



Accumulation and distribution of organochlorines (PCBs and DDTs) in various organs of *Stenella coeruleoalba* and a *Tursiops truncatus* from Mediterranean littoral environment (France)

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Abstract

The objective of the present study is to determine the levels of contamination by PCBs, DDT and its metabolites in dolphins failed on the coasts of the Mediterranean sea. Samples are represented by six *Stenella coeruleoalba* and a *Tursiops truncatus* collected in 2000 and 2003. The studies are achieved on the blubber, the heart, the liver, the kidney, the muscle and the lung. The concentrations of PCBs and DDT are very high in all tissues and organs analyzed. For the PCBs, the concentrations vary between 43,838 and 110,343 µg/kg lipid basis in the blubber, 601 and 39444 µg/kg dried weight in the liver, 1375 and 34512 µg/kg dried weight in the muscle, 3151 and 17082 µg/kg dried weight in the heart, 674 and 12365 µg/kg dried weight in the kidney and finally between 648 and 4118 µg/kg dried weight in the lung. These values are comparable to those previously obtained in our laboratory and by other authors during the years 1990 on the Mediterranean environment.

Significant differences in concentrations are noted in tissues and organs, neither according to the age, nor according to the gender.

In all the analyzed samples, the contents in PCBs are higher than those of DDT. The average ratios of pp'-DDE/ΣDDT are close to 0.6 which shows the metabolization of these compounds along the years.

The examination of the profiles of congeners shows that the hexachlorinated molecules are dominating in all tissues and organs which supposes the different animals were especially exposed to Pyralen-type compounds of transformer (Dp6).

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1. Introduction

Several organochlorine compounds, especially polychlorinated biphenyls and pesticides such as DDT, were measured in various organs of dolphins from the Mediterranean Sea. These marine mammals have a reduced capacity to decompose hydrophobic, persistent chemicals such as polychlorinated biphenyls and DDT and therefore accumulate high levels of these compounds through the food chain (Nakata et al., 1998; Tanabe et al., 1994a,b; Williams et al., 1992). They can amplify great amounts of toxic contaminants by feeding and also transmit them in large quantities from one generation to the next one through lactation. Small cetaceans have a number of biological characteristics that make them particularly prone to accumulate high levels of these pollutants. PCBs congeners as well as pesticides are lipophilic, extremely stable, chemically heterogeneous and are differentially bioaccumulated, biodegraded and partitioned when absorbed by mammals. Unfortunately, these animals have a small capacity for degradation of these contaminants (Poster and Simmonds, 1992; Tanabe et al., 1994a,b; Tanabe et al., 1981).

The dolphin in the Mediterranean sea has suffered a drastic decrease in its distribution during the last few decades (Borrell et al., 2001). These mammals are protected and most information about them refers to those failed or inopportunely trapped in fishing nets.

The degradation of their habitat must contribute to this decrease. In this regard, dolphins and other marine mammals are the focus of attention due to the occurrence of high concentration of organochlorine residues in their bodies (Tanabe and Watanabe, 1991). Thus, marine mammals are among the organisms vulnerable to long-term toxic effects of persistent organochlorine like PCBs (Tanabe et al., 1988).

In this study, congeners specific concentrations of PCBs, DDT and its by-products (DDD and DDE) were measured in different organs (liver, kidney, lung, heart, blubber, muscle) in some Striped Dolphins and Bottle-nosed Dolphin collected from various locations from French Mediterranean littoral, to evaluate the degree of the contamination, then to compare the results obtained before by our own laboratory. Comparative studies of the contents in organochlorinated compounds according to the age and the sex are carried out.

2. Materials and methods

2.1. Samples

Seven specimen of dolphins used in this study were found dead or entangled in fishing nets along the Mediterranean coasts in the period between 2000 and 2003 (Fig. 1). Collection of organs and tissues was made by Frank Dhermain and several searchers from the “Groupe d’Etude des Cétacées de Méditerranée” (GECCEM) and the “Réseau Echouage Méditerranée”. All the specimens were placed on ice and sent to the laboratory, where they were weighted, measured, and dissected. The organs and tissues were frozen and stored at $-20\text{ }^{\circ}\text{C}$ until the analysis. The different organs (blubber, muscle tissue, liver, kidney, heart, lung) were removed and lyophilized. Then, each organ was pulverized and homogenized. The glassware used was washed with soap and water, heated at $300\text{ }^{\circ}\text{C}$ overnight, and then rinsed three times with pesticide grade hexane.

Whatman cellulose thimbles ($22\times 80\text{ mm}$, no. 350211, Schleicher and Schuell) used for extraction of samples were pre-extracted for 12 h in a soxhlet apparatus with hexane Pestipur (Pestipur 99%, SdS, Peypin, France), in order to remove any organochlorine contamination.

2.2. Extraction and quantitative analysis

About 1 g of freeze dried sample was extracted with hexane in the thimbles in a soxhlet apparatus for 16 h minimum. The solution obtained was concentrated to 10 mL. The extract was purified with concentrated sulfuric acid, following the procedures described by (Murphy, 1972), and the resulting extract was concentrated to 6 mL. Then the extract underwent liquid chromatography on a column containing florisil that had been dried for 4 h at $110\text{ }^{\circ}\text{C}$. Analyses were performed with a HP 6890 series gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) at $300\text{ }^{\circ}\text{C}$ and an automatic injector on-column, HP 6890 series. The column used was a DB5 J and W ($60\text{ m}\times 0.32\text{ i.d.}\times 0.25\text{ }\mu\text{m}$). The carrier gas was helium. The temperature of injection was $60\text{ }^{\circ}\text{C}$ and was programmed to increase up to $250\text{ }^{\circ}\text{C}$ at $100\text{ }^{\circ}\text{C}/\text{min}$. The column temperature was $60\text{ }^{\circ}\text{C}$ and programmed to increase first up to $160\text{ }^{\circ}\text{C}$ ($10\text{ }^{\circ}\text{C}/\text{min}$) and then up

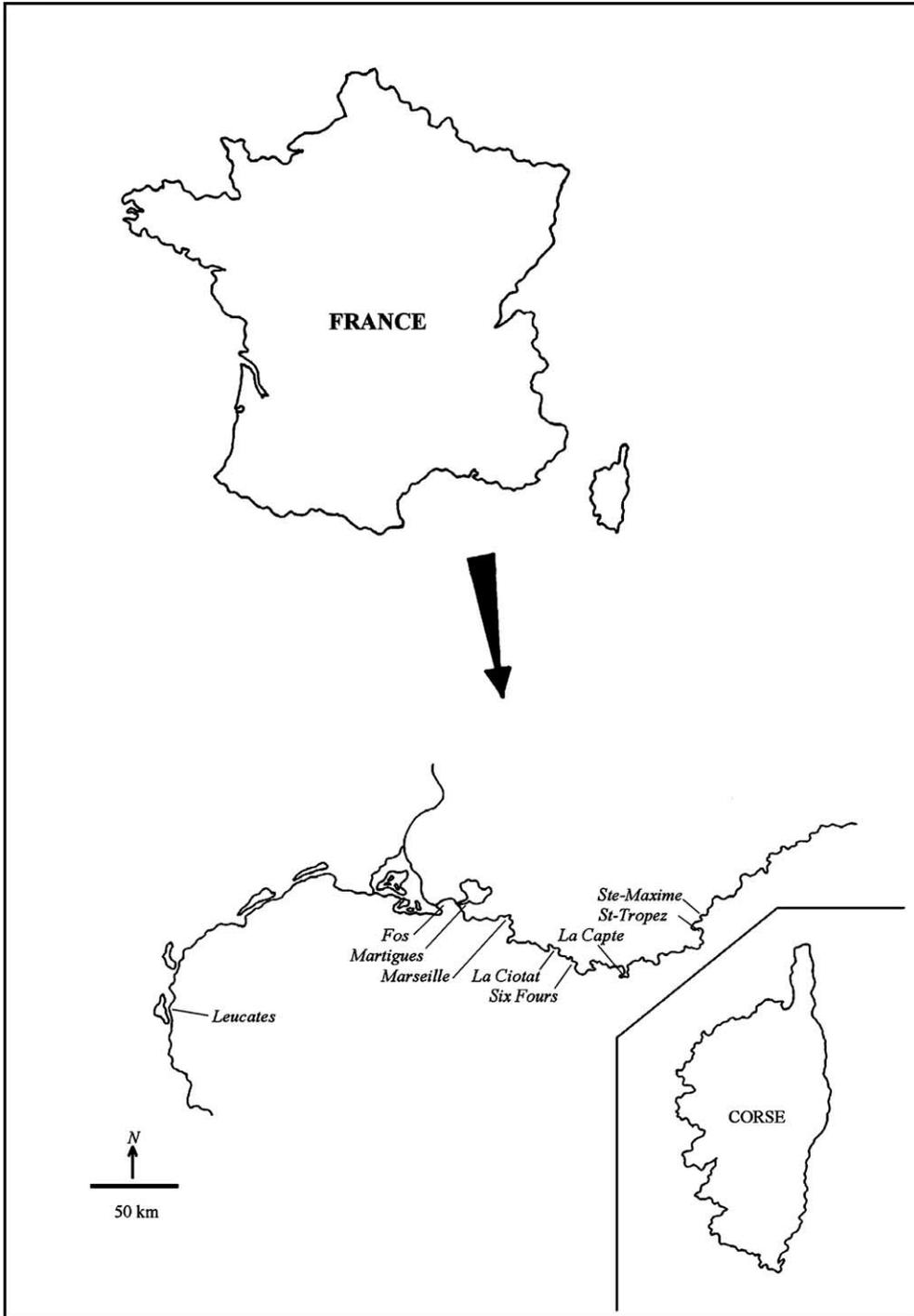


Fig. 1. Map of the sampling location.

280 °C at 2 °C/min. The variation in the response of the ECD detector was corrected by a daily calibration with a standard solution of PCBs congeners, and also for pp'-DDT, pp'-DDD, pp'-DDE. Two internal standards, OCN and Mirex were used. The sample response was matched to that of the standard solution by dilution or concentration of the sample.

2.3. Data analysis

The samples were analyzed for the following compounds: pp'-DDT, pp'-DDD, pp'-DDE, and PCBs. The total DDT (Σ DDT) was calculated as the sum of DDT, DDD, and DDE compounds analyzed. The confirmation of the presence of DDT was made by dehydrochlorination with alcoholic potassium hydroxide of selected samples.

The samples and blank were analyzed for content of the following congeners denoted by their IUPAC numbers: 18, 20, 28, 31, 44, 52, 95, 60, 92, 84, 101, 99, 87, 136, 81, 77+110, 151, 135, 123+149, 118, 153, 114, 105, 141, 138, 187, 126, 183, 128, 174, 167, 177, 156, 157, 169, 180, 170, 189, 201, 196, 195, 194 according to the numerotation proposed by (Ballschmitter and Zell, 1980).

Forty-four standards solutions were used for calibration and a DP6 equivalent to Arochlor 1260 was used for comparison of the congeners proportions found in a commercial mixture and the different organs. Since the concentrations of chlorinated hydrocarbons in most samples were above 1 µg/kg, decimals have been omitted from the tables. The total amount of PCBs has been estimated as $PCB\ total = (CB118 + CB138 + CB153 + CB180) \times 100/41$. These congeners 118, 138, 153 and 180 are the major constituents of the reference DP6, which is the industrial component widely used in France and representing 41% of the total amount of PCBs.

Our Laboratory participated to an IAEA/UNEP intercomparison study for the chemical analysis of organochlorinated compounds in fish homogenate sample (IAEA 406), which was distributed to worldwide laboratories in January 2000 (Villeneuve et al., 2000). For Quality assurance and Quality control of the analyses, congeners 28, 52, 101, 118, 138, 153, 180 were analyzed. Each sample was analyzed in triplicate, and analyte recoveries ranged from 85% to 98%.

As major constituents of the commercial mixture and being very persistent in the marine environment, CB 153 and CB 138 are also considered as good indicators of the overall concentration in PCBs. The behavior of the different PCBs classes (with respect to the number of Cl atoms) has also been analyzed in each sample.

3. Results and discussion

Among the 7 studied mammals, 5 are young (including one new born (no. I), one little runt (no. III)), and 2 have reached the sexual majority (IV and V). The base of this classification is made according to the data of (Alzieu and Duguay, 1979). The sizes of these individuals as well as sexual maturity are presented in Table 1. In parallel, Table 2 shows the characteristics of the studied specimens in our laboratory in the pass.

We made the choice to study separately tissues and organs of these various animals and compare the behaviors of the PCBs and the pesticides.

The PCBs and pesticides determined levels indicate that concentrations of these pollutants in dolphins are high. PCBs concentrations were comparable to those of DDT. Table 3 shows the total amount of PCBs and DDT in different organs and tissues of the 7 studied dolphins.

The levels of total PCBs in the tissues decrease in the order:

Blubber: from 43,838 to 110,343 µg/kg,
Liver: from 601 to 39,444 µg/kg,
Muscle: from 1375 to 34,512 µg/kg,
Heart: from 3151 to 17,082 µg/kg,

Table 1
Characteristics of the studied specimens

Sample code	Sex	Length (cm)	Collection location	Sampling year	Species	Growth stage
I	M	94	La Ciotat	15/08/02	St. c.	New born
II	M	112	Martigues	17/09/02	St. c.	Young
III	F	98	Corse(Borgo)	25/06/01	T. t.	Little runt
IV	F	150	Saint Tropez	28/01/03	St. c.	Adult
V	F	210	Bandol	20/05/03	St. c.	Adult
VI	M	107	Leucate	21/10/02	St. c.	Young
VII	M	115	Marseille	01/03/00	St. c.	Young

St. c.: *Stenella coeruleoalba*; T. t.: *Tursiops truncatus*.

Table 2
Characteristics of the studied specimens of the dolphins between 1989 and 1990

Sample code	Sex	Length (cm)	Collection location	Sampling year	Species	Growth stage
VIII	F	170	Fos	29/12/88	St. c.	Adult
IX	M	175	Fos	11/01/89	St. c.	Adult
X	F	203	Six-Four	26/10/90	St. c.	Adult
XI	M	185	la Capte	18/10/90	St. c.	Adult
XII	F	183	Ste Maxime	11/10/90	St. c.	Adult

St. c.: *Stenella coeruleoalba*.

Kidney: from 674 to 12,365 µg/kg,

Lung: from 648 to 4118 µg/kg.

These levels observed are generally comparable to those found in others populations of the same species previously studied in our laboratory, Table 4.

In addition, the values obtained in blubber tissues are definitely in the same order of concentration as those obtained by other authors in the Mediterranean environment.

On a great selection of samples of *Stenella coeruleoalba* ($n=68$ subjects) (Marsili and Forcardi, 1997), obtained the following means (µg/kg d.w) in PCBs: 86,257 in the blubber; 21,424 in liver; 8102 in heart; 3293 in kidney; 2666 in muscle.

However these values are often higher than those obtained in blubber by other authors in the world on the organisms of the same species (Table 7).

Table 3
Total PCBs and DDT levels (µg/kg lipid wt. in blubber) and (µg/kg dried weight in others organs and tissues) of the studied dolphins

Organ	Sample code	I	II	III	IV	V	VI	VII
Muscle	PCB	4363	n.d.	1375	n.d.	4007	n.d.	34512
	DDT	702	n.d.	23	n.d.	54	n.d.	272
Lung	PCB	648	2979	n.d.	4118	3831	3137	3905
	DDT	89	180	n.d.	266	224	296	968
Kidney	PCB	2185	12365	674	6979	8362	5912	1990
	DDT	320	796	343	352	410	608	695
Liver	PCB	5810	16239	601	n.d.	10863	39444	8646
	DDT	503	962	51	n.d.	618	1346	1518
Heart	PCB	n.d.	17082	n.d.	n.d.	n.d.	3151	6215
	DDT	n.d.	877	n.d.	n.d.	n.d.	326	1633
Blubber	PCB	n.d.	n.d.	n.d.	110343	43838	n.d.	55754
	DDT	n.d.	n.d.	n.d.	6566	2707	n.d.	2850

n.d.: not determined.

Table 4
Total PCB (µg/kg lipid wt. in blubber and µg/kg dried weight in others organs and tissues) of the dolphins studied between 1989 and 1990

Sample code	VIII	IX	X	XI	XII	Means
<i>Organ</i>						
Muscle	2500	4300	3600	1200	8500	4020
Liver	2800	3700	27,200	2700	30,400	13,360
Blubber	83,200	62,200	12,400	2700	65,500	45,200
Melon	116,100	138,600	48,200	8100	76,000	77,400

In parallel, as indicated in Table 3, considerable concentrations in pesticides were detected.

Usually the pattern of contaminants is known to be related to the lipid content of the various organs (Gi Beum et al., 1996; Aguilar, 1985). In the majority of studies, it is observed that average content in organochlorinated decreases according to the content in lipid in the order: blubber>liver>heart>kidney>muscle (Marsili and Forcardi, 1997; Marsili, 2000). This distribution is reproduced in our study, however with a small modification: in our case, the contents of organochlorinated decrease according to: blubber>liver>muscle>heart>kidney>lung.

The contamination of the animals we have analyzed is very heterogeneous. For an individual, the content in PCBs is always higher in the blubber than those obtained in the other organs. The organochlorinated compounds have a very strong affinity for the triglycerides which constitute blubber (Kawai et al., 1988; Aguilar and Borrell, 1990). On the other hand, the distribution of these compounds in the other organs does not seem to follow a clearly determined criterion. In none of the studied dolphins we observed a linear relation between the percentage in lipids and the contents in PCBs and DDT for the organs of one single animal.

After blubber tissues, the organ which presents strong contents of PCBs is the liver (except case ref. III and ref. VII), and this organ is known to be a good indicator of the processes of accumulation and elimination of those compounds in the organism and hence characterizes the general contamination of the tissues.

The differences in concentrations are caused by the specific capacity of each tissue and organ subsequent to the exposure to PCBs and/or the specific kinetics of each species of PCB, depending itself on their physicochemical parameters and bio-availabilities.

Six of the seven studied animals belong to the *S. coeruleoalba* species and only one belongs to the *Tursiops truncatus* species (Table 1, sample III).

The *S. coeruleoalba* is a pelagic dolphin commonly encountered in the Mediterranean Sea, whereas the *T. truncatus* is a coastal species.

Comparison of data of *T. truncatus* to those of *S. coeruleoalba* shows that for the same organs *T. truncatus* contains less PCBs than *S. coeruleoalba*.

Nevertheless, this comparison must be tempered, because only one specimen of *T. truncatus*, was studied here. Moreover, it is a little premature runt, which was never suckled, and never caught preys, which is not the case of the specimens of *S. coeruleoalba*.

Similar observations on more representative samples were made by other authors (Alzieu and Duguay, 1979; Marsili and Forcardi, 1997), particularly in the liver, muscle, and kidney.

The ratio PCBs/ Σ DDT is largely higher than 1 in all tissues and organs. This result reflects that the use of DDT has been restricted in the Mediterranean basin since the seventies whereas PCBs are still used in great quantities.

The various tissues have different affinities for the metabolites of DDT, depending on the sampling location of the dolphins.

The sum of DDT is in high concentration in blubber (2707 to 6566 $\mu\text{g}/\text{kg}$); followed in decreasing order by the heart (325 to 1633 $\mu\text{g}/\text{kg}$), the liver (51 to 1518 $\mu\text{g}/\text{kg}$), the kidney (320 to 796 $\mu\text{g}/\text{kg}$), the lung (88 to 968 $\mu\text{g}/\text{kg}$), and the muscle (24 to 702 $\mu\text{g}/\text{kg}$).

The proportions in DDT and its metabolites are presented in Table 5.

This table shows that the dominating compound is DDE in all tissues and organs. It varies from 50.62% to 82.59% in the blubber, followed by the kidney (44.27% to 82.22%), the lung (39.81% to 79.07%), the liver (44.07% to 76.77%), the muscle (53.31% to 76.88%), and the heart (46.62% to 67.74%).

The ratios DDE/ Σ DDT are relatively high. They are in average equal to 0.72 in the blubber, 0.60 in the heart, 0.58 in the lung and the muscle, 0.56 in the kidney and 0.54 in the liver.

This preponderance with respect to the total DDT indicates old DDT input in the environment (Borrell and Aguilar, 1987; Aguilar, 1984; Marsili et al., 1992).

Marsili et al. (1992) show that in the tissues and organs of dolphins collected along the Italian coasts, the contents in pp' DDE represent 80% of

Table 5
Proportions of DDT and its metabolites in the different tissues and organs

	Sample code	I	II	III	IV	V	VI	VII
<i>Organs</i>								
Muscle	DDT	12.29	n.d.	23.67	n.d.	22.97	n.d.	20.68
	DDD	29.26	n.d.	1.07	n.d.	0.15	n.d.	26.01
	DDE	58.45	n.d.	75.26	n.d.	76.88	n.d.	53.31
Lung	DDT	34.05	14.68	n.d.	2.78	20.94	22.55	9.78
	DDD	26.52	41.1	n.d.	45.09	39.25	32.55	11.14
	DDE	39.44	44.22	n.d.	52.13	39.81	44.89	79.07
Kidney	DDT	20.61	20.48	2.88	22.92	19.17	13.14	13.09
	DDD	35.12	29.05	14.9	4.11	38.22	32.8	29.78
	DDE	44.27	50.47	82.22	72.97	42.61	54.06	57.13
Liver	DDT	13.61	21.44	6.29	n.d.	18.15	16.96	12.52
	DDD	14.44	32.37	16.94	n.d.	37.78	32.52	28.39
	DDE	71.95	46.19	76.77	n.d.	44.07	50.52	59.09
Heart	DDT	n.d.	7.14	n.d.	n.d.	n.d.	13.71	16.11
	DDD	n.d.	25.12	n.d.	n.d.	n.d.	39.67	24.8
	DDE	n.d.	67.74	n.d.	n.d.	n.d.	46.62	59.09
Blubber	DDT	n.d.	n.d.	n.d.	3.06	5.8	n.d.	9.23
	DDD	n.d.	n.d.	n.d.	14.36	43.58	n.d.	21.26
	DDE	n.d.	n.d.	n.d.	82.59	50.62	n.d.	69.51

n.d.: not determined.

the total of the DDT. (Prudente et al., 1997), give concentrations higher than 85% in pp'-DDE in the blubber of the marine mammals collected in the northern Pacific Ocean and the Indian Ocean.

Comparable ratios were found in marine mammals in various countries where DDT was prohibited (De Kock et al., 1994; Gauthier et al., 1997; Metcalfe et al., 1999; Storelli and Marcotrigiano, 2000; Yogui et al., 2003).

The fact that the DDT is still present in the organisms demonstrates some residual accumulation of these compounds in the Mediterranean coasts.

Recently, we undertook studies on Mediterranean sediments, and we obtained substantial contents in DDT, from 0.32 to 118 µg/kg (Wafo, pers. comm.). This fact confirms the omnipresence of these products in the Mediterranean environment.

3.1. Influence of age and sex

The results of Table 3 show that whatever the age, the contents in PCBs in the liver are higher than those in the kidney. In the same way, the contents in PCBs are generally higher in the kidney compared to those of the lung, except for case VII. By comparison with the other organs, no linear relation between the concentrations in PCBs of the mature and immature organisms may be established.

According to the sex and size, there is an increase of concentration of PCBs and pesticides in the lung of males.

In comparison, the two mature females of our sampling have concentrations of the same order of magnitude in the lung and the kidney. On the other hand, in the blubber, the oldest organism (V) shows a concentration twice as important as the youngest one (IV).

The organisms I and VII are young males. They contain more PCBs in their muscles than those of the old female organisms VIII, X and XII. This is probably a consequence of the cumulative factor of organochlorines in young organisms. It is generally established that old females tend to present a diminution of these compounds, especially during the pregnancy and lactation period. The studies achieved by Alzieu and Duguay (1979) on a mother and her fetus showed higher concentrations in the muscles of the fetus than those of the mother.

Table 6

Average of PCBs congeners and DDT concentrations in the different tissues and organs

	Muscle <i>n</i> =5	Lung <i>n</i> =5	Kidney <i>n</i> =5	Liver <i>n</i> =5	Heart <i>n</i> =5	Blubber <i>n</i> =3
Lipid content	36.13	34.47	35.63	38.11	45.33	87.18
pp'-DDT	40.09	45.21	81.28	134.74	123.42	206.90
pp'-DDD	69.14	78.81	138.90	249.20	251.47	909.55
pp'-DDE	153.57	195.41	283.29	449.17	570.20	2924.82
DDE/ ΣDDT	0.58	0.58	0.56	0.54	0.60	0.72
<i>PCB congeners</i>						
18	6	39	5	13	9	41
20	3	13	6	6	2	11
31	3	3	2	6	1	11
28	6	8	4	9	6	33
44	3	2	3	5	1	7
52	36	98	34	74	44	360
77+110	92	52	122	341	193	1351
81	29	57	33	102	50	174
84	26	33	39	153	92	580
87	29	122	24	59	31	164
92	23	23	30	80	51	454
95	103	36	59	172	120	1098
99	136	50	82	226	161	1455
101	115	39	77	248	130	1199
105	96	59	74	206	120	1059
114	34	36	47	183	133	951
118	158	127	105	349	190	1795
123+149	189	143	350	868	783	4487
126	52	105	111	262	203	649
128	111	47	75	237	134	1535
135	33	26	56	168	104	827
136	24	32	40	90	71	396
138	1553	294	586	1513	972	8358
141	24	618	13	40	20	178
151	198	697	118	306	232	2160
153	1757	384	849	2014	1345	10,906
156	79	27	59	142	89	950
157	10	8	21	48	33	249
167	39	24	64	256	133	1214
169	3	1	3	10	6	56
170	115	78	233	589	260	3531
174	440	51	116	287	206	2007
177	167	58	135	346	236	2327
180	1069	294	714	1699	1109	7632
183	188	60	144	347	227	2430
187	602	208	510	1145	710	6943
194	88	37	87	190	107	1155
195	26	11	27	51	31	291
196	87	40	95	226	120	1205
201	99	52	123	272	180	1587

The results of Table 4 show that in all tissues and organs mean concentrations of PCBs are relatively higher for females in comparison with males, though they are of the same order of magnitude (except for liver). These concentrations can be considered as abnormal for females but may be explained by different geographic origins of the animals. Furthermore, these animals have probably not reproduced yet and consequently have kept their stock of PCBs.

Because of both the variability intra- and inter organisms, and the relatively low number of analyzed samples, one can draw no conclusion about the influence of the age and the sex on the general contamination by PCBs and pesticides of our samples. (Aguilar et al., 1999; Borrell et al., 2001), make similar observations in their studies.

Other authors (Tanabe et al., 1987; Aguilar and Borrell, 1994; Borrell et al., 1995) showed differences in accumulation in organochlorinated compounds according to the sex, age and size in the various organisms. This accumulation begins at the fetal life,

which is a period where the contents in the fetus can reach those of the maternal organs. These contents amplify for the newborn babies by lactation, to stabilize and even decrease with the age, especially for the females whose contents are often weak during gestation (Tanabe et al., 1982; Alzieu et al., 1982).

3.2. Distribution of the congeners

Isomers specific analysis of PCB was performed for representative samples of dolphin organs and tissues to examine the distribution of congeners. Table 6 shows the average concentrations of congeners in tissues and organs.

The dominating congeners are: CB153, CB180, CB138, CB187. This distribution of congeners is comparable to that obtained by the majority of authors, on various types of marine mammals (Corsolini et al., 1995; Minh et al., 1999, 2000; Storelli and Marcotrigiano, 2000; Yogui et al., 2003).

The profiles of the congeners of PCBs in all tissues and organs are all similar. Fig. 2 shows an example of

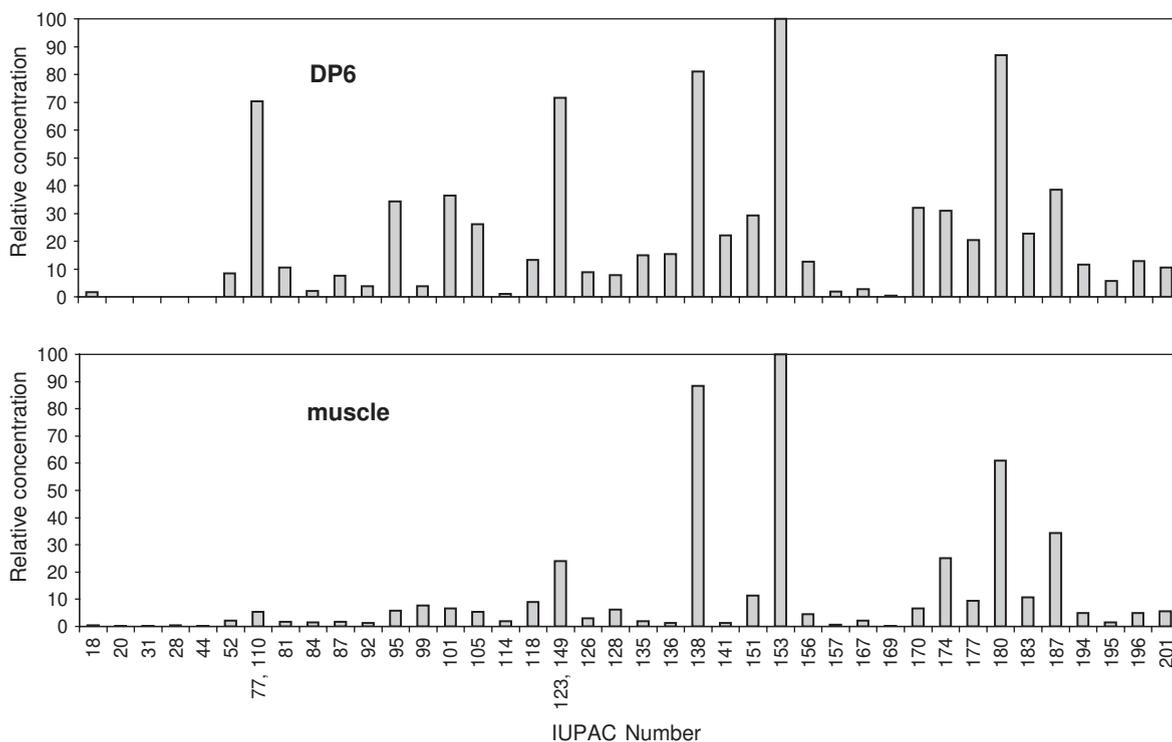


Fig. 2. Relative percentage of the main PCBs congeners in Dp6 and in the muscle chlorine number.

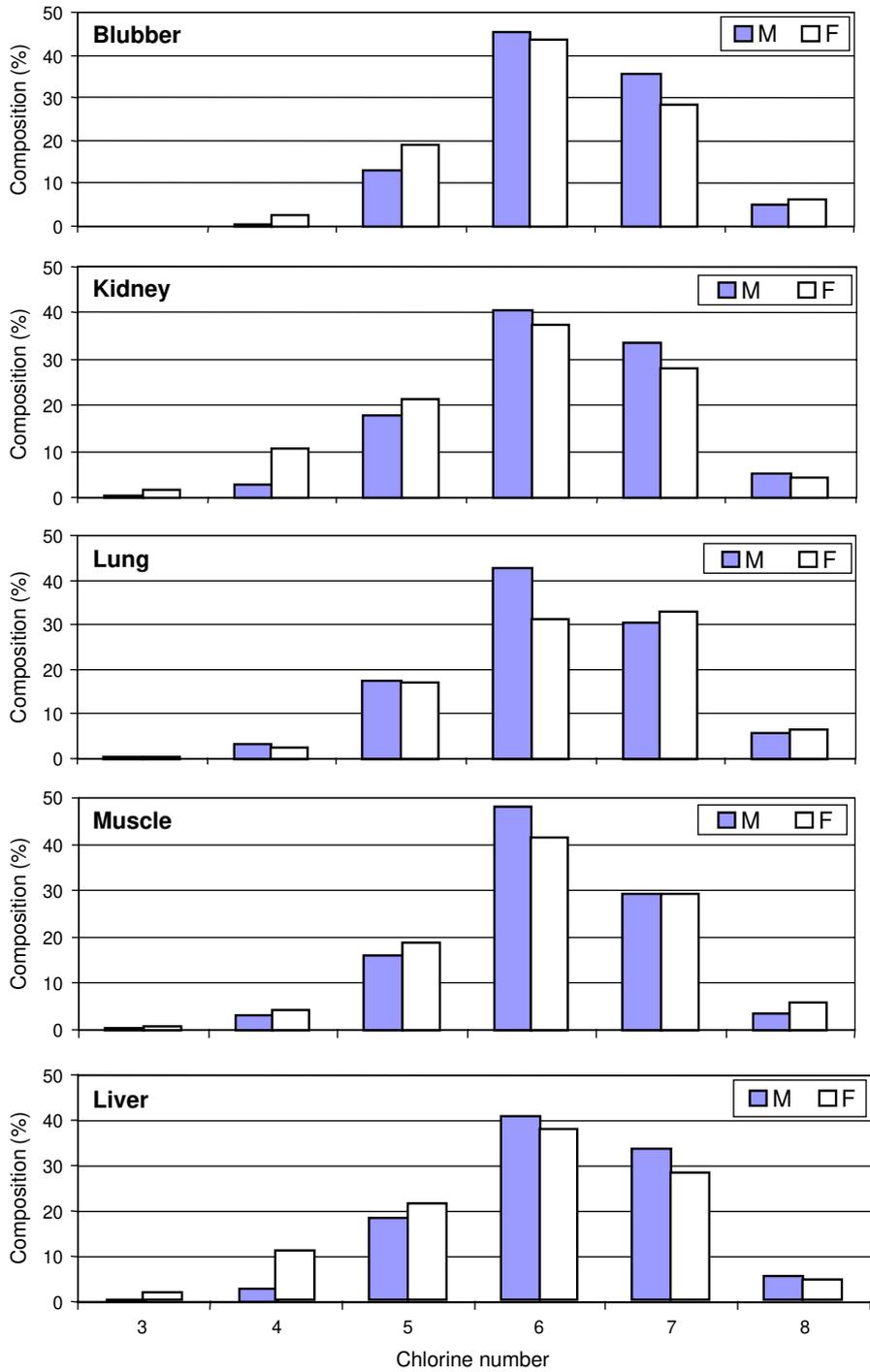


Fig. 3. Composition of PCBs in relation with chlorine number in different organs of males and females.

distribution of different congeners in the technical mixture DP6 and the muscle.

The different organs mainly accumulate the compounds with 5, 6, and 7 chlorines. They constitute 13% to 21.25%, 31.25% to 47.88% and 27.9% to 32.81% of total PCBs, respectively. The compounds with 3, 4, and 8 chlorines are in small proportions and constitute 0.15% to 1.65%; 0.6% to 10.8%; and 3.36% to 6.55% of total PCBs, respectively. This distribution is similar in all tissues and organs studied (Fig. 3).

In Dp6, which is the industrial compound the most used in France, the proportions of compounds with 5, 6, and 7 chlorines are respectively 25%, 37%, and 24% of total PCBs, while the proportions of compounds with 3, 4, and 8 chlorines are respectively 0.17%, 9%, and 4% of total PCBs. Thus there is a similarity of behavior between the DP6 and the contents in the samples. Examples of proportions in PCBs in the DP6 and the organs are presented in Fig. 4.

The PCBs are little metabolized (Bonn et al., 1992) and consequently, they accumulate in tissues and organs.

Many studies have shown that marine mammals have the capacity to concentrate organochlorinated compounds such as PCBs (Fossi et al., 1992; Sawhney, 1986), but have a weak potentiality to decompose them especially with regard to the strongly chlorinated congeners.

The congeners 153 and 138 are very stable and dominating in technical mixtures and their behavior gives the index either of the evolution or of the reduction of these compounds in the considered environment (water, sediment, organisms). Works carried out by (Monod et al., 1995; Perez et al., 2003;

Table 7

Comparison of total PCB (mg/kg lipid wt.) in the blubber of *Stenella coeruleoalba* from several areas in the world

Location	n	PCB (ppm, l.b.)	References
USA (Atlantic)	3	59	Taruski et al., 1975
Mediterranean, France	27	266.9	Alzieu and Duguy, 1979
Japan	49	14	Fukushima and Kawai, 1981
Japan	4	29	Tanabe et al., 1983
West Pacific (1986)	8	28	Loganathan et al., 1990
Western Mediterranean sea	10	390	Kannan et al., 1993
Gibraltar	3	67	Borrell, 1993a
Wales	7	39	Borrell, 1993b
Italy	64	205	Marsili and Forcardi, 1996
Italy (Biopsies)	61	70	Marsili, 2000
Mediterranean, France	6	70	This study

and Wafo, pers. comm.) showed that in water, the ratio 153/138 is lower than unity, on the other hand in the sediment it is equal or higher than 1, while in the organisms it is higher than 1. We observe this behavior in all tissues and organs of our samples.

In the technical mixture DP6, the tri-chlorine-biphenyl compounds are practically non-existent, except the congener 18 (Fig. 1). On the other hand in all analyzed samples, we find a certain number of these tri-chlorinated congeners (cong. 20, 18, 28, 31, 44). This could either represent a process of metabolism, or means that the animals were exposed to other types of PCBs, for example pyralene of condenser (DP3, equivalent to the arochlor 1243).

Studies we have conducted on the sediments in littoral Mediterranean environment show both high

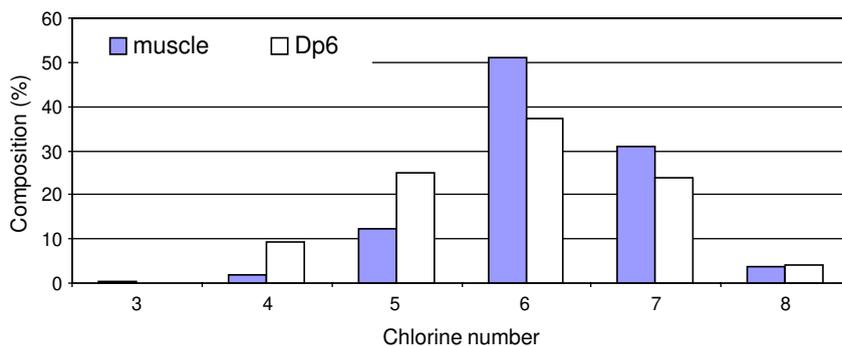


Fig. 4. Proportions of PCBs in relation to chlorine number in the muscle and DP6.

proportions of congeners strongly chlorinated, and the presence of these tri-chlorinated and tetra-chlorinated congeners (Wafo, pers. comm.).

4. Conclusion

The results of this study and their comparison with other data show the persistence of the organochlorinated compounds in the environment. Among the 7 studied animals, 6 belong to the same species: *S. coeruleoalba*, and only 1 *T. truncatus*. For the same organs studied for the two species, *T. truncatus* seems to be less contaminated than *S. coeruleoalba*, though it is hazardous to conclude from only one specimen analyzed. However similar results were obtained by Alzieu and Duguay (1979) and Marsili and Forcardi (1997) on numerous samples.

Important concentrations are found in all studied tissues and organs. The results obtained are of the same order of concentration as those obtained in our laboratory during the nineties. The results obtained in blubber tissues are relatively similar to those of other authors on the Mediterranean environment, but often higher than those recorded in the rest of the world (Table 7).

This observation confirms consequently low capacity of detoxification of these animals (Watanabe et al., 1989). Among the compounds of DDT, pp'-DDE is the most dominating molecule, which demonstrates the old age of these compounds in the environment.

The values of the ratio PCB/ Σ DDT are largely higher than 1 in all tissues and organs. This is due to the fact that DDT is prohibited in the majority of the European countries since the seventies.

The analysis of our results does not show any significant difference of concentration related to the age or the sex and from one animal to another. The profiles of the congeners are similar from one organ to another and similar for all the animals.

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References

- Aguilar A. Relationship of DDE/tDDT in marine mammals to the chronology of DDT input into the ecosystem. *Can J Fish Aquat Sci* 1984;41:840–4.
- Aguilar A. Compartmentation and reliability of sampling procedures in organochlorine pollution surveys of cetaceans. *Res Rev* 1985;95:91–114.
- Aguilar A, Borrell A. Patterns of lipid content and stratification in the blubber of fin whales (*Balaenoptera physalus*). *J Mamm* 1990;71(4):544–54.
- Aguilar A, Borrell A. Reproductive transfert and variation of body load of organochlorine pollutants with age in fin whales, *Balaenoptera physalus*. *ArchEnvironContamToxicol* 1994;27: 546–54.
- Aguilar A, Borrell A, Pastor T. Biological factors affecting variability of persistent pollutant levels in cetaceans. *J Cetacean Res Manag* 1999;(Special issue 1):83–116.
- Alzieu C, Duguay R. Teneurs en composés organochlorés chez les cétacées et les pinipèdes fréquentant les côtes Françaises. *Oceanol Acta* 1979;2(1):107–20.
- Alzieu C, Duguay R, Babin P. Pathologie des Delphinidae: contamination fœtale et néo-natale par les PCB Lésions cutanées ulcératives. *Rev Tr Inst Pêch mar* 1982;46(2):157–66.
- Ballschmiter K, Zell M. Analysis of polychlorinated biphenyls by glass capillary gas chromatography. *Fresenius Z Anal Chem* 1980;302:20–31.
- Bonn JP, Van Arnhem E, Jansen S, Kannan N, Petrick G, Schulz D, et al. The Toxicokinetics of PCBs in marine mammals with special reference to possible interactions of individual congeners with the cytochrome P450-Dependent Monooxygenase System: an overview. In: Walker CH, Livingstone DR, editors. *Persistent Pollutants in Marine Ecosystems*. Oxford: Pergamon; 1992. p. 119–59.
- Borrell A. Dinamica dels contaminants organoclorats en la Balena d'Aleta, el Cap d'Olla d'Alerta Llarga i el Dofi Llistat d'Aigües Atlàntiques i Mediterrànies, Testi di Doctoral, Departament de Biologia Animal. Facultat de Biologia, Universitat de Baecelona 1993a:1-398.
- Borrell A. PCB and DDTs in blubber of cetaceans from the North-Eastern North Atlantic. *Mar Pollut Bull* 1993b;26(3):146–51.
- Borrell A, Aguilar A. Variation in DDE percentage correlated with total DDT burden in the blubber of fin and sei whales. *Mar Pollut Bull* 1987;18(2):70–4.
- Borrell A, Bloch D, Desportes G. Age trends and reproductive transfert of organochlorine compounds in long-finned pilot whales from the Faroe Islands. *Environ Pollut* 1995;88: 283–92.
- Borrell A, Cantos G, Pastor T, Aguilar A. Organochlorine in common dolphins from the Atlantic and Mediterranean waters of Spain. *Environ Pollut* 2001;114:265–74.

- Corsolini S, Forcaldi S, Kannan K, Tanabe S, Borrel A, Tatsukawa R. Congener profile and toxicity assessment of polychlorinated biphenyls in dolphins, shark, and tuna collected from Italian coastal waters. *Mar Environ Res* 1995;40:33–53.
- De Kock AC, Best PB, Cockcroft V, Bosma C. Persistent organochlorine residues in small cetaceans from the east and west coasts of southern Africa. *Sci Total Environ* 1994;154:153–62.
- Fossi MC, Marsili L, Leonzio C, Notarbartolo di Sciarra G, Zanardelli M, Forcardi S. The use of non destructive biomarker in Mediterranean cetaceans: preliminary data on MFO activity in skin biopsy. *Mar Pollut Bull* 1992;24(9):459–61.
- Fukushima M, Kawai S. Variation of organochlorine residue concentration and burden in striped dolphin (*Stenella coeruleoalba*) with growth. In: Fujiyama T, editor. *Studies on the Levels of Organochlorine Compounds and Heavy Metals in the Marine Organisms*. Okinawa: University of the Ryukyus; 1981. p. 97–114.
- Gauthier JM, Metcalfe CD, Sears R. Chlorinated organic contaminants in blubber biopsies from northwestern Atlantic baleenopterid whales summering in the gulf of St. Lawrence. *Mar Environ Res* 1997;44:201–23.
- Gi Beum K, Jong SL, Tanabe S, Iwata S, Ryo S, Tatsukawa R, et al. Specific accumulation and distribution of Butyltin compounds in various organs and tissues of the Steller sea lion: comparison with Organochlorine accumulation pattern. *Mar Pollut Bull* 1996;32(7):558–63.
- Kannan K, Tanabe S, Borrell A, Aguilar A, Forcardi S, Tatsukawa R. Isomer-specific analysis and toxic evaluation of Polychlorinated Biphenyls in striped dolphin affected by an epizootic in the western Mediterranean Sea. *Arch Environ Contam Toxicol* 1993;25:227–33.
- Kawai S, Fukushima M, Miyazaki N, Tatsukawa R. Relation Ship between lipid composition and organochlorine levels in the tissue of striped dolphin. *Mar Pollut Bull* 1988;19(3):129–33.
- Loganathan BG, Tanabe S, Tanaka H, Watanabe S, Miyazaki N, Amano M, et al. Comparison of Organochlorine residue levels in striped dolphin from the western North Pacific, 1978–79 and 1986. *Mar Pollut Bull* 1990;21:435–9.
- Marsili L. Lipophilic contaminants in marine mammals: review of the results of ten years' work at the Department of Environmental Biology, Siena University (Italy). *Int J Environ Pollut* 2000;13(1–6):416–52.
- Marsili L, Forcardi S. Organochlorine levels in subcutaneous blubber biopsies of fin whales (*Balaenoptera physalus*) and Striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea. *Environ Pollut* 1996;91(1):1–9.
- Marsili L, Forcardi S. Chlorinated Hydrocarbon (HCB, DDTs, and PCBs) Levels in Cetaceans Stranded Along the Italian Coast: an Overview. *Environ Monit Assess* 1997;45:129–80.
- Marsili L, Forcardi S, Cuna D, Leonzio C, Casini L, Bortolotto A, et al. Chlorinated hydrocarbons and heavy metals in tissues of striped dolphins (*Stenella coeruleoalba*), stranded along the Apulian and Sicilian coasts (summer 1991). *Eur Res Cetaceans* 1992;6:234–7.
- Metcalfe C, Metcalfe T, Ray S, Paterson G, Koenig B. Polychlorinated biphenyls and Organochlorine compounds in brain, liver and muscle of Beluga whales from the Arctic and St Lawrence estuary. *Mar Environ Res* 1999;47:1–15.
- Minh TB, Watanabe M, Nakata H, Tanabe S, Jefferson TA. Contamination by persistent organochlorines in small cetaceans from Hong Kong Coastal waters. *Mar Pollut Bull* 1999;39:383–92.
- Minh TB, Nakata H, Watanabe M, Tanabe S, Miyazaki N, Jefferson TA, et al. Isomer-specific accumulation and toxic assessment of Polychlorinated Biphenyls, including Coplanar congeners, in cetaceans from the North Pacific and Asian coastal waters. *Arch Environ Contam Toxicol* 2000;39:398–410.
- Monod JL, Arnaud PM, Arnoux A. PCB Congeners in the Marine Biota of Saint Paul and Amsterdam Islands, Southern Indian Ocean. *Mar Pollut Bull* 1995;30(4):272–4.
- Murphy PG. Sulfuric acid for the cleanup of animal tissues for analysis of acid-stable chlorinated hydrocarbon residues. *J - Assoc Off Anal Chem* 1972;55:1360–2.
- Nakata H, Kannan K, Jing L, Thomas N, Tanabe S, Giesy JP. Accumulation pattern of organochlorine pesticides and polychlorinated biphenyls in southern sea otters (*Enhydra lutris nereis*) found stranded along coastal California, USA. *Environ Pollut* 1998;103:45–53.
- Perez T, Wafo E, Fourt M, Vacelet J. Marine sponge as biomonitor of polychlorobiphenyl contamination: concentration and fate of 24 congeners. *Environ Sci Technol* 2003;37:2152–8.
- Poster X, Simmonds M. Proceedings of the Mediterranean striped dolphins mortality international workshop, Greenpeace International Mediterranean Sea project, Palma de Mallorca, Spain, November 1992; 4–5; 1992. p. 190.
- Prudente M, Tanabe S, Watanabe M, Subramanian A, Miyazaki N, Suarez P, et al. Organochlorine contamination in some odontoceti species from the North Pacific and Indian Ocean. *Mar Environ Res* 1997;44:415–27.
- Sawhney BL. Chemistry and properties of PCBs in relation to environmental effects. Waid JS, editor. *PCBs and the environment*, vol. 1. Boca Raton: CRC press; 1986. p. 48–61.
- Storelli MM, Marcotrigiano GO. Persistent organochlorine residues in Risso's dolphins (*Grampus griseus*) from the Mediterranean (Italy). *Mar Pollut Bull* 2000;40:555–8.
- Tanabe S, Watanabe R. Persistent organochlorines in marine mammals. In: Jones KC, editor. *Organics Contaminants in the Environment, Environmental Pathways and Effects*. London: Elsevier Aoolied Science; 1991. p. 275–89.
- Tanabe S, Tanaka H, Murayama K, Tatsukawa R. Ecology and bioaccumulation of *Stenella coeruleoalba* Elimination of chlorinated hydrocarbons from mother striped dolphins (*Stenella coeruleoalba*) through parturition and lactation. In: Fujiyama T, editor. *Studies on the Levels of Organochlorine Compounds and Heavy Metals in the Marine Organisms*. Okynawa: University of the Ryukyus; 1981. p. 15–21.
- Tanabe S, Murayama K, Tatsukawa R, Miyazaki N. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. *Agric Biol Chem* 1982;46:1249–54.

- Tanabe S, Mori T, Tatsukawa R, Miyazaki N. Global pollution of Marine Mammals by PCBs, DDTs and HCHs. *Chemosphere* 1983;12:1269–75.
- Tanabe S, Loganathan BG, Subramanian AN, Tatsukawa R. Organochlorine residues in short-finned pilot whales Possible use as tracers of biological parameters. *Mar Pollut Bull* 1987; 18:561–3.
- Tanabe S, Watanabe S, Kan H, Iwata H, Tatsukawa R. Capacity and mode of PCB metabolism in small cetaceans. *Mar Mamm Sci* 1988;4:103–24.
- Tanabe S, Iwata H, Tatsukawa R. Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Sci Total Environ* 1994a;154:163–77.
- Tanabe S, Sung JK, Choi DY, Bara N, Kiyota M, Yoshida K, et al. Persistent organochlorine residues in northern fur seal from the Pacific coast of Japan since 1971. *Environ Pollut* 1994b;85: 305–14.
- Taruski AG, Olney CE, Winn HE. Chlorinated Hydrocarbons in Cetaceans. *J Fish Res Board Can* 1975;32:2205–9.
- Villeneuve, J.P., de Mora, S.J., Cattini, C. World-wide and Regional Intercomparison for the determination of organochlorine compounds and petroleum hydrocarbons in fish homogenate IAEA-406, Marine Environment Laboratory 2000, B.P. 800, MC-98012 MONACO.
- Watanabe S, Shimada T, Nakamura S, Nishiyama N, Yamashita N, Tanabe S, et al. Specific profile of liver Microsomal Cytochrome p-450 in dolphin and whales. *Mar Environ Res* 1989; 27:51–65.
- Williams LL, Giesy JP, Galan DE, Verbrugge N, Tillitt DA, Ankley DE, et al. Prediction on concentrations of 2378-TCDD equivalents from total concentrations of PCB in fish fillets. *Environ Sci Technol* 1992;26:1151–9.
- Yogui GT, de Oliveira Santos MC, Montone RC. Chlorinated pesticides and polychlorinated biphenyls in marine tucuxi dolphins (*Sotalia fluviatilis*) from the Cananéia estuary, south-eastern Brazil. *Sci Total Environ* 2003;312:67–78.