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Heavy Metals and Methylmercury in Tissues of Risso's Dolphin *(Grampus griseus)* and Cuvier's Beaked Whale *(Ziphius cavirostris)* Stranded in Italy (South Adriatic Sea)

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Heavy metals acquired through the food chain or absorption via the water as a result of marine pollution are a potential threat to dolphins. In fact, marine mammals are known to accumulate these contaminants with extremely high concentrations because they occupy a top trophic position in the ecosystem. Mercury is one of the heavy metals to cause most concern in the marine ecosystem because its elevated toxicity, especially when present in the organic form(s). Since toxic heavy metal and methylmercury concentrations can vary considerably among and within species depending upon factors such as growth, lactation, parturition, tissue analysed, etc, it is important to determine baseline data for both species.

In spite of many documents relating heavy metal pollution in *Stenella coeruleoalba* and *Tursiops truncatus* (Law et al. 1992; Augier et al. 1993; Wood & Van Vleet 1996; Beck et al. 1997; Storelli et al. 1998), studies dealing with *Grampus griseus* and *Ziphius cavirostris* are meagre. In the Mediterranean, the relatively scantiness of beached *Grampus griseus* and *Ziphius cavirostris* respect to other dolphins of the basin, do not allow to draw useful data on the presence, distribution and habits of these two species. Nevertheless, generally the Risso's dolphin *(Grampus griseus)* and Cuvier's beaked whale *(Ziphius cavirostris)* are very widely distributed over the world's oceans, from tropical to temperate waters. They seem to prefer deep offshore waters and their diet consists largely of squid and occasionally of fish (Duguy & Robineau 1982).

The present study was conducted to determine the concentration of heavy metals (Hg, Se, Cd, Pb and Cr) and methylmercury in various organs and tissues of *Grampus griseus* and *Ziphius cavirostris* stranded along South Italy coast (South Adriatic Sea). Results from post-mortem investigations made on the animals are also presented.

MATERIALS AND METHODS

Between June and September 1996, 2 *Grampus griseus* specimens and 1 *Ziphius cavirostris* specimen (biological information for each of the three cetaceans are given in table 1) were found beached along the Apulian coast (South Adriatic

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Sea). Both post mortem studies and the collection and preservation of tissue samples were conducted according to previously established protocols (Myrick 1986; Law 1994). Muscle, lung, liver and kidney were used for determination of the tissue distributions of total mercury, methylmercury, selenium, cadmium, lead and chromium. 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-$HClO_4$(8:3) for Cd, Pb and Cr (Ciusa and Giaccio 1984) and with 10 ml of the mixture H_2SO_4-HNO_3(1: 1) for Hg and Se (G.U. 1990). For Cd, Pb and Cr determination, a graphite furnace (HGA-500 Perkin Elmer) was used. Hg was determined by the cold vapour technique after reduction by $SnCl_2$(A.V.A. Thermo Jarrel Ash Corp.), while Se was measured as volatile hydrides after reduction by $NaBH_4$ (MHS-10 Perkin Elmer. Methylmercury was determined following the method described by Hight and Corcoran (1987). Homogenized samples of the tissue (1 g wet weight) were prewashed 3 times with 10 ml of acetone and once with 10 ml of benzene. The prewashed tissue was acidified with 5 ml HCl-H_2O (l+l) and extracted 3 times with 10 ml of benzene. After centrifurgation, the combined benzene extracts were concentrated in Kuderna-Danish glassware. The extracts were diluted to 25 ml with benzene, mixed with 5 g Na_2SO_4, and analyzed by gas chromatography (Carlo Erba model HRGC-5300) equipped with a ^{63}Ni electron capture detector (ECD-400), and splitless injection technique was used. The column consisted of a fused silica capillary SPB-5 Supelco (length = 30 mt, inside diameter = 0.50 mm, 5 µm film). The coefficients of variation (CV%) of replicate subsamples were below 10% both for metals and methylmercury. Acid washed glassware, analytical grade reagents and double distilled deionized water were used in the tissue analysis. In order to check on the purity of the chemical used, a number of chemicals blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using TORT-l Lobster Hepatopancreas (National Research Council of Canada) (Table 2). All data were computed on a mg/kg wet weight basis.

RESULTS AND DISCUSSION

Heavy metal and methylmercury concentrations in the muscle, lung, liver and kidney of *Grampus griseus* and *Ziphius cavirostris* are shown in table 3.

Total mercury highest concentration in *Ziphius cavirostris* were found in the liver, followed by kidney, lung, and muscle. A similar distribution pattern was found in both *Grampus griseus* with the highest total mercury level in liver, followed by lung, kidney, and muscle. Marine mammals are top predators in the food chain and are long-lived, and tend to have much higher mercury concentrations than other marine organisms. It is well known that mercury is highly accumulated in the liver of marine mammals. Extremely high concentrations of mercury have been reported in the livers of striped dolphin from the Mediterranean coast of France and Italy (Andre et al. 1991; Storelli et al. 1998). The source of the mercury is believed to be natural deposits in the Mediterranean basin (Aubert et al. 1983; Bacci 1989). Other marine species from the Mediterranean such as

bluefin tuna *(Thunnus thynnus),* striped mullet *(Mullus barbatus)* and mussels *(Mytilus galloprovincialis)* also contain very high concentrations of total mercury in edible tissues (Law et a1. 1992). The limit of tolerance for mercury in mammalian hepatic tissue seems to be within the range 100-400 mg/kg wet weight. Total mercury levels in the liver of both species were higher or within the above range where hepatic damage can occur (Wageman & Muir 1984). High values of total mercury were found in the present work, also in the lung of both *Grampus griseus.* In this respect the lung could also play a role in detoxification of mercury (Augier et al. 1990), a role which is generally played by the liver.

The variability of total mercury concentration in the different organs examined was greater than that of methylmercury. The concentration of methylmercury was slightly higher in liver and in muscle than in other tissues. Methylmercury is formed in aquatic sediments by bacterial methylation of inorganic mercury. Most of the methylmercury so formed enters biological material because of its high affinity for sulfhydryl groups and lipids. As a result, an accumulation of mercury, mainly as methylmercury, in aquatic organism occurs with concurrent bioamplification phenomena through the trophic chain. In marine mammals, methylmercury is by far the most predominant form of mercury present in muscle tissues, while in liver its content is only a few percent of the total. In the present work in fact, the ratio organic: total mercury, expressed as a percentage, was highest in muscle (aver. 60 %), followed by kidney (aver. 9.3%), lung (aver. 5%) and liver (aver. 2.3%). In muscle, methylmercury is firmly bound through carbon-mercury and sulphydryl linkages, which could account for the high ratio of organic mercury in tissue (Gaskin 1982), while in liver the low percentage of organic mercury observed supports the hypothesis of a demethylating activity in this organ.

The concentrations of selenium in *Ziphius cavirostris* were also the highest in liver, followed by kidney and lung, which show similar levels and by muscle. Maximum values of selenium were also found in both *Grampus griseus* liver, followed by lung, kidney and muscle. Selenium is an essential element and marine mammals appear to have a homeostatic mechanism for retaining trace amounts and excreting the excess material. Toxic effects can occur when the rate of intake exceeds the excretory capacity (Schroeder et al 1970). Elevated hepatic selenium levels have been shown to cause toxic effects in mammals (Glenn et al. 1964), although this does not always occur, due in part to the binding of selenium with other metals (Koeman et al. 1973,1975). Ridlington and Whanger (1981) suggest that the mechanism by which this occurs is that the selenide form of selenium binds to a sulphydryl group of a pre-existing protein in which the selenium group subsequently binds the metal. In addition to this diversion of metal binding, the selenide form could also react with metals to form insoluble selenides, thus reducing metal toxicities. In liver the Hg:Se molar ratios for the two species were of ca 1: 1, reflecting the detoxification of methylmercury and its immobilization as mercuric selenide (Palmisano et al. 1995). For striped dolphins from the coast of Italy Palmisano et al (1995) and Storelli et al. (1998) indicated a threshold mercury concentration of ca 100 mg/kg wet weight, above which the co-

accumulation of selenium with an equimolar ratio is observed, due to the formation of HgSe.
In *Ziphius cavirostris,* the highest cadmium concentration was in kidney, followed by liver, lung and muscle. The same trend was observed in *Grampus griseus.* Elevated concentrations of cadmium are usually ascribed to diet (Law 1996). In fact, high concentrations of cadmium have previously been reported in the liver and especially kidney of marine mammals which inhabit areas remote from pollution sources, but which consume prey species which are themselves relatively rich in cadmium (Law 1996). Squid, an important component of the diet of these species, are known to have high levels of cadmium (Caurant & Amiard-Triquet 1995; Storelli & Marcotrigiano, in press). In addition, the retention of cadmium in kidney and liver of mammals is related to its selective storage or sequestration in the protein metallothionein. As accumulation increases there is a spillage of cadmium to other proteins, after which signs of toxicity appear (Underwood 1977). Fujise et al. (1988) and Honda (1985) indicate that renal dysfunction attributable to cadmium can occur in marine mammals with liver cadmium concentrations greater than 20 mg/kg wet weight. None of the animals analysed in our study exhibited such high concentrations, thought the liver of *Ziphius cavirostris* showed concentrations near to that limit.

In *Ziphius cavirostris* the highest lead concentration was observed in liver, followed by kidney and muscle, while in lung the value was below the detection limits. In all organs of one *Grampus griseus* lead concentrations were below the detection limits. In the other one lead levels were very low in all organs. Lead is a non-essential element and higher levels can occur in animals close to anthropogenic sources (Law et al. 1991).

Chromium concentrations in *Ziphius cavirostris,* were almost uniform among the four tissues with values slightly higher in kidney and lung, followed by muscle and liver. In *Grampus griseus* a different distribution among the various tissues was observed. The highest concentration of chromium in *Grampus griseus* were in muscle and in lung of one of the animals examined, while in the other organs of both chromium levels were lower and of similar amount. As for lead, exposure to sources of chromium can lead to an elevation in tissue concentration. Levels found in marine mammals are usually below 1.0 mg/kg wet weight (Thompson 1990).

Post- mortem investigation on *Ziphius cavirostris* evidenced different tissue and organ damages, due principally to chronic inflammations and parasitoses. On the skin a parasitic infection by *Pennella* sp. common among most cetaceans species (Raga et al. 1982) resulted in fibrosis which extended to the subcutaneous with a large numbers of microhaemorragic and necrotic areas.
The digestive system showed a chronic inflammation; particularly, the stomach presented a massive mucosal hyperplasia, with eosinophilic infiltration in submucosal tissue due to the presence of *Anisakis* sp.
The kidneys, massively infested by *Crassicauda giliakiana,* were notably altered. The parasites of Crassicauda genus, exclusive of marine mammals, are in fact

located mainly at the level of the genital-urinary system (Raga et al. 1982; Raga 1987). Kidney histological examination showed marked thickening of Bowman's capsules with atrophic and shrunken glomerulae and most of the capsular space was taken up with a protein-rich fluid.
The liver was macroscopically congested and sclerotic and histologically, it presented steatosis and fibrosis, while the lung showed a pulmonary oedema characterised by the presence of inflammatory exudate in the airway.
As for *Grampus griseus* both animals revealed alterations of the same nature.
Gastric nematodes *(Anisakis simplex)* occurred in the stomach and were responsible for the formation of fibrosis in the wall of the cardiac stomach.
The liver was swollen and histological examination showed an acute inflammation characterised by infiltrated lymphocytes,
In these animals, the kidneys, macroscopically congested, revealed histologically a membranoproliferative glomerulonephritis.
The lung, congested too, evidenced large numbers of nodules composed of a fibrous capsule containing either calcified cores or *Halocercus* sp. as found in other cetaceans (Sweeney et al. 1975; Troncone et al. 1994). As for the other *Grampus griseus*, the lung showed a nodule 10 cm in diameter containing a purulent-like material of piogenic nature, surrounded by a great connective capsule.

In general, liver accumulated the highest levels of total mercury together with lung, while kidney showed maximum concentrations of cadmium. Concentrations of lead and chromium were similar in all tissues examined. Muscle tissue were generally characterised by the lowest concentrations of the metals analysed. In addition to these inter-tissue variations, inter-species differences in metal concentrations were observed. In fact the concentration of metals analysed in all tissues of *Ziphius cavirostris* were lower than those of *Grampus griseus*, except for cadmium which showed higher levels in kidney and liver in *Ziphius cavirostris* with respect to *Grampus griseus.* In this respect, considering the high cadmium values found in liver (18.49 mg/kg) together with high cadmium values in kidneys (27.39 mg/kg) it is likely to hypothesise a renal dysfunction in accordance to Fujise et al. (1988). These conditions associated to an acute pathology of kidney may have contributed to mortality.
As for both *Grampus griseus* high mercury levels found in liver (1002, 14 mg/kg; 478.32 mg/kg), which were above the range of tolerance hypothesised by Wageman & Muir (1984) may have contributed to the occurrence of the acute inflammation observed in this organ.

Establishing databases on contaminants in marine mammals will help in understanding their role in mortality events and provide a basis for investigating, predicting and mitigating these events. As post-mortem examination data becomes available a better understanding of the correlation between the concentrations of heavy metals and their impact on marine mammals health can be established.

Table 1. Biological and biometric data for three cetaceans from Adriatic sea.

Specimen N°	Sex	Length (cm)	Weight (kg)
Grampus griseus 1	F	299	410
Grampus griseus 2	F	311	456.3
Ziphius cavirostris	F	530	-----

Table 2. Metal concentrations in reference material (TORT-l mg/kg), found values (mg/kg) coefficient of variation (CV), recovery (%) and detection limit (D.L. ng/g).

	Hg	MeHg	Se	Cd	Pb	Cr
TORT-1	0.33±0.06	0.13±0.01	6.88±0.47	26.3±2.1	10.4±2.0	2.4±0.6
found values	0.32±0.02	0.12±0.01	6.37±0.18	26.4±0.45	9.9±0.83	2.1±0.12
	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10
CV %	13.62	11.94	2.82	1.70	8.35	9.76
Recovery	97	92	93	100	95	88
D.L.	50	20	50	2	20	3

Table 3. Concentrations of trace metals, methylmercury (mg/kg wet wt) and % MeHg

	Tissue	Hg	MeHg	% MeHg	Se	Cd	Pb	Cr
G. griseus 1		26.52	15.68	59.1%	17.28	0.21	ND	0.24
G. griseus 2	Muscle	30.87	14.96	48.5%	6.46	0.09	0.05	1.11
Z. cavirostris		9.68	7.00	72.3%	2.71	0.14	0.07	0.19
G. griseus 1		270.97	5.84	2.1%	51.82	0.38	ND	0.20
G. griseus 2	Lung	145.79	7.41	5.1%	38.41	0.44	0.13	1.41
Z. cavirostris		45.80	3.61	7.9%	12.21	0.85	ND	0.30
G. griseus 1		1002.14	14.70	1.5%	266.42	6.00	ND	0.28
G. griseus 2	Liver	478.32	7.35	1.5%	113.19	8.42	0.13	0.41
Z. cavirostris		259.26	9.85	3.8%	110.61	18.49	0.28	0.12
G. griseus 1		60.71	5.80	9.5%	23.31	7.34	ND	0.15
G. griseus 2	Kidney	48.07	5.23	10.9%	9.72	15.88	0.11	0.43
Z. cavirostris		59.33	4.51	7.6%	12.31	27.39	0.10	0.34

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