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The use of a non-lethal tool for evaluating toxicological hazard of organochlorine contaminants in Mediterranean cetaceans: new data 10 years after the first paper published in MPB

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Abstract

In the Mediterranean Sea, top predators, and particularly cetacean odontocetes, accumulate high concentrations of organochlorine contaminants and toxic metals, incurring high toxicological risk. In this paper we investigate the use of the skin biopsies as a non-lethal tool for evaluating toxicological hazard of organochlorines in Mediterranean cetaceans, presenting new data 10 years after the paper published by Fossi and co-workers [Mar. Poll. Bull. 24 (9) (1992) 459] in which this new methodology was first presented. Some organochlorine compounds, now with worldwide distribution, are known as endocrine disrupting chemicals (EDCs). Here the unexplored hypothesis that Mediterranean cetaceans are potentially at risk due to organochlorines with endocrine disrupting capacity is investigated. High concentrations of DDT metabolites and PCB congeners (known as EDCs) were found in the different Mediterranean species (*Stenella coeruleoalba*, *Delphinus delphis*, *Tursiops truncatus* and *Balaenoptera physalus*). In this paper we also propose benzo(a)pyrene monooxygenase (BPMO) activity in marine mammal skin biopsies (non-lethal biomarker) as a potential indicator of exposure to organochlorines, with special reference to the compounds with endocrine disrupting capacity. A statistically significant correlation was found between BPMO activity and organochlorine levels (DDTs, pp'DDT, op'DDT, PCBs and PCB99) in skin biopsies of males of *B. physalus*. Moreover a statistical correlation was also found between BPMO activity and DDT levels in skin biopsies of the endangered Mediterranean population of *D. delphis*. These results suggest that BPMO induction may be an early sign of exposure to organochlorine EDCs and can be used for periodic monitoring of Mediterranean marine mammal toxicological status.

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1. Introduction

Ten years ago, Fossi and coworkers suggested using biomarkers and chemical analysis, in skin biopsy material, as a non-lethal tool to evaluate toxicological hazard due to organochlorines in Mediterranean cetaceans (Fossi et al., 1992). This non-lethal approach has radically modified research methods (Fossi et al., 1992, 1997; Newman et al., 1994; Fossi and Marsili, 1997; Gauthier et al., 1997, 1999; Marsili et al., 1998; Boon et al., 2001; Bossart et al., 2002) providing an alternative

to using endangered marine mammals obtained by hunting (Folkow and Blix, 1992; Watanabe et al., 1998, 2002; O'Hara et al., 1999; Teramitsu et al., 2000; Severinsen et al., 2000). In this paper we reappraise the use of this non-lethal methodology in a wide range of species of Mediterranean cetaceans, presenting a large number of data 10 years after the original publication, in this journal, of the new methodology (Fossi et al., 1992).

The second main aim of this paper is to investigate the unexplored hypothesis that Mediterranean cetaceans are potentially at risk due to organochlorines with endocrine disrupting (ED) capacity. Endocrine disrupting chemicals (EDCs) have recently attracted much public and scientific attention (Colborn et al., 1993, 1996, 1998). This structurally diverse group of compounds

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may adversely affect the health of humans, wildlife and fisheries, or their progeny, by interaction with the endocrine system (Gillesby and Zacharewski, 1998). They include chemicals used heavily in the past, in industry and agriculture, such as polychlorinated biphenyls and organochlorine pesticides, as well as currently used chemicals, such as plasticizers and surfactants. Many known EDCs are estrogenic, affecting reproductive function in particular. These chemicals are especially detrimental during the embryonic, foetal and early postnatal periods because they mimic or interfere with hormones, growth factors and neurotransmitters (Colborn, 1998). Because of the persistent lipophilic nature of most xenobiotic estrogens and their metabolites, many bioaccumulate and biomagnify (Arukke et al., 1997). A wide range of man-made chemicals released into the aquatic environment are EDCs. Wildlife studies of fishes, alligators, turtles, gulls, terns, porpoises and whales link environmental contaminants with disturbed sex hormone production and/or effects. These effects have been associated with exposure to sewage industrial effluents, pesticides, ocean and river contamination, and aquatic food web (Arukke et al., 1997; Alleva et al., 1998).

Man-made EDCs range across all continents and oceans; some geographic areas, such as the Mediterranean Sea, are potentially more threatened than others. 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 (Aguilar et al., 2002). Mediterranean marine fauna could therefore be a target of EDCs. In this peculiar environment, top predators (such as large pelagic fish and marine mammals) tend to accumulate large quantities of organochlorine contaminants (OCs) and toxic metals (Corsolini et al., 1995; Marsili, 2002). The levels of OCs in a top predator of the Mediterranean, the striped dolphin (*Stenella coeruleoalba*), are 1–2 orders of magnitude higher than in Atlantic and Pacific dolphins of the same species (Marsili and Focardi, 1996). In stranded striped dolphins geometric mean levels of DDTs and PCBs from six Italian study areas were 101 ppm dry weight (d.w.) and 152 ppm d.w. respectively (Marsili, 2000). In other Mediterranean areas, levels of DDTs and PCBs in stranded striped dolphins were 71–456 ppm lipid basis (l.b.) and 67–846 ppm l.b. respectively (Alzieu and Duguay, 1979; Aguilar and Borrell, 1994). Levels of DDTs and PCBs in the Atlantic stranded specimens of the same species were 30–36 ppm l.b. and 39–59 ppm l.b. respectively (Taruski et al., 1975; Borrell, 1993a). In the same species stranded on Pacific coasts, DDTs levels were 21–43 ppm l.b. and PCBs 6–29 ppm l.b. (Tanabe et al., 1983; Loganathan et al., 1990). Without considering dolphins dying in Catalonia as result of *Morbilli-*

virus (Aguilar and Borrell, 1994), concentrations of these compounds in blubber of striped dolphins living in the Mediterranean are much higher than in specimens of the same species living in oceans.

All these information suggests that Mediterranean top predator species, and particularly cetacean odontocetes, are potentially “at risk” due to organochlorines with ED capacity. A warning of this hazard came from another group of Mediterranean marine organisms, a fish of commercial interest, the top predator swordfish (*Xiphias gladius*). This species was recently investigated by Fossi and collaborators (2001a, 2002) using vitellogenin (Vtg) and zona radiata proteins (Zrp) as diagnostic and prognostic biomarkers. Dramatic induction of typically female proteins (Vtg and Zrp) was detected by ELISA and Western Blot in some specimens of adult males of the species. It was also confirmed that Vtg and Zrp were more highly induced in adult male Mediterranean swordfish than in 25 Atlantic specimens. These results are a warning of the potential risk for the reproductive function of Mediterranean top predators, and suggest the need for continuous monitoring of this marine environment and particularly other Mediterranean top predator species such as marine mammals. If the scientific and ethical reasons imposed the use of only non-lethal methods, it is more difficult to investigate the ecotoxicological risk of free-ranging Mediterranean cetaceans. However we know that levels of organochlorines are 10–20 times higher in free-ranging striped dolphin of these seas (DDTs average value in blubber 26.65 µg/g a.w.; PCBs average value in blubber 46.67 µg/g f.w.; Marsili, 2000) than in swordfish (DDTs average value in blubber 2.13 µg/g f.w.; PCBs average value in blubber 1.97 µg/g f.w.; Ausili et al., 2000).

In order to understand the entity of the problem, in the present study we proposed the use of skin biopsy sampled with non-lethal technique as a biological material for hazard assessment of Mediterranean cetaceans exposed to organochlorines with ED capacity. Biopsy darting had been used in the Mediterranean since 1990 for several cetacean species (Fossi et al., 1992, 2000; Marsili and Focardi, 1996). The response of fin whales to the dart was studied and no change in behaviour was detectable in 80% of cases. Skin biopsy as certain advantages in toxicological studies (Fossi et al., 2001b):

- it enables a large number of samples to be obtained across a wide geographic range;
- it enables sequential samples to be obtained from the same animal if recognisable by photo-identification or other techniques;
- as biological material, it is suitable for residue analysis of polycyclic aromatic hydrocarbons (PAHs) and OCs, including dioxin group chemicals in blubber, and heavy metals in skin;

- it is suitable for analysis of biomarkers such as mixed function oxidase (MFO) induction by enzyme assay (BPMO), immunohistochemical assay (CYP1A1), DNA damage in skin;
- it is suitable for fibroblast cell cultures in skin.

2. Materials and methods

2.1. Sampling

In the summers from 1994 to 1998, subcutaneous blubber samples were obtained from Mediterranean cetaceans by the Tethys Research Institute and sent to the Department of Environmental Biology of Siena University for toxicological analysis. In the Ligurian Sea, samples of subcutaneous blubber were obtained from 33 free-ranging 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. To avoid the possibility of infection, the bolt tip was sterilised with alcohol before shooting. Biopsy specimens were taken in the dorsal area between the dorsal fin and the upper part of the caudal peduncle. 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. Eight striped dolphins were also sampled in the same sea. These dolphins 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 13 common dolphins (*Delphinus delphis*) and 7 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 reaction of cetaceans to sampling varied from a slight start to no reaction at all (Jahoda et al., 1996). The biopsy samples were immediately stored in liquid nitrogen.

2.2. Laboratory analysis

The small size of the biopsy samples (between 0.20 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. (1992). 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 N₂ and pulverizing in a Potter apparatus with ultrasound. BPMO activity was assessed using the incubation mix-

ture proposed by Kurelec 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 (AFU/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 and Focardi (1996). 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.

2.3. Data analysis

Data was processed using Statistica 5.0 (Microsoft). Differences between groups of data were detected by *t*-test for independent samples (significance level: $p < 0.05$), ANOVA (Kruskal–Wallis test; significance level: $p < 0.05$) and Kolmogorov–Smirnov test (significance level: $p < 0.1$). The Kruskal–Wallis test was applied to reveal any differences in variance. Because this test does not discriminate which groups differed or to what extent, the Kolmogorov–Smirnov test was used on pairs of samples. Precise information on the distribution of values was obtained by the Shapiro–Wilks *W* test of normal distribution. If the *W* test is significant ($p < 0.05$), the distribution cannot be normal. Although the variables had a non-normal distribution, we preferred the Pearson product-moment coefficient to estimate the linear relationship between two variables because theoretically, continuous random variables cannot be ranked. The non-parametric tests used to estimate possible correlations between our variables (e.g. Spearman rank order correlations) assume that the variables under consideration were measured on at least an ordinal (rank order) scale, that is, that the individual observations can be ranked in two ordered series. In other words this procedure uses the ranks of the data rather than the values themselves. We applied the Pearson test to find linear correlations between BPMO activity and ECDs in all samples. In samples with not showing linear correlation we applied the Spearman test to find other types of correlations. The Pearson and Spearman correlations were taken as significant at $p < 0.05$.

3. Results and discussion

Four types of organochlorine endocrine disruptors (Adami et al., 1995; Kelce et al., 1995; Vonier et al., 1996; Wong and Pessah, 1996; Hansen Larry, 1998;

Sohoni and Sumpter, 1998; Hilscherova et al., 2000) are commonly found in Mediterranean cetaceans (Aguilar and Borrell, 1994; Marsili, 2000) (Table 1): 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). Environmental estrogens are the most common and most widely studied EDCs (Colborn et al., 1993, 1996, 1998). The relative estrogenic power of these chemicals, identified by *in vitro* and *in vivo* screening methods (Safe, 1995; Environmental Agency, 1998) is rather weak (10^{-3} or less) compared with the reference power of 17-estradiol or DES (Miyamoto and Klein, 1998). However, the high levels of organochlorine compounds detected in marine mammals, particularly in pinnipeds and odontocetes, and consequently, the high levels of organochlorines with ED capacity, cannot be ignored.

Organochlorine concentrations (HCB, DDTs and PCBs) and BPMO activities, in the skin biopsies of odontocetes and mysticetes sampled in the Mediterranean Sea are reported as descriptive statistics (means and standard deviations) in Fig. 1A–D. Confirming literature data and results obtained in our lab before 1994 (Fossi et al., 1992; Borrell, 1993a,b; Marsili, 2000), marked differences in levels of all contaminants were recorded between fin whales and odontocete species (Fig. 1A–C).

The same was found for BPMO activity (Fig. 1D) but differences between fin whale and odontocete species were smaller. Only for striped dolphins was this difference remarkable. Different position in the food chain with odontocetes as terminal consumers and fin whales as macroplanktophages, is the main explanation for these results.

Storage differences in the analysed species were verified with two statistical tests: student's *t*-test (parametric) (significant for $p < 0.05$), and the non-parametric test of Kolmogorov–Smirnov (significant for $p < 0.1$) (Table 2). In terms of general contamination by OCs, the levels of DDT and PCB contaminants in fin whales were lower than in the odontocete species (Fig. 1B and C). Moreover a different interpretation of this data is possible if PCB congeners and DDT metabolites, with known endocrine disrupting properties, are separated into estrogenic and antiandrogenic on one hand and antiestrogenic and androgenic on the other. To do this, it is appropriate to express the data as percentages: pp/DDT, op/DDT, op/DDE and pp/DDE data as percentages of total DDT and 95, 99, 101, 118 and 153 congener data as percentages of total PCB. op/DDT and pp/DDE belong to both groups, but their principal activity is in the first group (Figs. 2A and B and 3).

It was interesting that the op/DDT% was much higher ($p < 0.05$) in fin whales than odontocetes. This DDT metabolite is a potent estrogen and antiandrogen and could theoretically interfere with the reproduction of this mysticete. Most large whales have a very low reproductive rate: females give birth to a single calf every 2–4 years. Considering the low calf production and the reproductive segregation of the Mediterranean fin whale population (Bérubé et al., 1998), estimated at about 3500 individuals (Forcada et al., 1996), even slight impairment of reproduction effectiveness could dramatically affect the status of the Mediterranean population.

In order to investigate the relationship between the BPMO activity and the different OCs with ED capacity, we valued the normal distribution of the variables with the Shapiro–Wilkes *W* test. Even though not all the variables distributions are normal, in order to estimate if a linear relationship between BPMO activities and OCs we used the Pearson product-moment coefficient. Later, in the samples non-linear correlated, we applied the Spearman test to estimate other types of possible correlations. The results are in Tables 3 and 4.

An interesting result with the Pearson test was a linear correlation between OCs known as endocrine disruptors and BPMO activity in striped dolphins and common dolphins. In striped dolphins a linear correlation was found between op/DDT/BPMO and PCB153/BPMO. In the common dolphin there were identified five linear correlations with the BPMO activity: DDTs, pp/DDE, op/DDT, PCBs and PCB153. Fig. 4A–E shows these correlations: total correlation (males and females continuous line), male correlation (broken line) and female correlation (dotted line). The main result in this species was non-induction of BPMO in females with increasing levels of contaminants. A similar result was obtained in fin whales sampled in the Ligurian Sea from 1992 to 1995 (Marsili et al., 1998). A statistically significant correlation was found between BPMO activity and organochlorine levels (DDTs/BPMO $p = 0.0319$; PCBs/BPMO $p = 0.0220$; DDTs+PCBs/BPMO $p = 0.0155$); in male skin biopsy specimens but not in females or males and females considered together. This difference in the inductive capacity of skin BPMO between males and females of this species is interesting but more research is required in order to explain it.

In the present paper, the only correlations found by the Spearman test were in fin whales between BPMO activity and DDTs, pp/DDT, op/DDT, PCBs and PCB99 (Table 4). These correlations were found in the whole group (males and females). There were only four male specimens and 14 females; the other was of unknown sex so males and females could not be analyzed separately as in previous papers (Fossi et al., 1999).

Correlations between OCs with ED capacity and BPMO activity in common dolphins and significantly higher percentage levels of op/DDT in fin whales than

Table 1
DDT metabolites and PCB congeners (IUPAC number—Ballschmiter and Zell, 1980) with known Endocrine disruptors capacity

	Activity	Activity references	Potency ^a	aER binding IC ₅₀ (μM) ^b	ER binding IC ₅₀ (μM) ^c	ER binding RBA (%) ^d
DDTs	Estrogen	Adami et al. (1995)				
pp'DDT	Estrogen Antiandrogen	Hilscherova et al. (2000) Adami et al. (1995) Wong and Pessah (1996) Nesaretnam et al. (1996)		>50*	>1000	
op'DDT	ER agonist Estrogen	Hilscherova et al. (2000) Adami et al. (1995) Hilscherova et al. (2000) Wong and Pessah (1996) Nelson (1974) Safe et al. (1991) Soto et al. (1995)	++	9.1	5	0.1
	Antiestrogen Antiandrogen	Sohoni and Sumpter (1998) Hilscherova et al. (2000) Sohoni and Sumpter (1998)	+ +++			
	ER agonist	Hilscherova et al. (2000)				
pp'DDE	Estrogen	Adami et al. (1995) Wong and Pessah (1996) Nesaretnam et al. (1996) Cooper and Kavlock (1997)	+	>50*	>1000	
	Antiestrogen	Hilscherova et al. (2000) Sohoni and Sumpter (1998)	+			
	Androgen Antiandrogen	Sohoni and Sumpter (1998) Hilscherova et al. (2000) Sohoni and Sumpter (1998) Safe (2000)	+ ++			
	ER agonist AR agonist AR antagonist	Hilscherova et al. (2000) Hilscherova et al. (2000) Hilscherova et al. (2000) Kelce et al. (1995)				
op'DDE	Estrogen	Wong and Pessah (1996) Safe et al. (1991)		37.25		
	ER agonist	Hilscherova et al. (2000)				
pp'DDD	ER agonist	Hilscherova et al. (2000)			>1000	
op'DDD	ER agonist	Hilscherova et al. (2000)		2.26		
PCBs		Adami et al. (1995) Hilscherova et al. (2000) Wong and Pessah (1996) Safe et al. (1991) Bergeron et al. (1994) Safe (1994)				
Arochlor 1260	Estrogen	Hilscherova et al. (2000) Soto et al. (1995) Matta et al. (1998)				
	Effect on sexual differentiation	Hilscherova et al. (2000) Soto et al. (1995) Matta et al. (1998)				
	Gonad abnor- malities	Hilscherova et al. (2000) Soto et al. (1995) Matta et al. (1998)				
95	Estrogen	Hansen Larry (1998) Sajjd (1996)	+			
99	Estrogen	Hansen Larry (1998) Sajjd (1996)	++			
101	Estrogen	Hansen Larry (1998)				<0.001

Table 1 (continued)

Activity	Activity references	Potency ^a	aER binding IC ₅₀ (μM) ^b	ER binding IC ₅₀ (μM) ^c	ER binding RBA (%) ^d
	Wolf et al. (1997)				
118	Antiestrogen Hansen Larry (1998) Wolf et al. (1997)	++			
153	Estrogen Hansen Larry (1998) Li et al. (1994)	+++			0.004

^a The most potent chemical for each activity has been assigned a potency of four plus (++++), and the potency of all the chemicals expressed relative to this (Hansen Larry, 1998; Sohoni and Sumpter, 1998).

^b Inhibitor concentrations necessary for 50% inhibition (IC₅₀) of [³H]17β-estradiol binding to estrogen receptors (aER) in alligators. aER binding IC₅₀ for 17β-estradiol was 0.0078 μM. *Compounds that inhibited [³H]17β-estradiol but were insoluble at concentrations necessary to achieve 50% inhibition (Vonier et al., 1996).

^c Inhibitor concentrations necessary for 50% inhibition (IC₅₀) of [³H]17β-estradiol binding to ER in rats. ER binding IC₅₀ for 17β-estradiol was 0.002 μM (Kelce et al., 1995).

^d Relative estrogen receptor-binding affinities (RBA). Competitive binding with estradiol in rat uterine ER preparations (Hansen Larry, 1998).

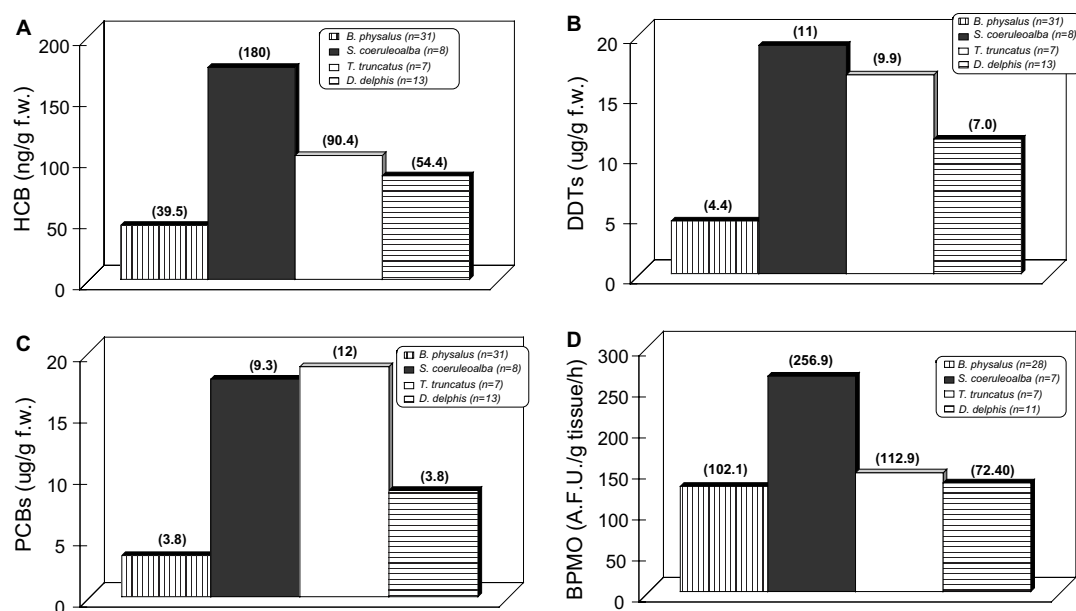


Fig. 1. (A–D) HCB, DDT and PCB concentration (ng/g and μg/g f.w.) and BPOM activity (AFU/g tissue/h) in skin biopsies from Mediterranean cetaceans. Arithmetic mean and S.D. in brackets; *n* = number of samples.

Table 2

Student *t*-test ($p < 0.05$) and Kolmogorov–Smirnov test ($p < 0.1$) for mean values of BPOM activity and OC contaminants between different pairs of cetaceans

	BPOM		HCB		DDTs		PCBs	
	T-student, <i>p</i>	Kol.–Smir., <i>p</i>	T-student, <i>p</i>	Kol.–Smir., <i>p</i>	T-student, <i>p</i>	Kol.–Smir., <i>p</i>	T-student, <i>p</i>	Kol.–Smir., <i>p</i>
<i>B. physalus</i> / <i>S. coeruleoalba</i>	0.035577*	<0.050*	0.000382*	<0.005*	0.000001*	<0.001*	0.000000*	<0.001*
<i>B. physalus</i> / <i>T. truncatus</i>	0.711982	>0.100	0.010531*	<0.100*	0.000007*	<0.005*	0.000000*	<0.005*
<i>B. physalus</i> / <i>D. delphis</i>	0.907899	>0.100	0.012318*	<0.100*	0.000210*	<0.005*	0.000285*	<0.001*
<i>S. coeruleoalba</i> / <i>T. truncatus</i>	0.288143	>0.100	0.356521	>0.100	0.663713	>0.100	0.858459	>0.100
<i>S. coeruleoalba</i> / <i>D. delphis</i>	0.137460	>0.100	0.119670	>0.100	0.086420	>0.100	0.007583*	<0.025*
<i>T. truncatus</i> / <i>D. delphis</i>	0.798742	>0.100	0.598688	>0.100	0.217835	>0.100	0.016249*	<0.100*

(*) = significant correlation.

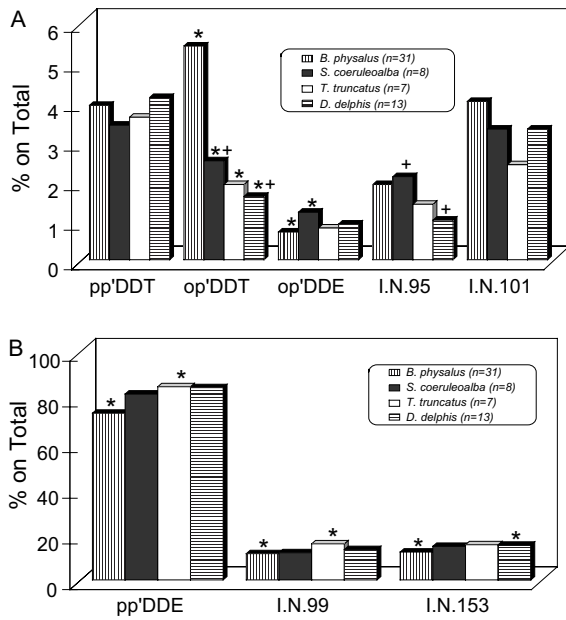


Fig. 2. (A and B) Organochlorine compounds (pp'DDT, pp'DDE, op'DDT, op'DDE, PCB95, PCB101, PCB99, PCB153) with known estrogenic and antiandrogenic activity (see Table 1) in skin biopsy specimens from Mediterranean cetaceans. Significant differences (Student *t*-Test, $p < 0.05$) between the species are indicated with * or +.

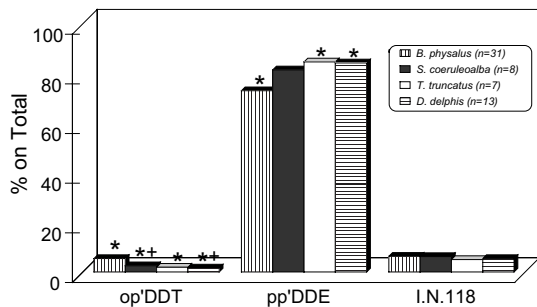


Fig. 3. Organochlorine compounds (pp'DDE, op'DDT, PCB118) with known antiestrogenic and androgenic activity (see Table 1) in skin biopsy specimens of Mediterranean cetaceans. Significant differences (Student *t*-Test, $p < 0.05$) between species are indicated with * or +.

odontocetes are signals of a potential toxicological stress in these two species even though total levels of these OCs were lower than in the other species studied. This hypothesis can only be verified when the real sensitivity of these species to these compounds is known. To obtain this information, it will be necessary to develop in vitro tests, because for ethical and conservation reasons, these mammals cannot be studied in vivo with lethal approach. Evaluation of oestrogen receptor (ER) capacity binding of different chemicals and detoxication enzyme responses of each species in fibroblast cultures could indicate the real levels of toxicological stress to which these species are subject (Fossi et al., 2000; Marsili et al., 2000).

Some general considerations on potential hazard to these Mediterranean species can be drawn from comparison of the data of the present paper 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*) (Fujise et al., 1998). Tumours and reproductive problems are documented in beluga whales of the St. Lawrence estuary, now among the most contaminated animals on earth (De Guise et al., 1995; Martineau et al., 2002). De Guise et al. (1994) reported a true hermaphrodite beluga whale. Here it is worth noting that levels of PCBs found in Mediterranean free ranging odontocetes sampled in the period 1992–1999 (striped dolphin, bottlenose dolphin and common dolphin, mean value = 54 587; 44 924; 25 032 ng/g l.w. respectively) (Fossi et al., in press) are similar or lower to those detected in the population of beluga whales of the St. Lawrence estuary in which was detected a hermaphrodite specimen (mean value = 78 900 ng/g l.w.) (Muir et al., 1996). Moreover in stranded striped dolphins the levels of PCBs are dramatically higher (mean value = 194 715 ng/g l.w.; Marsili, 2000) than the levels

Table 3

Pearson product-moment correlations between OCs with endocrine disruptors capacity and BPMP activity in the different species of cetaceans

	<i>B. physalus</i>			<i>S. coeruleoalba</i>			<i>T. truncatus</i>			<i>D. delphis</i>		
	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>
DDTs	27	-0.2875	0.146	7	0.6685	0.101	7	-0.3741	0.408	11	0.8040	0.003*
pp'DDT	27	-0.2700	0.173	7	0.6147	0.142	7	-0.2175	0.639	11	-0.0411	0.905
op'DDT	27	-0.2777	0.161	7	0.8210	0.024*	7	-0.6360	0.125	11	0.7610	0.007*
op'DDE	27	-0.2110	0.291	7	0.1408	0.763	7	0.1434	0.759	11	0.3248	0.330
pp'DDE	27	-0.2746	0.166	7	0.6648	0.103	7	-0.3681	0.417	11	0.8371	0.001*
PCBs	27	-0.2115	0.290	7	0.6766	0.095	7	-0.3784	0.403	11	0.6529	0.029*
95	27	-0.0968	0.631	7	0.0168	0.971	7	-0.2125	0.647	11	0.3506	0.290
99	27	-0.2432	0.222	7	0.6891	0.087	7	0.0539	0.909	11	0.4223	0.196
101	27	-0.1717	0.392	7	-0.0770	0.870	7	-0.2331	0.615	11	-0.0013	0.997
153	27	-0.1642	0.413	7	0.8665	0.012*	7	-0.5592	0.192	11	0.7239	0.012*

Correlations marked * were significant for $p < 0.05$. *n* = number of samples; *r* = Pearson correlation coefficient; *p* = significance level.

Table 4

Spearman rank order correlations between OCs with endocrine disruptors capacity and BPMO activity in the different species of cetaceans

	<i>B. physalus</i>			<i>S. coerulealba</i>			<i>T. truncatus</i>			<i>D. delphis</i>		
	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>
DDTs	27	-0.438339	0.022194*	7	0.000000	1.000000	7	-0.500000	0.253170	11	X	X
pp'DDT	27	-0.393162	0.042479*	7	0.250000	0.588724	7	-0.285714	0.534509	11	0.263636	0.433441
op'DDT	27	-0.411477	0.032973*	7	X	X	7	-0.642857	0.119392	11	X	X
pp'DDE	27	-0.325397	0.097675	7	0.000000	1.000000	7	-0.035714	0.939408	11	0.109091	0.749509
PCBs	27	-0.411477	0.032973*	7	0.178571	0.701658	7	-0.500000	0.253170	11	X	X
95	27	-0.156288	0.436291	7	-0.250000	0.588724	7	-0.142857	0.759945	11	0.172727	0.611542
99	27	-0.383394	0.048372*	7	0.178571	0.701658	7	-0.142857	0.759945	11	0.363636	0.271638
101	27	-0.322955	0.100365	7	-0.214286	0.644512	7	-0.285714	0.534509	11	-0.009091	0.978836
153	27	-0.278388	0.159702	7	X	X	7	-0.571429	0.180202	11	X	X

Correlations marked * were significant for $p < 0.05$. n = number of samples; r = Spearman correlation coefficient; p = significance level. X = significant correlation according to Pearson test.

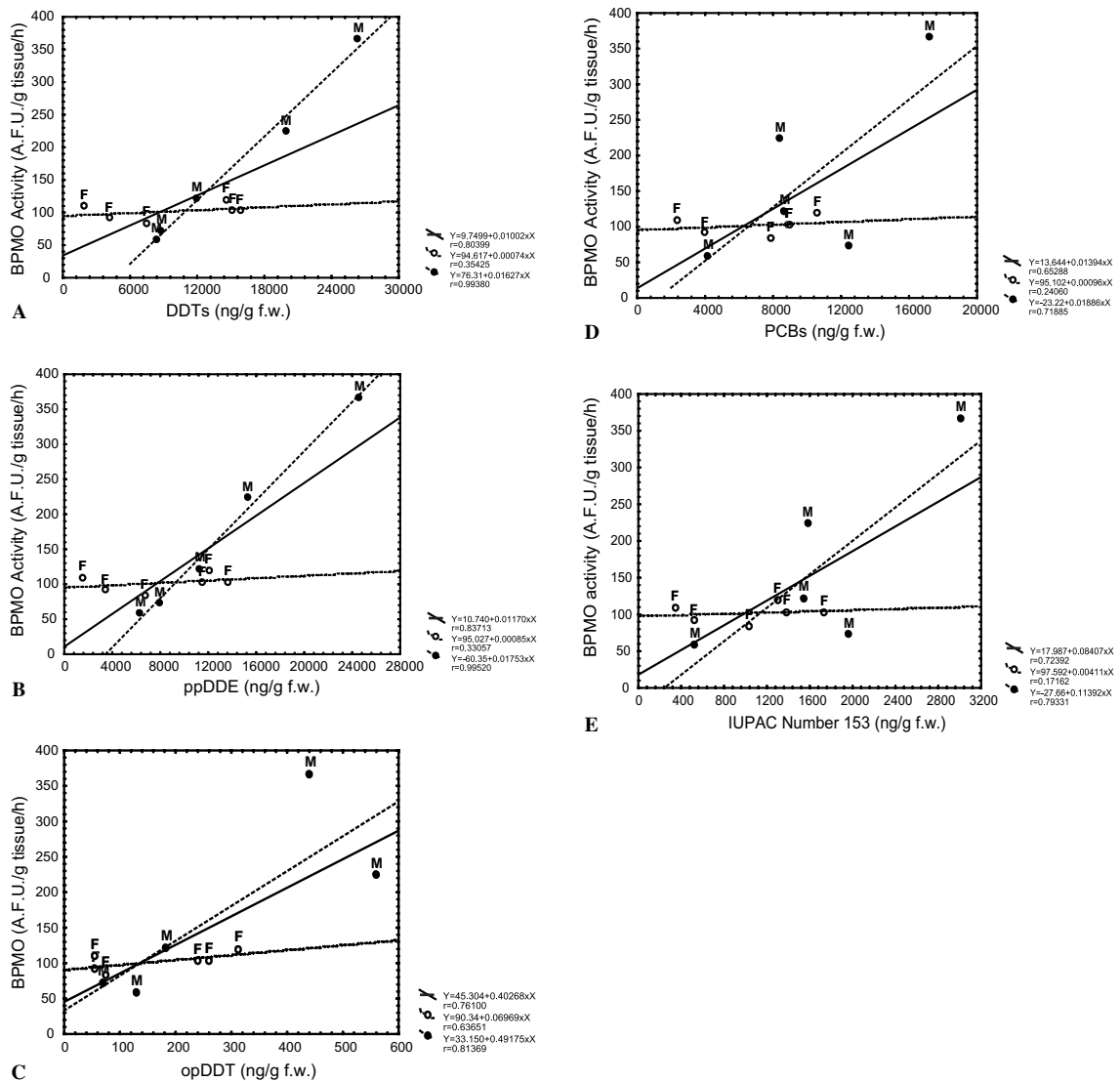


Fig. 4. (A–E) Linear correlations (Pearson test) in the common dolphin between BPMO activity and Total DDT, pp'DDE, op'DDT, Total PCB and PCB153. Total correlation (males and females, continuous line), male correlation (broken line) and female correlation (dotted line).

found in beluga whales of the St. Lawrence estuary. Levels of PCBs detected in Mediterranean free ranging fin whales in the same period (mean value = 7331 ng/g l.w.) (Fossi et al., in press) are approximately 10 times higher than those found in the population of bowhead whales (*Balaena mysticetus*) in which were detected pseudohermaphroditism and other reproductive dysfunctions (mean value = 610 ng/g l.w.) (Tarpley et al., 1995; Hoekstra et al., 2002a). This observation suggests the potential risk to which these species are exposed in the Mediterranean Sea. Future research on stranded animals will help to clarify potential effects of these chemicals on gonad integrity.

4. Conclusion

Some general conclusions can be drawn from the present results.

- Significant differences in total levels of organochlorine with ED capacity were found between odontocetes and mysticetes. Highest levels were found in striped dolphins, followed by bottlenose dolphins and common dolphins. Differences in organochlorine bioaccumulation, BPMO induction and consequently potential risk due to compound with endocrine disruptors capacity were primarily related to different positions in the marine food chain: of the Mediterranean cetaceans investigated, striped dolphins showed the highest levels of OCs and the highest biomarker response. Interspecies differences in susceptibility to OCs are the main question to consider: high levels of contaminants and high biomarker responses do not necessarily mean high risk for the species.
- Levels of the different OCs with endocrine disrupting properties need to be explored separately. The high percentage levels of op'DDT detected in fin whale samples is a finding worthy of further investigation (Fig. 2A). This DDT metabolite is a potent estrogen and antiandrogen and could affect the already low reproductive rate of this mysticete. Comparison of total DDT levels found in bowhead whales from Barrow, Alaska, in which two specimens showed pseudohermaphroditism (Tarpley et al., 1995), and those found in fin whales of the Mediterranean Sea is further evidence of the threat to these marine mammals. Indeed, the mean value of total DDT in 71 specimens of bowhead whales sampled in Barrow, Alaska, was 410 ng/g wet weight (Hoekstra et al., 2002b), whereas it was 5169 ng/g wet weight in 63 fin whales sampled in the Mediterranean Sea in 1992–1999 (Fossi et al., in press).
- One reason for this research was the preliminary observation that species such as the common dolphin have almost completely disappeared from the Medi-

terranean Sea. The high statistical correlation between BPMO activity and pp'DDE, op'DDT and PCB153 levels found in male common dolphins suggests that OCs with ED capacity may be one stress factor for common dolphin populations in the Mediterranean Sea.

- Interspecies differences in susceptibility to OCs with ED capacity are a major aspect to consider in this context. Future studies are planned into the role of detoxification enzymes and ER receptors in interspecies susceptibility to OCs contaminants. We are currently investigating fibroblast cultures of different species for testing interspecies differences in susceptibility to the main Mediterranean OCs with ED capacity (Marsili et al., 2000).
- 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 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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