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Environmental Contamination in Bottlenose Dolphin *(Tursiops truncatus):* Relationship Between Levels of Metals, Methylmercury, and Organochlorine Compounds in an Adult Female, Her Neonate, and a Calf

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Bottlenose dolphin *(Tursiops truncatus)* occurs in all cold temperate to tropical seas worldwide. It appears to be common in the Mediterranean Sea. This species is primarily coastal in its distribution and it forms small groups of 10-100 individuals. These dolphins grow to a maximum length of ca 3 m, with a weight of around 300 kg and have a longevity of at least 30 years. They feed on a wide variety of fish available in their habitat, with a large fraction consisting of mullet. squid and octopus. Sexual maturity is attained between 5-12 years for females and from 10 years for males, According to estimates of the reproductive cycle, females give birth every two years. The pregnancy lasts 12 months while lactation lasts 12-18 months (Duguy & Robineau, 1982).
These marine mammals, since they are inshore organisms. have been considered to be subject directly to pollution because coastal areas, though highly productive systems providing important nursing zones and habitats, are also the repository for toxic agents and hazardous materials from industrial. agricultural and urban sources such as heavy metals and organochlorine compounds. This, together with the consideration that dolphins are typical end-points in the biomagnification of persistent pollutants in the pelagic food chain led to many studies on the levels and effects of contaminants in marine mammals (Beck et al. 1997: Wood and Van Vleet 1996: Gauthier et al.. 1998). Since organochlorine compounds and methylmercury may be transferred from mother to offspring (Tanabe et al.. 1982: Ridgway and Reddy, 1995) it is-important to gather information for a particular species in different stages of life if possible.
The present study reports the changes in concentrations of metals, methylmercury, polychlorinated biphenyls and organochlorinated pesticides in various tissues and organs of a female, her neonate and a calf of bottlenose dolphin *(Tursiops truncatus)* species.

MATERIALS AND METHODS

One *Tursiops truncatus* female (length 262 cm) with the umbilical cord still tied to her neonate. (male length 111 cm). and a calf (female length 138 cm) were found dead along Apulian coast (South Adriatic Sea) in June 1996 and May 1997. respectively. Following necropsy, brain, heart, lung liver, intestine and kidney were removed and kept in a deep freeze at -20 °C until chemical analysis. Placenta and uterus were analysed from the adult female only.
Homogenized samples (l-3 g wet weight) for quantitative analysis of heavy metals by atomic absorption spectrophotometer (Perkin Elmer 5000) were digested into the reaction flask with 11 ml of the mixture HNO_3-HC104 (8:3) for Cu and Zn (Ciusa & Giaccio 1984). and with 10 ml of the mixture H_2SO_4-HNO_3 (1: 1) for Hg and Se (G.U. 1990). Cu and Zn were determined in a flame atomic absorption spectrophotometer. Hg was determined by the cold vapour technique after reduction by $SnC1_2$ (A.V.A. Thermo Jarrel Ash Corp.), while Se was measured as volatile hydrides after reduction by $NaBH_4$ (MI-IS-10 Perkin Elmer). Methylmercury was determined following the method described by Hight and Corcoran (1987), already reported elsewhere (Storelli et al.. 1998). and analyzed by gas chromatography equipped with a ^{63}Ni electron capture detector (ECD-400).
Analytical quality control was achieved using TORT-l Lobster Hepatopancreas (National Research Council of Canada) (Table 1). All data were computed on a mg/kg wet weight basis and the coefficients of variation (CV%) of replicate subsamples were below 10%.

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Table 1. Total Hg, MeHg, (as Hg), Se, Cu and Zn concentrations in reference material (TORT-l). recovery (%) and detection limit (D.L.).

	Hg	MeHg	Se	Cu	Zn
TORT-1 (mg/kg)	0.33±0.06	0.128±0.014	6.88±0.47	439±22	177±10
found values (mg/kg)	0.32±0.02 n = 10	0.123±0.14 n = 10	6.37±0.18 n = 10	426±15 n = 10	166±12 n = 10
Recovery %	97	92	93	100	100
D.L. (ng/g)	50	20	50	10	10

To determine chlorobiphenyl (PCBs = sum of 11 congeners) and chlorinated pesticide (POC = -p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD. o,p'-DDD) concentrations the following method was used. Aliquots (2-5 g) of the homogenised samples were ground with anhydrous sodium sulphate in a mortar. The mixture was extracted with petroleum ether according to Erney's procedure (Emey, 1983). The extracts were then concentrated and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot of the remaining extract was mixed with H_2SO_4 conc. for the clean up, following the procedure described by Murphy (1972). Analyses were made on a Carlo Erba HR gas chromatograph 5300 Mega Series with automatic injection system and with an electron capture detector ECD-400, Ni^{63}(temperature: 310 °C). The GC was connected to an IBM PS/2 55SX PC equipped with System Gold version 6.1 software program for integration purposes (Beckman). For all the analyses a fused-silica capillary column SPB-608 Supelco (length = 30 mt. inside diameter 0.25 mm and film thickness 0.25 µm), was used. Helium at a flow rate of 1 ml/min was used as gas carrier, nitrogen as make-up gas 60 ml/min. Temperature was programmed according to the following sequence: injection at 50 °C. Oven steady for the first 1 min and then an increased from 50 to 180 °C at a rate of 15 °C/min. Oven maintained at steady temperature for 1 min and then increased from 180 to 220 °C at a rate of 4 "C/min: oven maintained at steady temperature for 20 min and then increased from 220 to 275 °C at a rate of 5 °C/min: from this point until the end of the analytical nm. the column remained isothermal at a temperature of 275 °C. The eleven individual PCB congeners were 8. 20. 28. 35. 52. 101, 118. 138. 153, 180 and 209 IUPAC numbering system (Ballschmiter & Zell. 1980) determined against the corresponding individual standards obtained from ULTRA Scientific. Inc. (chemical purity 99%). The identity of the DDT group compounds was confirmed by an alkali conversion to their respective olefins and re-analysis by GLC. Analytical data, as for DDT group compounds were obtained by a comparison between sample peak area and external standards peaks area (POCs mixture, bought from Supelco). Recoveries are determined by adding known amounts of PCBs and POCs standards to empty samples and found to be within 80-110%. The limits of quantification were from 0.1 to 0.4 ng/g on a wet wt basis for the pesticides and the PCB congeners. Quantification was done within the linear range of the detector. Non detected constituents were assigned a value of zero. Residues in 10% of the samples were confirmed by gas-liquid chromatography-mass spectrometry (Fisons MD 800). Concentrations of PCBs and POCs are presented as ng/g on a lipid weight basis.

RESULTS AND DISCUSSION

Table 2 and 3 show metal. methylmercury concentrations and % MeHg in tissues and organs of the adult female dolphin. her neonate and a calf of *Tursiops truncatus.*

In the adult female high concentrations of mercury were found in liver followed by kidney. brain. intestine. heart, uterus. lung and placenta. In the calf and neonate too, the highest mercury levels were observed in liver. Slightly higher levels in the neonate than in the calf were detected in addition to liver. also in lung. brain and heart.

As for selenium. in the adult female. the highest concentrations were detected in liver followed by kidney. lung, brain. intestine and heart. The lowest concentrations were found in uterus and placenta. In the neonate the highest levels were discovered in liver. while the other organs and tissue showed the same levels. In the calf the concentrations of selenium were approximately the same in all the organs and tissues analysed.

Table 2. Mean concentrations of total Hg. Se. Cu and Zn (mg/kg w.w.) and standard deviation in different organs and tissues of bottlenose dolphin.

Organ and Tissue		Total Hg	Se	Cu	Zn
Liver	Mother	393.36±1.32	129.35±1.12	8.29±0.16	52.82±0.65
	Neonate	9.21± 0.90	5.17±0.51	80.65±0.98	64.12±8.76
	Calf	3.95±0.10	1.94±0.07	19.34±0.71	62.91±1.60
Kidney	Mother	34.58±1.80	13.54±0.19	2.78±0.21	28.73±1.22
	Neonate	1.62±0.04	1.93±0.16	6.94±0.16	30.78±1.51
	Calf	1.21±0.43	1.88±0.23	5.32±0.21	25.91± 2.67
Lung	Mother	10.92±0.48	8.45±0.44	1.12±0.13	17.71±0.92
	Neonate	1.42±0.03	1.29±0.09	5.53±0.37	32.10±1.13
	Calf	0.33±0.03	1.05±0.11	0.87±0.11	21.87±0.35
Brain	Mother	25.84±0.65	5.12±0.12	4.30±0.01	14.86±1.02
	Neonate	1.61±0.03	1.00±0.18	3.11±0.47	22.53±0.25
	Calf	0.39±0.01	0.59±0.21	1.33±0.03	12.60±0.00
Intestine	Mother	21.60±0.71	4.29±0.53	1.12±0.03	25.47±0.95
	Neonate	0.98±0.02	1.21±0.30	5.04±0.08	46.84±0.60
	Calf	0.57±0.09	1.09±0.08	2.80±0.15	22.06±0.76
Heart	Mother	13.30±0.85	4.28±0.06	4.10±0.41	29.76±1.33
	Neonate	1.42±0.13	0.90±0.04	5.13±0.31	29.31±1.15
	Calf	0.46±0.01	0.88±0.06	3.37±0.01	23.05±0.17
Uterus	Mother	12.24±0.08	3.06±0.61	1.10±0.11	39.34±0.30
Placenta	Mother	1.98±0.35	1.56±0.45	1.91±0.23	15.98±0.03

Table 3. Mean concentrations of total mercury, methylmercury, (mg/kg w.w.) standard deviation and % MeHg in different organs and tissues of bottlenose dolphin.

		Total Hg	MeHg	%MeHg
Liver	Mother	393.36±1.32	38.31±0.30	9.7
	Neonate	9.21± 0.90	9.30±0.28	100
	Calf	3.95±0.10	ND	---
Kidney	Mother	34.58±1.80	25.32±0.29	73.2
	Neonate	1.62±0.04	ND	---
	Calf	1.21±0.43	ND	---
Lung	Mother	10.92±0.48	3.73±0.36	34.1
	Neonate	1.42±0.03	ND	---
	Calf	0.33±0.03	ND	---
Brain	Mother	25.84±0.65	20.95±0.14	81.0
	Neonate	1.61±0.03	ND	---
	Calf	0.39±0.01	ND	---
Intestine	Mother	21.60±0.71	3.84±0.52	17.7
	Neonate	0.98±0.02	ND	---
	Calf	0.57±0.09	ND	---
Heart	Mother	13.30±0.85	9.44±0.47	70.9
	Neonate	1.42±0.13	0.87±0.03	61.2
	Calf	0.46±0.01	ND	---
Uterus	Mother	12.24±0.08	9.26±0.18	75.6
Placenta	Mother	1.98±0.35	N.D.	---

Mercury is a well-known and persistent marine pollutant that is capable of being biomagnified along the food chain. Mercury accumulates mainly in liver and its toxicity depends on its chemical form with the organic form methylmercury, having greater toxicity than inorganic mercury. Organic mercury concentration in liver varies with the total amount of this metal, with the larger proportion of the total being the less toxic inorganic form (Palmisano et al.. 1995; Storelli et al., 1998). In fact in the liver of the adult female dolphin the levels of methylmercury were lower than the amount of total mercury with a ratio of MeHg/total Hg. expressed as a percentage. of 9.7%. This supports the hypothesis of a detoxifying activity taking place in this organ, whereby MeHg is converted to the less toxic inorganic. Inorganic mercury is complexed with selenium and precipitated as mineral granules composed of mercuric selenide. identified in the liver of some dolphins by Martoja & Berry (1980). Granules are highly insoluble, thus representing a form in which Hg is immobilized and prevented from exerting cytotoxic effects. In fact the inorganic Hg/Se molar ratios occurred in the liver of the adult female was approximately 1. In the other organs and tissues of the adult female. on the contrary, methylmercury percentages were higher ranging from 17.7 to 81%. In the neonate methylmercury high levels, compared with the amounts of total mercury present, were observed in liver and heart while in the other organs and tissues methylmercury was below the instrumental detection limit. In neonate liver, mercury was entirely in the methylated form in agreement with a recent study on mercury speciation in *S. coeruleoalba* showing that for low total mercury concentrations, methylmercury percentage was very high (Storelli et al. 1998). It has been suggested that in dolphins methylmercury can be transferred from mother, through the placenta. and accumulate in foetus (Andrè et al.. 1990). On the contrary, other researchers (Law et al. 1992) reported for porpoises and common dolphin that the transplacental transfer from mother to foetus is neither a major accumulation route for the offspring, nor a significant method of elimination for the mother. In the present study MeHg concentrations found in the liver and heart of the neonate were 9.30 mg/kg and 0.87 mg/kg respectively. relative to concentrations of 38.31 mg/kg and 9.44 in his mother. These results. though relative to liver and heart solely. indicate that methylmercury can cross the placental barrier and accumulate in the foetus.

In the adult female the highest copper concentrations were discovered in liver. Relatively high concentrations of copper were also found in brain. It is known that copper in this organ is associated with Cu-protein as albocuprein I and II (Kimura and Araki, 1981). As for the other organs and tissues the accumulation order was the following: heart>kidney>placenta>lung= intestine=uterus. A similar distribution pattern was observed by Honda et al. (1982) in adult females of striped dolphin from Japan. In the neonate dolphin the highest concentration occurred in liver followed by kidney, lung, intestine and heart which showed similar levels. while in brain was the lowest. For the calf too. the highest concentrations of copper were in liver. followed by kidney. heart. intestine, brain and lung. A discrepant accumulation of copper in liver, differing from that of the adult female was observed. The concentration of copper in the liver of the neonate was markedly high (80.65 mg/kg wet wt.). compared with that in the adult female (8.29 mg/kg wet wt). Likewise in the liver of the calf. copper concentration was higher than that observed in the adult female but sensibly lower than that found in the neonate. In most mammalian species, including man, liver copper concentrations are higher in newborn than in adult females (Underwood 1971). Aaseth and Norseth (1986) report copper values in newborn human livers of 30 mg/kg wet weight decreasing to 5-10 mg/kg wet weight within the first year of life. Significantly higher copper concentrations have also been reported in liver tissue of neonate harp seals than in adult female *(Phoca groenlandica)* (Wagemann and Muir,1984; Wagemann et al. 1988). In the present work the calf showed a liver copper value twice higher than that of the adult female and this appears to be a result of the initial decrease in copper during the first year of life followed by maintenance of a fairly constant concentration throughout the lifespan of the animal. The neonate showed a liver copper concentrations over 10 times greater than that found in the mother. The same ratio was observed in a mother and foetus of Dall’s porpoise *(Phocoenoides dalli)* from the north Pacific Ocean (Fujise et al., 1988) and in a mother and foetus of common dolphin *(Delphinus delphis)* from the Irish Sea (Law et al., 1992). Luckey & Venugopal (1977) reported that the livers of newborn animals contain a cystine-rich copper binding protein. which is thought to have a either a detoxifying or storage function. Mammalian milk is known to be low in copper so the neonate animals that consume only milk must rely on copper acquired prior to parturition (Robbins. 1983).

The concentrations of zinc in the adult female dolphin *Tursiops truncatus* were higher in liver. and decreased in the order: uterus > heart > kidney > intestine > lung > placenta >brain. As for the neonate. the highest levels were found in liver and intestine followed by lung. kidney, heart and brain. High zinc concentrations were found in the rear part of the foetal intestine and in the intestine contents of Dall's porpoises also by Fujise et al. (1988). They suggest that a large amount of zinc is excreted by the meconium during foetal periods, and also that its excretion mechanism is important for understanding the turnover rates and accumulation process of zinc in foetus. The calf showed the same zinc concentration in liver as the neonate. while for the other tissues and organs the levels were lower but comparable with those of the adult female. Zinc is a bioessential element. so the animals maintain the concentration within a specific range by homeostasis (Falconer et al.. 1983). Literature data compiled by Law et al. (1991) show a range of liver Zn concentrations of 27-66 mg/kg in grey and common seals from the North sea and the Wadden Sea: 18-87 mg/kg in porpoises and 16-210 mg/kg in dolphins. The liver concentrations of zinc in this study are within the range reported by Law et al.. (199 1) for dolphins with values close to the lowest level to established range.

Table 4 and 5 show PCBs. organochlorine and PCB congeners concentration (ng/g lipid weight) respectively in tissues and organs of the adult female dolphin. her neonate and the calf of *Tursiops truncatus.*

In the adult female PCBs were present in higher concentrations in kidney followed by heart. uterus, placenta. liver, intestine lung and brain. In the neonate the higher levels of PCBs were observed in kidney followed by lung intestine. liver. heart and brain. In the calf PCBs were present in concentrations higher m liver followed by kidney>lung>heart>intestinc and brain.

The major congeners in the adult female were CB153>CB138>CB180>CB118>CB101>CB52. A similar congener profile was found in the neonate with the following accumulation order: CB138≈CB153>CB180>CB118>CB101>CB52. Little variation w-as found in the calf with the following pattern: CB153≈CB138≈CB118>CB101>CB180 >CB52. The other congeners. including some with both less 3 and more 9 chlorine (8, 20, 28, 35 and 209) were below the detection limits in all samples. The isomer composition reflected the prevalence of highly chlorinated PCB congeners. The richness of a PCB isomer in a given organism is the result of the composition of PCB present in the environment modulated by the decomposition processes that occur from their entrance into the environment to the accumulation in the organism. PCBs with higher chlorine content are usually more difficult to metabolise (Sawhney. 1986). and therefore their biomagnification potential is higher. The dolphins are situated at the end of long food webs. for which reason PCBs have undergone substantial metabolization before ingestion by dolphin. This contributes to explain the prevalence of highly chlorinated congeners in their tissues.

As for DDTs the accumulation order in the adult female was the following: heart > liver = uterus > intestine > placenta > lung> kidney > brain. The p,p'-DDE concentration was the highest among the DDT compounds with percentages of 87,3% followed by p,p'-DDD (5.7%). o,p'-DDT (2.8%). o,p'-DDD (2.5%). and by p,p'-DDT (1.7%). As regards DDTs the accumulation order in the neonate was the following: kidney, intestine. lung. liver. heart and brain. The p,p'-DDE concentration was the highest among the DDT compounds with percentages of 71.8% followed by o,p'-DDT e p.p'-DDD which showed the same percentages (10.2%).o,p'-DDD (4.2%). and by p,p'-DDT (3,6%). In the calf DDTs were present in concentrations higher in liver followed by kidney>lung>heart>intestine and brain. Among DDT metabolites. p,p'-DDE was dominant in all the tissues representing 85,3% of the total DDT burdens. followed by o,p'-DDD (4.6%). o,p'-DDT (4.5%). p,p'-DDD (3.8%). and by p,p'-DDT (1.8%). Although p,p'-DDE was a major component of DDTs. the proportion of other isomers differed more or less according to the organ and tissue. Particularly. p,p'-DDT in the liver showed lower concentration. implying active metabolism of p,p'-DDT in this organ. The lowest levels of PCBs and DDTs in the three individuals examined were observed in brain. This may be explained by the effect of the hematoencephalic barrier which blocks the passage of the pollutants to a certain degree. or else by the molecular structure of the phospholipids. basic constituents of the cerebral lipids. which are characterized by a greater

Table 4. Mean concentrations of PCBs and POC compounds (ng/g lipid) in different organs and tissues of bottlenose dolphin.

Organ and Tissue		% lipid	PCBs	p,p'DDE	o,p'DDT	p,p'DDT	o,p'DDD	p,p'DDD	DDTs
Liver	Mother	13.8	2109	4783	116	145	239	521	5804
	Neonate	8.37	2832	1231	108	48	84	154	1625
	Calf	2.33	247854	115064	4678	1674	4206	4635	130257
Kidney	Mother	2.85	7895	1123	ND	ND	105	70	1298
	Neonate	5.90	5085	4000	915	186	220	713	6034
	Calf	4.31	157587	74037	1392	2158	4501	2181	84269
Lung	Mother	0.63	1111	2222	ND	ND	ND	ND	2222
	Neonate	0.63	4646	1496	79	ND	157	315	2047
	Calf	1.29	143799	72093	1318	1318	3876	2326	80931
Brain	Mother	7.69	325	286	221	182	169	286	1144
	Neonate	7.95	1082	340	63	12	50	88	553
	Calf	3.78	13862	4894	1614	1243	3413	3807	14471
Intestine	Mother	0.29	1725	3448	ND	ND	ND	ND	3448
	Neonate	3.03	4125	3861	495	330	133	330	5149
	Calf	1.08	28981	9074	7130	ND	ND	556	16760
Heart	Mother	3.02	3576	6225	530	199	232	695	7881
	Neonate	2.81	1922	1210	71	36	71	107	1495
	Calf	4.88	64201	42520	635	492	1045	963	45655
Uterus	Mother	0.12	3333	5833	ND	ND	ND	ND	5833
Placenta	Mother	1.20	2500	2833	ND	ND	ND	167	3000

Table 5. PCB congeners concentrations (ng/g lipid) in the various organs and tissues of bottlenose dolphin.

		52	101	118	153	138	180
Liver	Mother	58	159	138	783	536	435
	Neonate	72	179	370	705	1219	287
	Calf	3948	6824	12747	97554	84292	42489
Kidney	Mother	211	456	702	2070	2070	2386
	Neonate	68	424	424	2169	1203	797
	Calf	1856	4269	19722	56752	46891	28097
Lung	Mother	ND	ND	ND	476	318	317
	Neonate	79	236	472	1260	2047	552
	Calf	698	8760	10000	40853	54186	29302
Brain	Mother	ND	26	65	91	117	26
	Neonate	13	38	88	264	591	88
	Calf	370	317	1323	4894	4577	2381
Intestine	Mother	ND	ND	ND	690	690	345
	Neonate	33	330	297	1683	1221	561
	Calf	ND	1389	5370	7130	10648	4444
Heart	Mother	99	232	397	1358	828	662
	Neonate	ND	142	178	605	712	285
	Calf	1066	2377	512	26045	20041	14160

polarity than the remaining lipids and therefore, less capacity for retention of substances of apolar nature such as PCBs or DDTs, or by a combination of both factors (Aguilar. 1985).

In marine mammals, organochlorine loads tend to increase with age during the juvenile stage. both in males and females. because the uptake of contaminant usuallv exceeds metabolization and excretion. In adult males, this pattern continues and their contaminant levels increase with age although levels often reach a plateau in older individuals (Tanabe *et al.,* 1985: Martineau *et al.*. 1987; Aguilar & Borrell. 1988). However, in sexually mature females concentrations often decrease (Gaskin *et al,* 1982. 1983; Tanabe *et al.,* 1985: Aguilar & Borrell, 1988). because they transfer appreciable quantities of these compounds to their offspring during pregnancy and to a larger extent, during lactation (Fukushima & Kawai. 1981: Tanabe *et al.,* 1982).

In the present study high level of organochlorine compounds were found in the calf due to the transfer of these substances across the milk which containing an high percentage of triglycerides ranging 70-80% (Kawai *et al.,* 1988) is the principal route of elimination. Miyazaki *et al.,* (1998) reports that the major elimination rate of DDTs and PCBs in adult females of the striped dolphin estimated to be 91% and 88% respectively occurs in lactation. while notably lower percentages of PCBs and DDTs equal to 4.2 % and 3.8% respectively are eliminated in parturition. A contrasting pattern was discernible with regard to PCBs and DDTs levels in different organs and tissues between the mother and her neonate. In their livers PCBs concentrations were of the same order of magnitude. in kidney and heart were observed levels of about twice higher in the mother, while in the other organs concentrations were greater for the neonate. As for DDTs higher levels were observed in the neonate intestine and kidney with respect to the mother. comparable in lung and higher in the mother than in the neonate in liver. brain and heart. Presence of these contaminants in concentration equal or higher in the neonate versus her mother seem to demonstrate transplacental transfer.

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