocrine disruptors including organochlorines, in marine mammals as previously suggest by other authors (Fossi et al., 2003; Stegeman, Miller, Beyer, Moore, & Goksøyr, 1998; Stegeman, 2000).

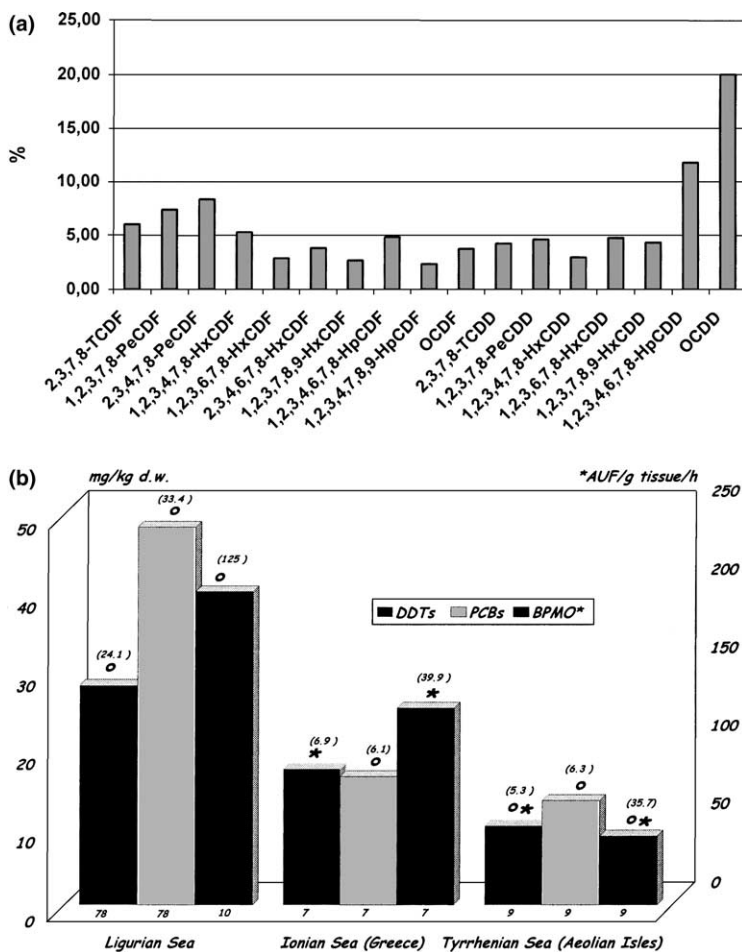


Fig. 1. (a) Percentage contribution of 2,3,7,8-substituted PCDDs and PCDFs in skin biopsy from striped dolphins (*Stenella coeruleoalba*). (b) Mean levels of total DDT and total PCB (mg/kg dry weight) and CYP1A (BPMO) activity (AUF/g tissue/h) in the biopsies of striped dolphins sampled in three different areas of Mediterranean Sea (Marsili, 2000). The significant differences (p -level < 0.05) between Ligurian group and the other two groups are marked with \circ and the significant differences (p -level < 0.05) between Ionian group and the other two groups are marked with $*$. Standard deviation is in brackets and number of samples are in the bottom.

In conclusion skin biopsies emerged as a suitable material for assessing the toxicological status of the various Mediterranean striped dolphins groups.

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