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Baseline

PCBs and DDTs in *Stenella coeruleoalba* dolphins from the French Mediterranean coastal environment (2007–2009): Current state of contamination

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ABSTRACT

Organochlorinated compounds including PolyChloroBiphenyles, Dichloro-DiphenylTrichloroethan and metabolites are determinated in *Stenella coeruleoalba* ($n = 37$) stranded on the french Mediterranean coasts from 2007 till 2009. Studies are carried out on lung, muscle, kidney, liver, and blubber. The sought-after compounds are all detected to variable levels in each tissue and organ. In general, total PCBs are the most abondant, followed by total DDTs. The concentration (in ng g^{-1} of lipid weight) in blubber of *S. coeruleoalba*, varied from 2,052 to 158,992 for PCBs and from 1,120 to 45,779 for DDTs. The ratios DDE/tDDTs are higher than 80% in almost all samples. The overall results of this work, compared to previous studies concerning the Mediterranean Sea, seems to confirm the tendency to a decrease of the contamination by organics compounds for the cetaceans in the Western Mediterranean Sea.

Due to their toxicity and their classification as persistent organic pollutants (Drinker et al., 1937), DDTs and PCBs use was regulated, in France, since 1973. After their release in the natural environment, most of pesticides, just as PCBs, are strongly accumulated by living organisms (Nakata et al., 1998; Tanabe et al., 1994a,b). Thus, Organisms at the top of the food chain such as cetaceans can be considered as actual indicators of the level of contamination of their natural environment.

In this work, we report levels of PCBs and DDTs in 37 specimens of *Stenella coeruleoalba* found stranded along the French Mediterranean coast between 2007 and 2009 years. Fig. 1 indicated the stranding places as well as the main oceanic currents in the Mediterranean Sea. The map shows that the coastal Liguro-Provencal current baths the north of the Genoa Gulf, the French Riviera and the Rhône gulf and carries pollution from Italian industrial areas heavily polluted, such as the Neapolitan coast (Magnani et al., 1991; Piérard et al., 1996; Wafo et al., 2006).

The collection of tissues and organs was performed by the French Mediterranean Cetacean Study Group (GECEM). Since the dolphins' teeth were not sampled by the GECEM, the sexual maturity has been determinated according to the data of Alzieu and Duguy, 1979; Cardellicchio et al., 2002. There are 19 males, 13 females and 5 young dolphins. The size and sexual maturity of these individuals as well as the date and place of stranding are presented in Table 1. The studies are achieved on the blubber, the liver, the muscle, the kidney, and the lung of the cetaceans.

One of the objectives of this study is to assess the current status of the dolphins'contamination by organochlorine compounds, in the French Mediterranean coastline region. We have also examined the repetition of the contamination in the different organs studied and compared the level of contamination according to age and sex using variance analysis (for statistical analyses, the level of Statistical Significance is $p < 0.05$ (normal distribution)). Evolutions in the profiles of congeners (PCBs and DDTs) have been studied depending on the different organs.

Freeze-dried sample was soxhlet extracted with hexane and the extract underwent liquid chromatography on a column containing silica gel and alumina following the procedures described by Wafo et al., 2005. All the analyses were performed in our laboratory (COFRAC accreditation n°1–1234 since 2001). The laboratory participates regularly to interlaboratory comparison exercises and data from our laboratory were in good agreement with those of reference materials. For this study, we used, as certified material, an

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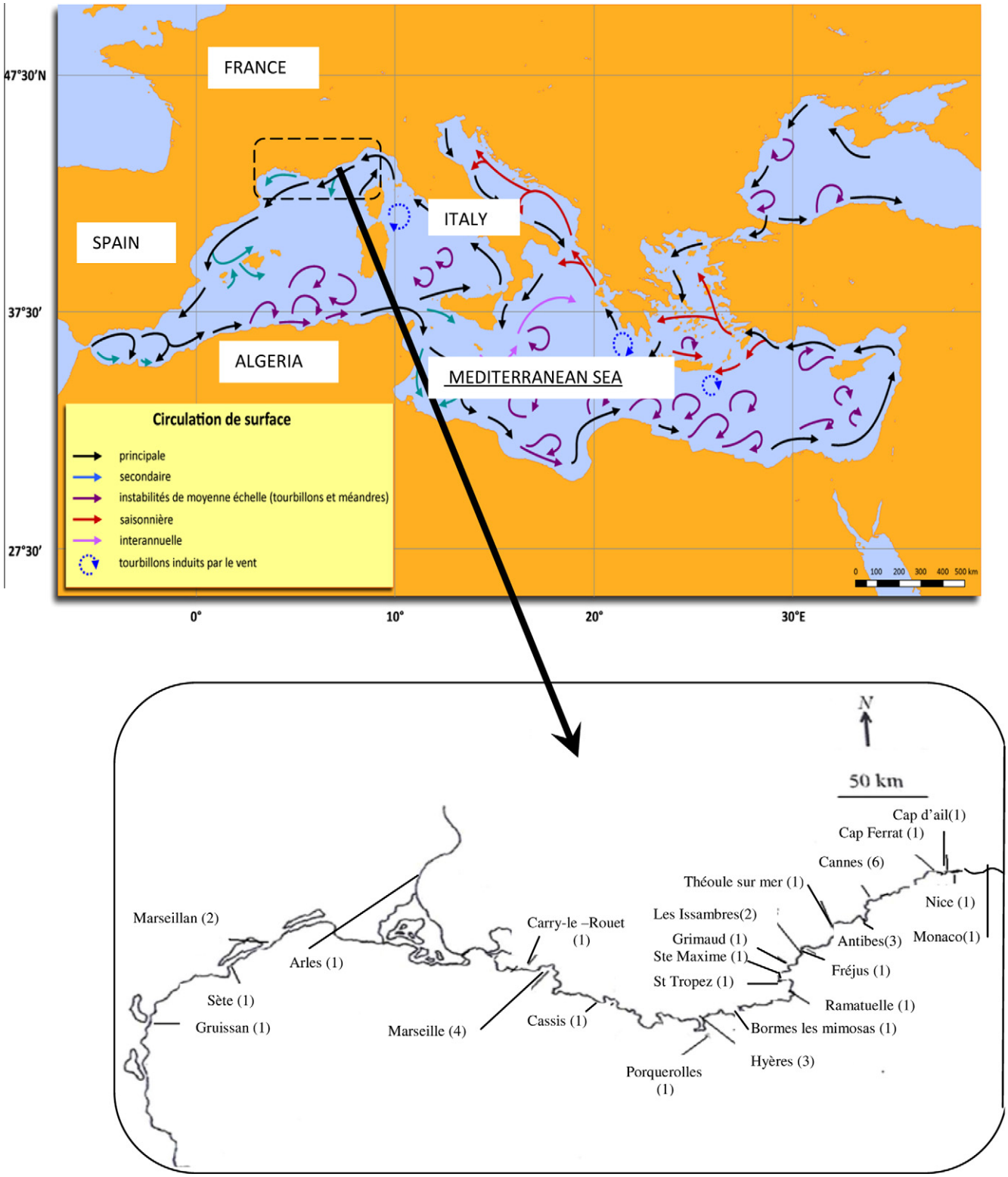

Fig. 1. Map showing the main sampling locations (main ocean currents according to Millot, 2005).

IAEA/UNEP intercomparison sample of Tuna homogenate (IAEA-435), which was distributed to world-wide laboratories in October 2004 (Villeneuve et al., 2006). In each batch, a blank and a certified material were systematically introduced in order to validate the results obtained. For quality insurance and quality control, 7 IUPAC congeners (28, 52, 101, 118, 138, 153 and 180) were analyzed as well as all the pesticides detailed previously. The results obtained for the reference materials were used to plot control charts and to decide upon acceptance or rejection of the data produced for each sample batch. Rejected batches were reanalyzed. Ten replicated of IAEA 435 were conducted on the same day, in order to determine the average recovery for each PCBs'congener and each pesticide: results for the PCBs' congeners varied from 89 to 93 percent and for pesticides from 85 and 101 percent. The detection limits range

Table 1
Codes and characteristics of the *Stenella coeruleoalba* (total = 37) M = Male, F = Female, Y = Young.

Codes	Stranding Years	Stranding Areas	Area codes	Sex	Length (cm)
63	2007	Nice	06000	M	163
61	2007	Cannes-Bocca	06150	F	165
62	2007	Cap d'Ail	06320	F	208
36	2007	Cannes	06400	F	200
58	2007	Cannes	06400	F	143
59	2007	Cannes	06400	F	212
60	2007	Antibes	06600	M	177
16	2007	Marseille	13000	M	180
21	2007	Marseille	13000	M	210
19	2007	Marseillan	34340	M	155
55	2007	Ramatuelle	83350	Y	105
40	2007	Les Issambres	83380	F	220
37	2007	Hyeres	83400	M	167
39	2007	Hyeres	83400	F	200
38	2007	Frejus	83600	F	130
68	2008	Cap Ferrat	06230	F	145
43	2008	Cannes	06400	M	155
71	2008	Cannes	06400	M	207
69	2008	Theoule	06590	F	144
65	2008	Antibes	06600	M	208
28	2008	Gruissan	11430	M	155
24	2008	Marseille	13000	M	221
33	2008	Marseille	13000	Y	99
56	2008	Arles	13200	M	197
30	2008	Cassis	13260	Y	110
25	2008	Carry	13620	M	195
26	2008	Sete	30200	F	147
29	2008	Marseillan	34340	M	197
57	2008	Ste Maxime	83120	Y	110
46	2008	Bormes Mimosas	83230	M	150
42	2008	Grimaud	83310	Y	100
45	2008	Les Issambres	83380	F	190
41	2008	Hyeres	83400	M	163
31	2008	Porquerolles	83400	M	193
51	2008	St Tropez	83990	F	190
70	2008	Monaco	98000	M	150
72	2009	Antibes	06600	M	160

from 0.01 ng g^{-1} for PCB congeners to 0.2 ng g^{-1} for pesticides. Results in the blubber are expressed in ng.g^{-1} lipid weight (ng g^{-1} lw) while, in all other tissues and organs, they are expressed in ng.g^{-1} dried weight (ng g^{-1} dw). Since the concentrations of chlorinated hydrocarbons in most samples were above 1 ng g^{-1}, decimals have been omitted from tables. Commercial standard solutions were used for calibration and, in the case of PCB, a sample of Dp6 (a commercial mixture equivalent to Arochlor 1260) was used for comparison of the proportions of the congeners in the different organs. The total amount of DDTs (tDDTs) was calculated as the sum of the amounts of pp′-DDT, pp′-DDD, and pp′-DDE. The PCBs congeners 118 (CB118), 138 (CB138), 153 (CB153) and 180 (CB180) were the major constituents of the reference Dp6, and represented 41 percent of the total amount of PCBs. The total amount of PCBs (tPCBs) was estimated as tPCBs = (CB118 + CB138 + CB153 + CB180) × 100/41 (Monod et al., 1995; Perez et al., 2003).

Organochlorines are very lipohilic compounds so they are mainly accumulated in the blubber (Aguilar and Borrell, 1990; Gi Beum et al., 1996). Results concerning total PCBs (tPCBs), total DDTs (tDDTs) in blubber are presented in Table 2. Considering the standard deviation for all the samples analyzed, average levels of tPCBs and tDDTs are 57,336 ± 46,232 ng.g^{-1} lw and 15,995 ± 13,268 ng.g^{-1} lw, respectivly. These results show a high contamination both for tPCBs and tDDTs in all the samples analyzed. The levels measured are of the same order of magnitude as other populations of *S. coeruleoalba* previously analyzed in our laboratory (Wafo et al., 2005) or in other sudies concerning the

Table 2
Lipid content (%) and concentrations (ng g^{-1} lw) of the *Stenella coeruleoalba*.

Code	Lipid (%)	tPCBs	tDDTs
29	26.0	12,011	6,179
51	30.7	2,052	1,120
58	35.7	55,256	23,488
41	36.4	44,300	11,938
72	39.6	7,896	5,368
70	46.5	106,699	8,686
42	46.5	135,673	55,174
71	47.0	38,281	14,141
56	48.3	87,075	30,369
59	51.3	158,992	45,779
40	53.1	17,327	3,924
33	53.1	40,513	3,630
36	55.9	24,430	7,397
68	56.9	47,066	12,556
28	56.9	65,018	27,531
30	63.0	2,372	1,822
55	63.6	69,027	22,525
63	63.7	23,424	11,532
21	66.8	167,516	7,519
43	67.0	58,049	15,360
69	67.1	24,685	8,344
25	69.5	65,460	17,368
57	69.7	130,232	35,386
31	70.6	25,992	11,687
26	72.7	32,711	7,704
24	79.3	48,687	19,352

Mediterranean environment (Marsili and Forcardi, 1997). Any clear relationship appears between the level of tPCBs (or tDDTs) and the lipid content of the blubber. This can be explained by the indivual history of each dolphin with, for some of them, phases of starvation or of disease with a direct impact on lipid level (but not on the contamination level of the bubbler).

Considering the sex and the sexual maturity (Fig. 2), the average concentrations of tPCBs and tDDTs for young dolphins were 77,198 ± 57,395 and 24,003 ± 22,407 ng g^{-1} lw respectively. The average levels of tPCBs and tDDTs, for males, were 57,724 ± 41,900 and 14,387 ± 7,403 ng g^{-1} lw, respectively. For females, they were 45,315 ± 45,689 and 13,789 ± 13621 ng g^{-1} lw, respectively. So, from all these results, it emerges two main observations:

- The levels for both tPCBs and tDDTs were higher for young dolphins than for adult ones,
- Among adults, males seemed more contaminated with tPCBs than females; by contrast, no significant difference appeared in levels of tDDTs between males and females (significance level $p < 0.05$).

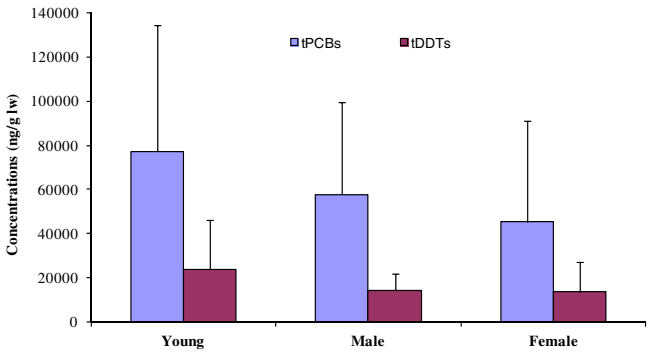

Fig. 2. Average concentration (with standard deviation) for tPCBs and tDDTs, respectively, in the blubber for young, male and female *Stenella coeruleoalba*.

These results are in agreement with those of several studies (Tanabe et al., 1987; Aguilar and Borrell, 1994; Borrell et al., 1995) which have showed similar accumulation mechanisms. The accumulation of organics compounds in the organisms begins during the fetal life. At this stage, contaminations levels in the fetus' organs can reach those of the maternal organs. In the newborn dolphins, these levels then increase during the phase of feeding throught the breastmilk. Then, contaminants continue to accumulate throughout life, except for females who eliminate organics contaminants during gestation and nursing because of the organics compounds are transferred to their baby (Tanabe et al., 1982; Alzieu et al., 1982), levels of organic contaminants can also decrease. Previous work has shown that these transfers may be important: Stockin et al. (2010) have indicated, for the DDTs, ratios of approximately 6 percent transferred from the mother to the fetus and 4 percent for the tPCBs (calculated from 45 congeners of PCBs). These authors have also indicated that this transfer of contaminants, via the placenta, might lead to an "imbalance" of the levels between the mother and the fetus.

Regardless of the gender and age, the results showed a clear predominance of the level of tPCBs on the level of tDDTs in the bubbler of the studied dolphins (Fig. 2). This can reflect the aging of DDTs in relation to the PCBs in the natural environment. Indeed, the use of DDTs has been banned in the Mediterranean basin since the late 1970s. During the same period, the use of PCBs was spreading, and even augmentated. It is only in 1986 that the manufacture and marketing of products or equipment containing PCB has been strictly forbidden in France. However, existing stockpiles are still substantial, and visibly continue to contaminate the environment. Borrell and Aguilar (1987, 2007), have reported that between 1987 and 2002, the tDDTs has decreased by a factor of 23. During the same period, the tPCBs has decreased by a factor 6 only. Others results confirm these observations: Wafo et al., 2006; Wafo, Pers. Comm.

Considering all the studied organs, the analyzed samples showed heterogeneous contents for each compound analyzed. This heterogeneity might be mainly linked to the own "life-history"of each dolphin. Thus, interpretation of data was based on the average values. Fig. 3 shows the average distribution of tPCBs and tDDTs in the various organs, for the young dolphins, the males and the females, respectively.

The average levels of tPCBs and tDDTs respectively, in the organs decreased in the order: Liver > kidney ≈ Muscle > lung.

Overall, these values were lower than those obtained in the blubber.

These observations were consistent with the various studies on the Mediterranean *Stenella* (Aguilar and Borrell, 1990; Marsili and Forcardi, 1997; Marsili, 2000; Wafo et al., 2005) from various geographic origins: Italy, Spain and France.

The almost constant distribution of the tPCBs levels in the organs of each subject was explained by the highly lipophilic nature of these compounds. As we have shown, the ultimate accumulation took place in blubber; nevertheless during their progression in the organism, the contaminants distributed in the different tissues and organs. The affinity between organic compounds and organs might be correlated to the lipid content.

Furthermore, depending on the organs, there might an accumulation through mobilization of residues previously accumulated or on the contrary, of decontamination process via metabolism. The distribution of the contamination between the organs is therefore linked to complex mechanisms, difficult to characterize.

The dolphin contamination by the DDTs naturally followed the same mechanism as the contamination by the PCBs. The DDTs levels were, however much lower than the PCBs concentrations: 9-fold, 4-fold and 3-fold decrease in young dolphins, males and females respectively. In order improve distribution of the organochlorinated contaminants in the different organs of dolphins, the level of tPCBs in blubber; lung, muscle, and kidney were plotted v.s. the level of tPCBs in liver, for males, on the one hand, and for females, on the other hand. The same correlations were plotted for the level of tDDTs. Each correlation took into account all the dolphins for which both organs considered to have been analyzed (Fig. 7).

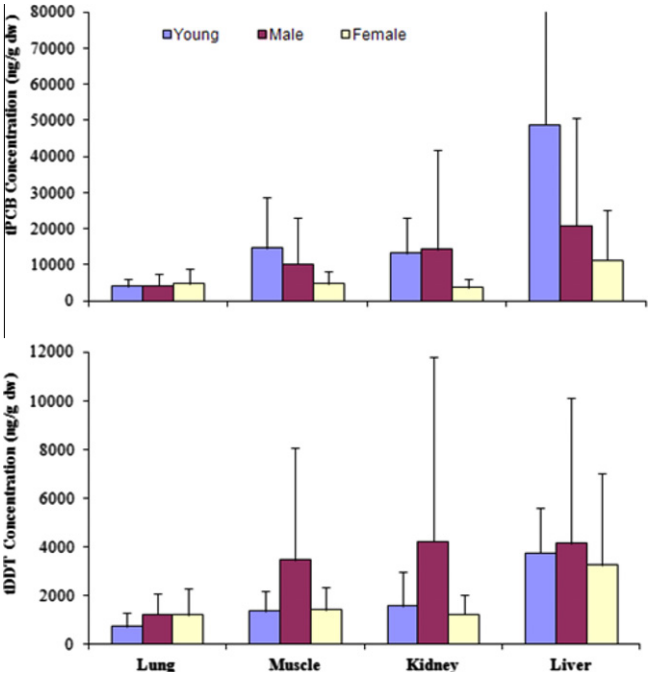

Fig. 3. Average concentration (with standard deviation) for tPCBs and tDDTs, respectively, in organs other than the blubber for young, male and female *Stenella coeruleoalba*.

In the case of the muscle, no significant correlation between organs could be highlighted.

For tPCBs, positive correlations pointed out between liver and blubber, liver and kidney, and liver and lung. For tDDts, positive correlations can be pointed out only for liver and kidney (for male) and liver and lung.

The calculated correlations show that tPCBs contents in the liver were about 3 times lower than in the blubber, 3 times higher than in the kidney, and 3 times higher than in the lung of the females and 6 times higher for the males. tDDTs contents in the liver are about 3 times higher than in the lung and 2 times higher than in the kidney (only for male). Thus we confirmed the previous results on the distribution of organochlorinated compounds according to organs.

The Fig. 4A (Females) and Fig. 4B (Males) represent the variation of the congener composition of PCBs in the various organs for males and females respectively, as compared with the Dp6. In Dp6, the congeners with 5, 6, and 7 atoms of chlorine represented 20 percent; 41 percent and 27 percent of tPCBs respectively. The molecules having 3, 4, and 8 atoms of chlorine represent only small proportions: 0.2 percent; 1 percent and 11 percent of tPCBs, respectively. The profiles appeared broadly similar in all organs and similar to the profile of the Dp6. However, some substantial differences could be found. The proportion of octochlorinated compounds was in general much lower for all of the organs relatively to that of the Dp6. This difference was even more pronounced for the liver (especially for females) and for the blubber (especially for males). In parallel, the proportion of hexachlorinated compounds

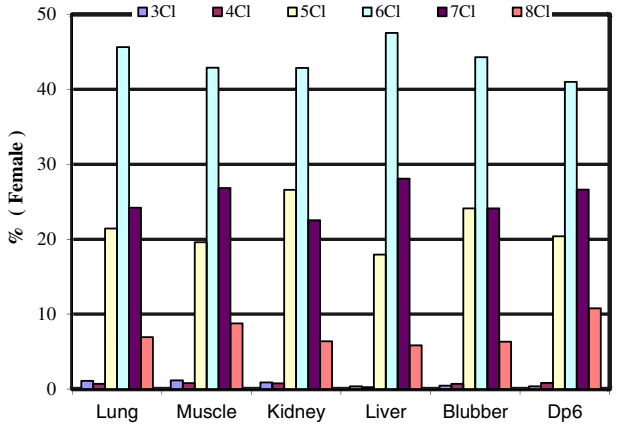

Fig. 4A. (Female) Composition of PCB in relation with chlorine number in the different organs of the females.

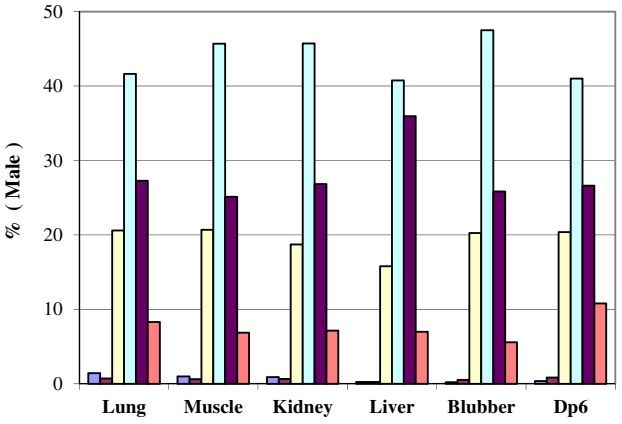

Fig. 4B. (Male) Composition of PCB in relation with chlorine number in the different organs of the males.

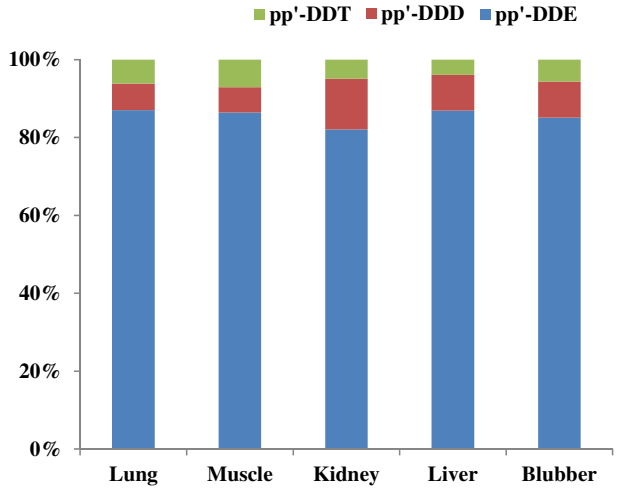

Fig. 5. Average relative proportions of DDT, DDD and DDE in all the tissues and organs studied for *Stenella coeruleoalba*.

was dominant for the whole organs in comparison with that of the Dp6. For the other "classes" of congeners, variable differences according to organs appeared: especially, a relatively high proportion of the heptachlorinated compounds were found in the livers of the male dolphins. Morever, the proportion of hexachlorinated congeners (41 percent in the technical mixture Dp6) varied, depending on the tissues and organs, from 39 to 48 percent among the males, and from 41 to 48 percent among the females. At the same time, the proportion of octochlorinated compounds (11 percent in the technical mixture Dp6) varies from 3 to 8 percent in the various tissues and organs, letting to suspect that the "dechlorination" of the most chlorinated compounds took place. Therefore, despite the apparent similarity of the profile of PCBs in the various samples, variation happened in the relative proportions of congeners in comparison with the distribution in initial industrial mixtures. However, it was impossible to assert if the variation were caused by the coumpound metabolism within the organism or if dechlorinations took place in the natural environment before absorption of PCBs by the dolphins.

Fig. 5, shows the average proportions of DDT, DDD and DDE in all the tissues and organs of the studied *S. coeruleoalba*. DDE was always the major component in the different organs. DDD and DDT were in much lower proportions. In fact, DDE has been shown to be the ultimate stage of degradation of DDT, and this stable derivative has been considered as less toxic than DDT. DDE/tDDTs represent the state of degradation of the DDTs; i.e. the "aging" of the contamination by these compounds: higher is the ratio, older is the pollution by the DDT. Fig. 6 shows that these ratios were relatively stable from one organ to another for the same individual and from one specimen to another. They varied from 78 to 96 percent regardless of the organ considered and independently of the size and the sex of the dolphin. In living organisms, most of the detoxification pathways (leading to the DDE from the DDT and DDD) generally are taking place in the liver. In the present study, we would have expected the ratios DDE/tDDTs to be lower in the liver than the other organs. However, homogeneous distribution of the DDT, DDE and DDD were measured in all the studied organs. Thus the bulk of the degradation of DDT into DDE must have been initiated in the natural environment, even before this compound could penetrate the organism of the dolphins. These results are in agreement with most of the studies on the cetaceans in the Mediterranean Sea (Borrell and Aguilar, 1987). In particular, Marsili et al. (1992) have shown that in the tissues and organs of the dolphins collected along the Italian coastline, DDE represents 80 percent of tDDTs. This proportion differed with results obtained for cetaceans from the Indo-Pacific coasts where DDE represents 68 percent of tDDTs: in this region of the world, restrictions concerning the use of DDT were less drastic or the restriction of use was later than in Europe (Fossi et al., 2003; Jefferson, 2006). The ratios DDE/tDDTs close to 100 percent clearly means that DDTs were present in the natural environment of the Mediterranean Sea for a long period of time and were not widespread any more. These results are in agreement with the prohibitions of the use of these compounds, particularly in France and in the Mediterranean pool since the 1970s.

The ethological conditions vary from one marine environment to another. So, dolphins could be classified both by kind and by geographical areas. Fig. 8 presents a comparison between the present results and results obtained previously by other authors for *S. coeruleoalba* from the Mediterranean Sea.

Data from Alzieu and Duguy (1979) have shown relatively low levels of tPCBs and tDDTs as compared with the other studies which have been published between 1993 and 1996. However, taking into account the improvement of the analytical techniques for detection and determination of organochlorined compounds since the 1980s, the data of Alzieu and Duguy's are hardly comparable to more recent results (since 1990) and are presented here only for information.

The study of Borrell (Borrell, 1993) has concerned the region of Gibraltar where important exchanges between the Mediterranean Sea and the Atlantic Ocean has been taking place. It has shown

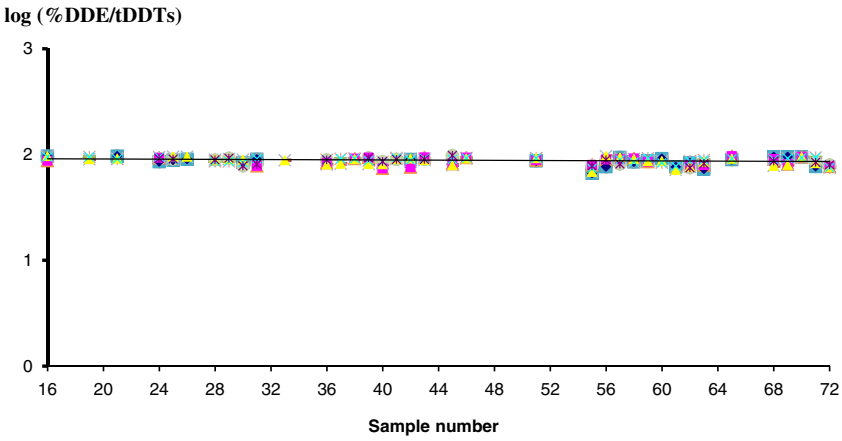

Fig. 6. Ratios DDE/tDDTs expressed as log (% DDE/tDDTs), for all the samples studied (different organs) for *Stenella coeruleoalba*.

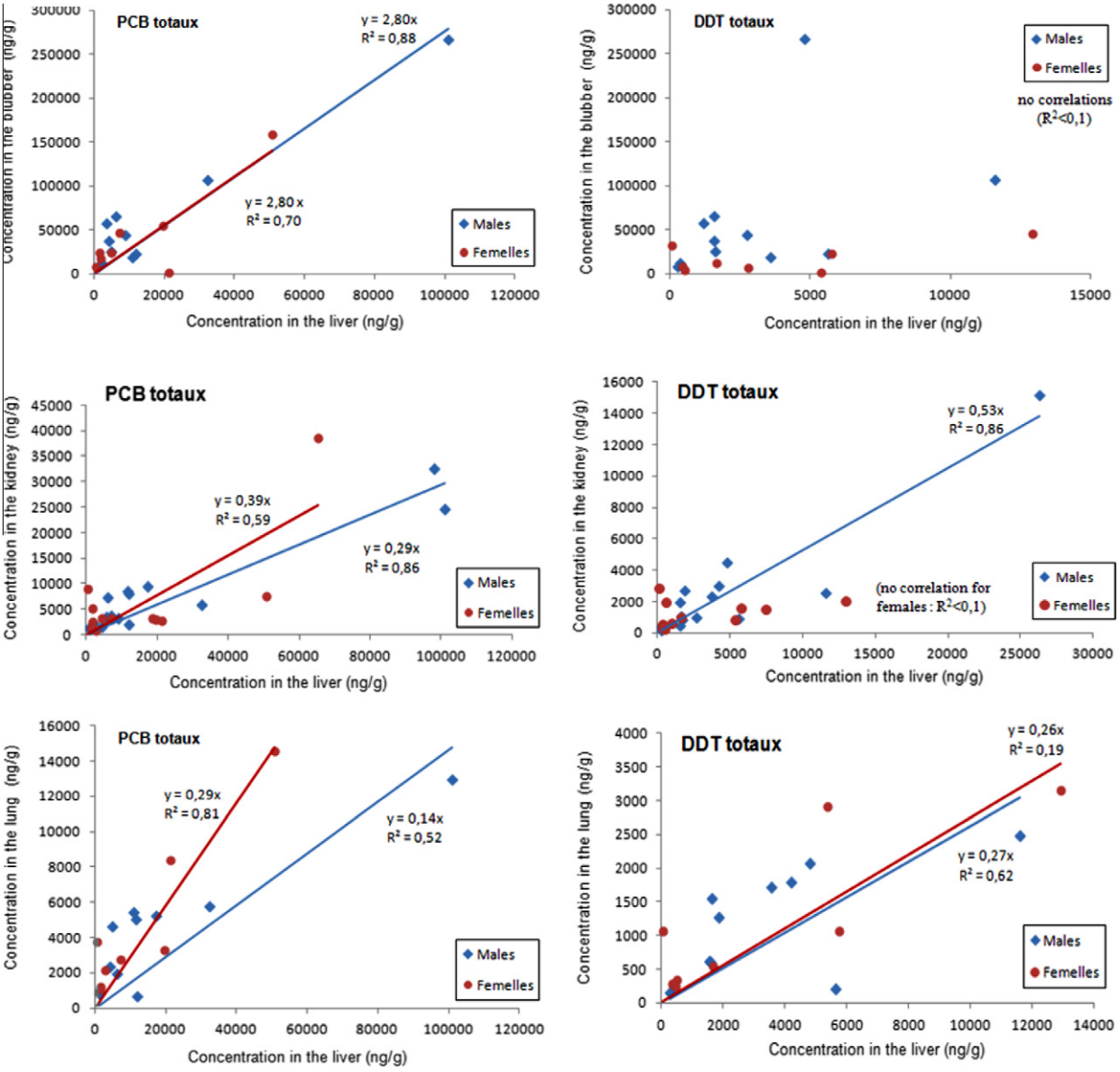

Fig. 7. Correlations between the liver and the other organs (the bubbler, the kidney and the lung respectively) for tPCBs and tDDTs concentrations respectively.

lower values for tDDTs and tPCBs than the other studies carried out in the « inner » Mediterranean Sea. Except the study mentioned previously, variable but relatively high levels had always reported concerning the different areas of the Mediterranean Sea in the

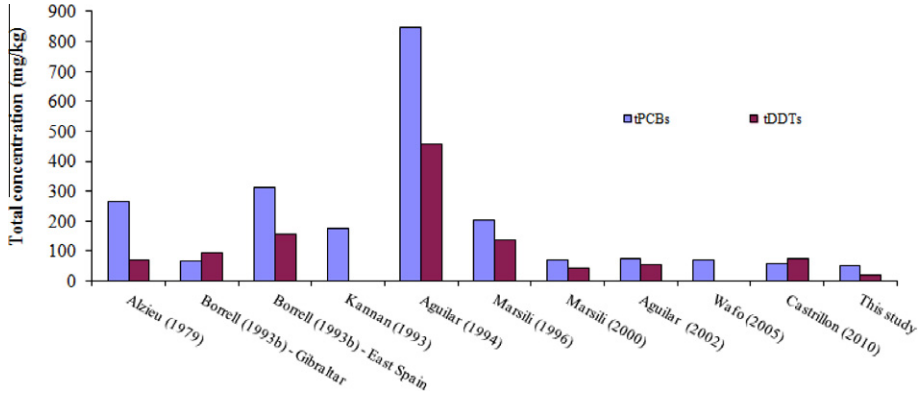

Fig. 8. Temporal trends concerning tPCBs concentrations and tDDTs concentrations respectively, in the blubber of cetacean from Mediterranean Sea between 1979–2010.

1990s (150–450 μg g^{-1} lw for the tDDTs and 150–800 μg g^{-1} lw for the tPCBs). For the most recent works, since 2000s, a net tendency to decrease has been detected for both tDDTs and tPCBs.

So, the overall results of this work, compared to previous studies concerning the Mediterranean Sea, seems to confirm the tendency to a decrease of the contamination by organics compounds for the cetaceans in the Western Mediterranean Sea.

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