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Theoretical models to evaluate hazard due to organochlorine compounds (OCs) in Mediterranean striped dolphin (*Stenella coeruleoalba*)

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Abstract

Many studies document the chemical stress related to organochlorine (OC) xenobiotics in Mediterranean cetaceans. The aim of this study was to establish a theoretical model to evaluate the hazard to Mediterranean striped dolphins (*Stenella coeruleoalba*) due to HCB, DDTs and PCB congeners. Differences in OC levels in blubber of stranded and free-ranging specimens enabled us to evaluate the hazard associated with different chlorinated xenobiotics, taking the live population as control sample, assumed to be in good health. For the most toxic compounds, with teratogenic, mutagenic, carcinogenic and endocrine disrupting capacity, we indicate levels beyond which there can be toxicological hazard for the striped dolphin. Using a mathematical formula derived from knowledge of the length and age of 62 stranded specimens, the age of dolphins was estimated and sexual maturity was identified at nine years. This evaluation was important for understanding differences in contaminant burden between males and females.

Keywords: Striped dolphin (*Stenella coeruleoalba*); Cetaceans; Chlorinated hydrocarbons; Mediterranean Sea; Hazard evaluation; Length/age correlation

1. Introduction

The striped dolphin (*Stenella coeruleoalba*) is the most common cetacean in the Mediterranean Sea, and therefore probably the most representative of this half-closed basin. This may also be the reason for the high frequency of strandings of this species along Italian coasts (Centro Studi Cetacei, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995). Using stranded specimens found in a good state of conservation, and sampling free-ranging dolphins by non-destructive methods, it has been possible to carry out many studies into the health status of Mediterranean striped dolphins from the toxicological point of view. Levels of contaminants such as organochlorines, polycyclic aromatic hydrocarbons and heavy metals have been measured in biological material of stranded and free-ranging specimens, and mixed function monooxygenase (MFO) activity has been measured in that of free-ranging dolphins (Aguilar and Raga, 1993; Aguilar and Borrell, 1994a,b; Marsili and Focardi, 1996, 1997; Marsili et al., 1996, 1998, 2001; Fossi and Marsili, 1997; Fossi et al., 1998, 1999, 2000; Reich et al., 1999; Marsili, 2000; Pertoldi et al., 2000). If this ecotoxicological data is not analysed together with parasitological, epidemiological, genetic and other studies, no conclusions can be made on the health status of the dolphins or on the cause of the death of stranded

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Table 1
Median levels (ng/g dry weight) of PCBs, PCB congeners, DDTs, DDT metabolites and HCB in free-ranging and stranded populations, and results of non-parametric Kruskal–Wallis test across the two groups (free-ranging and stranded populations)

Compounds	Stranded median (ng/g d.w.)	Free-ranging median (ng/g d.w.)	*p*-value	MCT	EDC
144 + 135	7734	369	<0.0001		
101	1383	492	<0.0001		+
128	1568	428	<0.0001		
206	411	112	<0.0001		
95	1577	470	<0.0001		+
196	2415	972	<0.0001		
174	3268	1272	<0.0001		
194	1408	512	<0.0001		
op′DDE	588	207	<0.0001	MCT	+
201	2035	784	<0.0001		
146	2449	1024	<0.0001		
195	1457	600	<0.0001		
180	11 508	5400	<0.0001	MCT	
151	1375	738	<0.0001		
178	1384	617	<0.0001		
170	7658	3485	<0.0001	MCT	
op′DDT	2640	1107	<0.0001	MCT	+
156 + 171 + 202	1390	567	<0.0001	MCT	
177	1837	912	<0.0001		
pp′DDT	4556	2442	<0.0001	MCT	+
153	15 219	7780	<0.0001	MCT	+
187	6010	2748	<0.0001		
PCBs	86 228	40 098	<0.0001		+
pp′DDD	3262	1639	<0.0001	MCT	+
DDTs	49 827	22 035	<0.0001	MCT	+
138	9181	5016	<0.0001	MCT	
pp′DDE	36 133	15 273	<0.0001	MCT	+
99	2202	1564	<0.0001		+
op′DDD	656	399	0.00020	MCT	+
199	208	138	0.0047		
HCB	314	185	0.0122		+
149 + 118	2741	2739	0.6799	MCT	+

Significance level for $p < 0.0001$. MCT = mutagenic, carcinogenic and teratogenic compound. EDC = endocrine disruptor compound.

dolphins without signs of external damage due to accidents with boats, fishing nets, etc. Other information is useful for understanding the history of a specimen; the following parameters are important: sex, age, nutritional status, reproductive status (and in the case of females, number of pregnancies and lactations), and health status. By measuring the levels of certain contaminants in organs and tissues of cetaceans, it is possible to establish to what extent these substances are accumulated by these mammals and their enzymatic response to contamination.

The aim of this study was to provide further information on the contamination status of Mediterranean striped dolphins due to organochlorines (HCB, DDTs and PCBs). Having verified the initial hypothesis, namely that OC levels in stranded specimens were significantly higher than in free-ranging dolphins (Table 1), we tried to establish possible hazard levels from OCs for this species in the Mediterranean, using appropriate statistical methods. To do this, we made the assumption that free-ranging dolphins were in good health and had OC levels reflecting background levels of the Mediterranean Sea (Impellizzeri et al., 1982; Geyer et al., 1984; Picer and Picer, 1991, 1994; Villeneuve et al., 1999).

2. Materials and methods

2.1. Free-ranging specimens

During the summers of 1990–1998, biopsy specimens of subcutaneous blubber of 102 Mediterranean striped dolphins, ranging free in the Ligurian, Tyrrhenian and Ionian Seas, were sent to the Department of Environmental Biology for toxicological analysis. The samples were obtained by the Tethys Research Institute with stainless steel cylindrical biopsy tips, containing a dentist's nerve retractor to retain the tissue, mounted on 2 m poles, and sterilised with denaturated ethyl alcohol before use. The dolphins were sampled in the dorsal area between the dorsal fin and the upper part of the caudal peduncle, from the prow of a boat while they rode the bow wave. Their reaction varied from a slight start to no reaction at all (Jahoda et al., 1996). All material was immediately stored in liquid nitrogen.

2.2. Stranded specimens

Most of the dolphins analysed were found dead along the Ligurian, Tyrrhenian and Ionian coasts in the period 1987–1994. Collection and transport of the carcasses was authorized and supervised by the Centro Studi Cetacei (Milan). The subcutaneous blubber used for this ecotoxicological study was from 89 striped dolphins.

2.3. Organochlorine contaminants

For analysis of HCB, DDTs and PCBs, the samples were freeze-dried and extracted with *n*-hexane in a Soxhlet apparatus followed by sulphuric acid clean-up and Florisil chromatography (Marsili and Focardi, 1997). The analytical method used was Perkin Elmer 8700 high resolution capillary gas chromatography (GC) with 63Ni electron capture detector and SBP-5 bonded phase capillary column (30 m long, 0.2 mm i.d.). The carrier gas was N_2 (head pressure 15.5 psi; split ratio 50/1) and the scavenger gas argon/methane (95/5; 40 ml/min). Oven temperature was 100 °C for the first 10 min, after which it was increased to 280 °C at 5 °C/min. Injector and detector temperatures were 200 and 280 °C respectively. A mixture of specific isomers was used to calibrate the system, evaluate recovery and confirm the results, which were expressed in ng/g or μg/g dry weight (d.w.). Recoveries were calculated by adding known quantities of standard to homogeneous replicates of the same sample. Extracted organic material (EOM%) was calculated in freeze-dried samples of stranded and free-ranging dolphins. The mean percentage of EOM% in blubber was 74% (SD = 20) in stranded striped dolphins and 88% (SD = 16) in free-ranging striped dolphins. The mean percentage water content in blubber was 35% (SD = 22) in stranded striped dolphins. The water content was calculated in the samples from stranded dolphins only, because the skin biopsy specimen was too small for this analytical procedure. Capillary gas-chromatography revealed the presence of *op*′- and *pp*′-isomers of DDT and its derivatives DDD and DDE, and about 30 PCB congeners.

2.4. Age determination of stranded dolphins

The age of 62 striped dolphins was determined by counting dentine growth layer groups in the teeth (Marsili et al., 1997). The teeth were prepared for examination as described by Lockyer et al. (1981) and Myrick et al. (1983).

2.5. Statistical analysis

The software used for statistical analysis was the SAS-System. The data was processed using multivariate and univariate statistical methods. The first aim of analysis was to check which HCB, PCB congeners and DDT compounds showed significant differences between free-ranging and stranded groups and between male and female stranded specimens. We used the Kruskal–Wallis test because normal distribution of the data could not be assumed. The Kruskal–Wallis test is used to test the null hypothesis that the sample groups are identical, against the alternative hypothesis that some of the sample groups generate observations that are larger than others. Empirically, in Table 1 we observe the *p*-values: smaller values indicate the rejection of the null hypothesis.

The second aim of analysis was to identify boundaries between the two groups of specimens (stranded and free-ranging). Since no assumptions were made about the distribution within each group we used canonical discriminant analysis (CDA), based on kernel normal probability densities (Klecka, 1980; Hand, 1982), to estimate group-specific densities. The canonical correlation coefficient, a measure of multiple correlation between the set of discriminant variables (the organochlorine contaminants selected for each of four groups of contaminants) and the corresponding canonical function, can have values between zero and one, larger values indicating increasing degrees of association. The squared canonical correlation represents the proportion of variation in the discriminant function explained by groups. Thus a high coefficient indicates a strong relationship between groups. The resubstitution summary table (classification matrix) is an indirect measure of the power of the canonical functions to discriminate between the two groups. However, because we classified the same entities that we used to derive the classification functions, the correct classification rates and chance-corrected classification criteria in Tables 2–5 and 7 are all biased upward. To assess the power of discriminant analysis we therefore used a jacknife validation based on resampling of the original data (see cross-validation summary in tables) (McGarigal et al., 2000). The prior probabilities equal to 50% represent the random chance of belonging to one of the two groups. The number of correct classifications divided by the total number of entities gives a measure of classification accuracy: the greater the correct classification rate with respect to the prior probabilities, the better the classification based on the discriminant variables.

The third aim of analysis was to forecast the variable "age" using available information on stranded dolphin

Table 2
Canonical discriminant analysis (CDA) using normal Kernel density for DDTs + PCBs

Group	Free-ranging	Stranded
Resubstitution summary		
Free-ranging	97%	3%
Stranded	46%	54%
Cross-validation summary		
Free-ranging	89%	11%
Stranded	53%	47%
Correct classification rate	0.74	
Classification Summary for striped dolphins sampled in 1990–1991		
	Free-ranging 1991	Stranded 1990–91
Free-ranging 1991	100%	0
Stranded 1990–91	14%	86%
Correct classification rate	0.93	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

Table 3
Canonical discriminant analysis (CDA) using normal Kernel density for OCs with known mutagenic, carcinogenic and teratogenic activity

Group	Free-ranging	Stranded
Resubstitution summary		
Free-ranging	100%	0
Stranded	2%	98%
Cross-validation summary		
Free-ranging	98%	2%
Stranded	37%	63%
Correct classification rate	0.80	
Classification summary for striped dolphins sampled in 1990–1991		
	Ionian Sea free-ranging	Ionian Sea stranded
Ionian Sea free-ranging	100%	0
Ionian Sea stranded	50%	50%
Correct classification rate	0.75	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

body length. Because age is related to length for complete cases (dolphins for which age and length were both known), we used a deterministic imputation method based on a regression model to compensate for missing values of the variable "age" (Kalton, 1983; Little and Rubin, 1987; Schafer, 1997). A non-linear regression model was set up using the data of complete cases and then used to impute missing values (Ratkowsky, 1983).

Table 4
Canonical discriminant analysis (CDA) using normal Kernel density for OCs with EDC activity known

Group	Free-ranging	Stranded
Resubstitution summary		
Free-ranging	98%	2%
Stranded	0	100%
Cross-validation summary		
Free-ranging	89%	11%
Stranded	19%	81%
Correct classification rate	0.86	
Classification summary for Ionian Sea striped dolphins		
	Ionian Sea free-ranging	Ionian Sea stranded
Ionian Sea free-ranging	86%	14%
Ionian Sea stranded	50%	50%
Correct classification rate	0.69	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

Table 5
Canonical discriminant analysis (CDA) using normal Kernel density for Toxic OCs with ED activity

Group	Free-ranging	Stranded
Resubstitution summary		
Free-ranging	98%	2%
Stranded	11%	89%
Cross-validation summary		
Free-ranging	89%	11%
Stranded	34%	66%
Correct classification rate	0.81	
Classification summary for Ionian Sea striped dolphins		
	Ionian Sea free-ranging	Ionian Sea stranded
Ionian Sea free-ranging	86%	14%
Ionian Sea stranded	100%	0
Correct classification rate	0.46	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

3. Results and discussion

In this study we assumed that free-ranging striped dolphins were "in good health" or, at least with Mediterranean background levels of OCs. The first important result was the significant difference in the mean values of single organochlorine compounds analysed in blubber between free-ranging and stranded dolphins. The results of the Kruskal–Wallis test are shown in Table 1. Levels of each organochlorine compound were higher in stranded animal group than in free-ranging group. The OCs quantified in blubber of biopsied dolphins were grouped in four different ways. The first group, "PCBs + DDTs", was indicative of total OC burden of a given animal. HCB was not included because although it differed between stranded and free-ranging dolphins, it did not do so to the required level of significance ($p < 0.0001$). The other three groups were created on the basis of certain properties of the compounds. These properties were mutagenicity–teratogenicity–carcinogenicity and capacity to disrupt the endocrine system of mammals. OCs with the first of these properties (Fishbein, 1975; De Voogt et al., 1990; Høyer et al., 1998; Dorgan et al., 1999; Porta et al., 1999; Aronson et al., 2000; Holford et al., 2000; Demers et al., 2002) formed the "toxic OCs" group. The third group, "ED OCs", consisted of endocrine disruptors (Colborn et al., 1993; Hansen, 1998; Høyer et al., 1998). The fourth group, "toxic and ED OCs", consisted of OCs with mutagenic–teratogenic–carcinogenic and endocrine disrupting properties.

The results of CDA to establish boundaries between the two groups (free-ranging and stranded) are shown in Tables 2–5. In models estimated in these tables, assignment by CDA was better than random assignment, since the correct classification rate was always greater than the prior probabilities. In other words, the canonical discriminant function worked quite well as classification rule. The parameter PCBs + DDTs (Table 2), a measure of "total input" of significant OCs in blubber, gave excellent cross-validation results for free-ranging dolphins with 89% of specimens classified in the free-ranging group. However, about 50% of stranded dolphins classified in the free-ranging group and the rest in the stranded group. In interpreting this result, it should be borne in mind that there was no data on the cause of death, so this result was not surprising.

The last part of Table 2 shows the classification (using the estimated model) of a set of 14 dolphins (7 free-ranging and 7 stranded) sampled in 1990 and 1991, a period characterised by a major die-off of dolphins along the Mediterranean coasts (Bortolotto et al., 1992; Aguilar and Raga, 1993). A virus of the genus *Morbillivirus* was identified in dolphins stranded in this period, and was probably one cause of the die-off (Domingo et al., 1991). Since PCBs depress the immune system and are toxic to the liver, they are regarded as a promoting factor for disease (Aguilar and Borrell, 1994a). For dolphins sampled in 1990 and 1991, 100% of free-ranging animals classified in the free-ranging group and 86% of stranded animals in the stranded group. These results confirmed that OCs may have played a role in the die-off.

Other interesting results were obtained for cross-validation for the three canonical correlations of Tables 3–5. For free-ranging specimens classified in the free-ranging group, the results were 98% for toxic OCs, 89% for ED OCs and 89% for toxic and ED OCs. For stranded dolphins classified in the stranded group the results were 63%, 81% and 66% respectively. The last part of Tables 3–5, shows the classification summary, based on the estimated model, of some dolphins sampled in the Ionian Sea (7 free-ranging and 6 stranded) considered to be one of the less polluted among the Italian seas (Marsili and Focardi, 1997). This assumption is also confirmed by the MED POL programme, which involves more than 100 Mediterranean research and monitoring institutions and has monitored the Mediterranean sea for the following parameters: mercury, cadmium, copper and zinc, organohalogen compounds, organotin, organophosphorus compounds, herbicides and fungicides as well as persistent synthetic material. The results have shown that pollution in the Mediterranean sea is limited to coastal areas where sources, such as industries and towns, are situated. The higher pollutant concentrations recorded in the north-western Mediterranean may be due to natural sources (for mercury) and regional industrial development (Gabrielides, 1995). For toxic OCs, 100% of free-ranging dolphins classified in the free-ranging group and only 50% of stranded dolphins were in the stranded group (Table 3). For ED OCs, 86% of free-ranging animals classified in the free-ranging group and 50% of stranded dolphins in the stranded group (Table 4). For toxic and ED OCs, 86% of free-ranging dolphins classified in the free-ranging group and no stranded dolphins in the stranded group (Table 5). These results confirmed that dolphins stranded on the coasts of the Ionian Sea were only relatively affected by OC contamination, with levels of these pollutants very similar to Mediterranean background levels for striped dolphins.

Theoretical models using the canonical variables (indicated with CAN) for the four groups of OCs (DDTs + PCBs, Toxic OCs, ED OCs, toxic and ED OCs) were established to evaluate the hazard of OCs in specimens of Mediterranean striped dolphin (Table 6). The canonical variable for each dolphin was obtained as a linear combination of the variables used in CDA, e.g. $\mathrm{CAN} = \beta_1\mathrm{var}_1 + \beta_2\mathrm{var}_2 + \cdots \beta_p\mathrm{var}_p$ where the parameters $\beta_1, \beta_2, \ldots, \beta_p$ are estimated by the canonical analysis procedure. The empirical analysis shows that all canonical equations estimated are significantly ($p < 0.001$). These theoretical models pinpointed three arbitrary intervals, which establish three areas of toxicological stress for Mediterranean striped dolphins due to OCs: low toxic stress (Mediterranean background levels), moderate toxic stress (uncertainty) and high toxic stress (hazard). The upper limit of the first was the minimum value of CAN of stranded specimens, that of the second was the maximum value of CAN of free-ranging specimens, the third began above the upper limit of the second. The equations for the evaluation of potential

Table 6
Theoretical intervals for the evaluation of potential hazard to Mediterranean striped dolphins from PCBs + DDTs, toxic OCs, ED OCs, toxic and ED OCs

	Mediterranean background levels	Uncertainty	Hazard
DDTs + PCBs	CAN value < 0.47	CAN value = 0.47	CAN value > 0.47
Toxic OCs	CAN value < −1.16	−1.16 ⩾ CAN value ⩽ 0.89	CAN value > 0.89
ED OCs	CAN value < −0.60	−0.60 ⩾ CAN value ⩽ 1.25	CAN value > 1.25
Toxic and ED OCs	CAN value < −0.60	−0.60 ⩾ CAN value ⩽ 0.16	CAN value > 0.16

hazard for Mediterranean striped dolphins due to the four classes of contaminants are reported below:

1. DDTs + PCBs

$$\text{CAN (Potential Hazard)} = (9.51 \times 10^{-6} \times \text{PCBs} + 4.40 \times 10^{-6} \times \text{DDTs}) - 0.92$$

2. Toxic OCs

$$\begin{aligned}\text{CAN (Potential Hazard)} &= [2.04 \times 10^{-4} \times op'\text{DDE} \\ &+ 2.42 \times 10^{-4} \times op'\text{DDT} \\ &+ 3.44 \times 10^{-6} \times pp'\text{DDE} \\ &+ 4.84 \times 10^{-5} \times pp'\text{DDT} \\ &+ 1.21 \times 10^{-4} \times \text{PCB153} \\ &+ 1.17 \times 10^{-4} \times \text{PCB180} \\ &+ 1.45 \times 10^{-4} \times \text{PCB170} \\ &+ (-3.42) \times 10^{-4} \times pp'\text{DDD} \\ &+ (-2.77) \times 10^{-4} \times \text{PCB138} \\ &+ (-8.40) \times 10^{-4} \times \text{PCB156} + 171 + 202] \\ &- 0.55\end{aligned}$$

3. ED OCs

$$\begin{aligned}\text{CAN (Potential Hazard)} &= [2.35 \times 10^{-4} \times op'\text{DDT} \\ &+ 2.40 \times 10^{-5} \times pp'\text{DDE} \\ &+ 5.74 \times 10^{-5} \times pp'\text{DDT} \\ &+ 1.77 \times 10^{-3} \times \text{PCB101} \\ &+ 3.46 \times 10^{-6} \times \text{PCB95} \\ &+ (-9.82) \times 10^{-4} \times op'\text{DDE} \\ &+ (-3.13) \times 10^{-4} \times pp'\text{DDD} \\ &+ (-4.09) \times 10^{-5} \times \text{PCB153} \\ &+ (-2.72) \times 10^{-4} \times \text{PCB99}] - 0.83\end{aligned}$$

4. Toxic and ED OCs

$$\begin{aligned}\text{CAN (Potential Hazard)} &= [3.89 \times 10^{-4} \times op'\text{DDE} \\ &+ 1.81 \times 10^{-4} \times op'\text{DDT} \\ &+ 7.58 \times 10^{-6} \times op'\text{DDE} \\ &+ 1.62 \times 10^{-5} \times pp'\text{DDT} \\ &+ 1.27 \times 10^{-5} \times \text{PCB153} \\ &+ (-2.35) \times 10^{-4} \times pp'\text{DDD}] - 0.67\end{aligned}$$

In formulating the above models we did not consider morphological parameters such as sex, age, length, weight, etc. These parameters were known for many stranded specimens but not for biopsied dolphins. Some of these parameters, such as age and sex, are particularly important for the study of OC accumulation and distribution in tissues, because females lose more than 90% of their body burden of OCs during pregnancy and lactation (Tanabe et al., 1981, 1982). What we were able to do was to evaluate any differences in accumulation of OCs in blubber between stranded males and females, in relation to age, in other words differences due to reproduction by females. Males and females reach the sexual maturity at about 9 years (Bryden, 1986; Notarbartolo di Sciara and Demma, 1994). Using age data of 62 stranded specimens to obtain a dolphin growth curve (Marsili et al., 1997), a model for age determination when body length is known was obtained iteratively by a least squares procedure.

The model is

$$Y = 0.051\,e^{(0.028 \times X)}$$

where Y is age (years) and X is length (cm) (Fig. 1). The model fit quite well the experimental data. This model was used to impute the age of 64 dolphins analysed for OCs in blubber. In a further 25 dolphins analysed for OCs in blubber, age had been determined by counting dentine growth layer groups in teeth. OC levels in blubber, divided into the four groups DDTs + PCBs, Toxic OCs, ED OCs, Toxic and ED OCs, were then plotted against age, for 89 stranded specimens, taking sex as discriminant factor (Fig. 2A–D). The results confirmed that males and females had similar OC

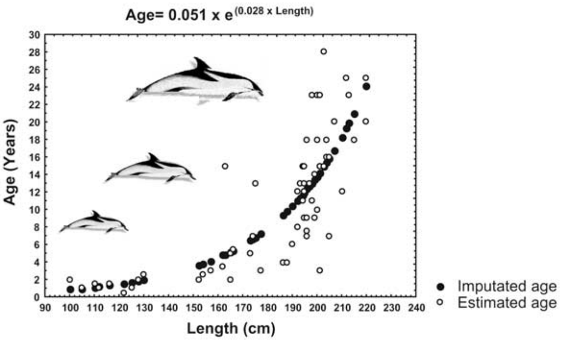

Fig. 1. Estimated model (full black points) for age determination in Mediterranean striped dolphins. Empty black points = age determined with the teeth.

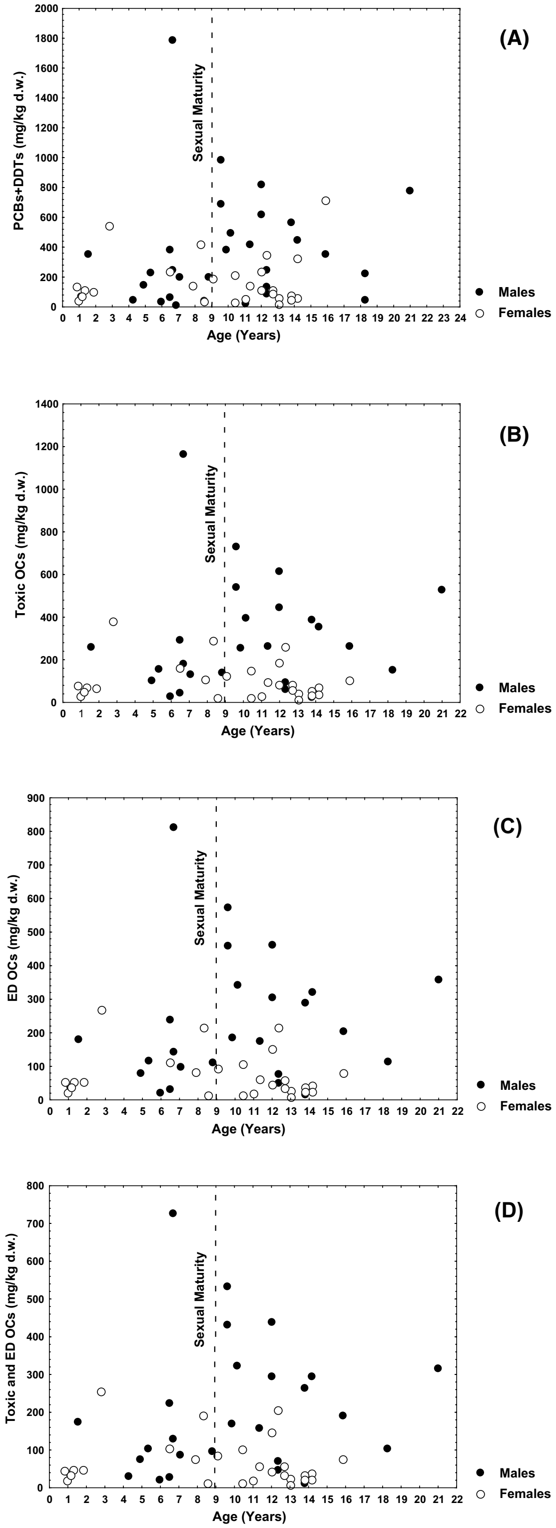

Fig. 2. A, B, C and D—plot of different group of OCs in relation to estimated age and sex. A = PCBs + DDTs; B = known toxicity; C = ED OCs; D = toxic and ED OCs.

accumulation until sexual maturity (9 years), above this age, males were generally located in the upper part of the distribution. A comment is warranted on the type of endocrine disrupting compounds composing the group of Fig. 2C, in relation to statistical analysis with sex as discriminant factor. *pp*′DDT, *op*′DDT, *pp*′DDE and *op*′DDE and PCB congeners 95, 99, 101 and 153 are EDCs with known estrogenic and anti-androgenic capacity, which could affect male reproductive processes (Adami et al., 1995; Wong and Pessah, 1996; Hansen, 1998; Sohoni and Sumpter, 1998; Hilscherova et al., 2000; Safe, 2000; Fossi et al., 2001). *pp*′DDE and *op*′DDT are also androgenic and anti-estrogenic and could affect female reproductive processes, but they are predominantly estrogenic and anti-androgenic (Sohoni and Sumpter, 1998; Hilscherova et al., 2000; Safe, 2000). These pollutants are major sources of hazard due to OCs in male striped dolphins. With regard to differences in the quantities and percentages of these compounds in males and females, again the Kruskal–Wallis test showed significantly different results in the two groups (besides higher levels in males than females) ($p < 0.005$).

The boundaries between the male and female groups for estrogenic and anti-androgenic compounds, analysed by CDA (Table 7), showed excellent cross-validation results for females with 82% of specimens classified in the female group, but not for males with only 42% of specimens classified in the male group. Male dolphins did not all seem to be affected in the same way by EDCs. This was to be expected, since their inputs of OCs varied with geographic area, age, nutritional status, reproductive status and so forth. We can also establish a theoretical model for these compounds for male striped dolphins. In this case, the females were considered to have Mediterranean background levels of estrogenic and anti-androgenic OCs. This model indicated that a CAN < 0.19 was associated with low stress for male dolphins due to estrogenic and anti-androgenic OCs whereas CAN < 0.19 was associated with hazard. The

Table 7
Canonical discriminant analysis (CDA) using normal Kernel density for OCs with estrogenic and anti-androgenic activity known

Group	Females	Males
Resubstitution summary		
Females	100%	0%
Males	0%	100%
Cross-validation summary		
Females	81%	19%
Males	62%	42%
Correct classification rate	0.62	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

equation for potential hazard to Mediterranean male striped dolphins from estrogenic and anti-androgenic compounds is

$$\begin{aligned}\text{CAN (Potential Hazard)} &= [1.29\times10^{-3}\times op'\text{DDE}\\ &\quad+(-1.60)\times10^{-4}\times op'\text{DDT}\\ &\quad+1.84\times10^{-5}\times pp'\text{DDE}\\ &\quad+(-1.42)\times10^{-4}\times pp'\text{DDT}\\ &\quad+(-3.47)\times10^{-5}\times\text{PCB153}\\ &\quad+6.83\times10^{-4}\times\text{PCB101}\\ &\quad+(-3.29)\times10^{-4}\times\text{PCB95}\\ &\quad+(1.15)\times10^{-4}\times\text{PCB99}]-1.18\end{aligned}$$

4. Conclusions

- Assuming the free-ranging population of striped dolphins to be in "good health", or at least with Mediterranean background levels of OCs, significant differences in mean values of single organochlorine compounds in blubber were found between free-ranging and stranded specimens. The results showed that OCs may have played a role in the epizootic which occurred in the Mediterranean in 1990 and 1991, and that the dolphins stranded in the Ionian Sea were relatively unaffected by OC contamination, showing OC levels very similar to Mediterranean background levels for Mediterranean striped dolphins.
- Four theoretical models were estimated to evaluate potential stress from OCs in Mediterranean striped dolphins. The respective canonical equations can be used to evaluate the potential hazard due to OCs for a generic Mediterranean striped dolphin, after analysis of subcutaneous blubber for these compounds.
- A model to evaluate the age of Mediterranean striped dolphins when length is known was developed: Age (years) $= 0.510\,e^{[0.0280\times\text{length (cm)}]}$. This model is clearly only valid for the juvenile age segment of the population. In adulthood, the correlation between age and length is obviously lost, making prediction unreliable. Here our concern was to determine whether dolphins were younger or older than 9 years (i.e. whether they had reached sexual maturity). The model was more than adequate for this purpose.
- Significant differences between males and females were found in stranded specimens for OCs with known estrogenic and anti-androgenic capacity. Their levels were significantly higher in males than females ($p < 0.05$). A theoretical model for the evaluation of the potential hazard for Mediterranean male striped dolphins due to estrogenic and anti-androgenic compounds was estimated.

In conclusion, the main result of the present study was to obtain a different key for interpreting data on OC accumulation in blubber of Mediterranean striped dolphins. This was made possible by combining classical toxicology with applied statistics. Since evaluation of health depends on many factors (such as nutrition, reproductive status, pregnancies, lactation, parasite load, age, diseases), not only on exposure to xenobiotics, clearly the only information that can be obtained from this multidisciplinary approach is a theoretical evaluation of stress to Mediterranean striped dolphins due to certain types of OCs. For dolphins sampled by means of biopsy, it is possible to determine whether a particular animal is in the range of OC contamination of most specimens of the Mediterranean population, or whether it is in state of hazard due to OCs. For dead dolphins, it is only possible to determine the extent to which OCs may have influenced the causes of death; they certainly cannot be regarded as the main cause. Again we stress that these models are only valid for Mediterranean striped dolphins. Indeed, various studies have shown that OC levels are much lower in ocean dolphins (Aguilar et al., 2002). This means that comparison of free-ranging ocean and Mediterranean dolphins would have indicated that the latter are in a state of toxicological hazard due to OCs. This is why we considered OC levels in Mediterranean free-ranging dolphins to be in the range of background contamination for the Mediterranean Sea. A task for future research will be to obtain models valid for striped dolphins in general, no matter where they live.

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