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Comparison of Total Mercury, Methylmercury, and Selenium in Muscle Tissues and in the Liver of *Stenella coeruleoalba* (Meyen) and *Caretta caretta* (Linnaeus)

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In the marine environment, mercury exists in both organic and inorganic forms. Methylmercury is by far the most toxic and the most commonly occurring organomercury compound. Anthropogenic emission of methylmercury is generally rare, but it 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 lipids. As a result, an accumulation of mercury, mainly as methylmercury, in aquatic organisms occurs with concurrent bioamplification phenomena through the trophic chains. Because methylmercury is lipid soluble while inorganic mercury is not, their distributions among animal tissues differ. Marine organisms have been shown to exhibit different proportions of these two forms of mercury, depending on such factors as trophic status, tissue analysed, size and adaptive abilities to biotransform organic mercury into inorganic mercury (Thompson et al. 1990).

There are many studies concerning total mercury and methylmercury accumulation in cetaceans (Itano et al. 1984; Palmisano et al. 1993 and 1995), while no information is available on the presence of methylmercury in marine turtles. The objectives of the present study are to provide data about total and methylmercury concentrations in the liver and in the muscle tissue of organisms belonging to different classes, *Stenella coeruleoalba* (Mammalia) and *Caretta caretta* (Reptilia), to analyse the distribution pattern among them and, finally, to determine selenium concentration and the existence of any correlation between it and mercury due to its possible detoxifying effect with respect to mercury.

MATERIALS AND METHODS

Between February-April 1991 and June-September 1995 were found beached along the Apulian coasts (South Adriatic Sea) 30 *Stenella coeruleoalba* specimens (length: 90-220 cm) and 7 *Caretta caretta* specimens (weight: 6.7-18 kg), respectively that were analysed for total mercury, methylmercury and selenium. All specimens were female. Liver, and muscle tissue were taken from samples and stored in plastic bags in accordance with FAO methods (FAO 1983). The tissues were dissected with plastic materials that were washed with HNO_3 and rinsed with distilled and deionized water, in order to avoid metal contamination. The

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samples were preserved at - 25 °C until their analysis. For analyses of total Hg and Se, homogenized samples of the tissue (1-3 g wet weight) were digested to a transparent solution with 10 mL of the mixture H_2SO_4-HNO_3(1:1) under reflux. The resultant solutions were then diluted to a known volume with deionized water (G.U. 1990), and the total Hg concentrations were measured in atomic absorption spectrophotometry (Perkin Elmer 5000) by the cold vapour technique after reduction by $SnCl_2$(A.V.A. Thermo Jarrel Ash Corp.), while the Se concentrations were 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 (1+l) and extracted 3 times with 10 mL of benzene. After centrifugation, 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). 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-1 Lobster Hepatopancreas (National Research Council of Canada) (Table 1). All data were computed on a mg/kg wet weight basis. The mean values of the ratio fresh/dry weight was 3.39±0.35 for liver and 3.99±0.39 for muscle.

RESULTS AND DISCUSSION

Total Hg, MeHg, Hg*(inorganic) = Hg total - MeHg and Se concentrations in the liver and in the muscle tissue of cetaceans and turtles are listed in tables 2 and 3.

All 30 dolphins studied showed the highest total Hg concentrations in the liver with values ranging from 0.58 to 966.31 mg/kg (aver. 277.40±246). In fact the liver is the organ where Hg is preferentially accumulated. This possibly relates to a combination of factors such as different distribution of specific proteins, transport of Hg on a differential basis to particular organs and peculiarity of transport through certain cellular barriers (Gaskin 1982). In the muscle tissue total Hg concentrations were much lower than in the liver with values ranging from 0.56 to 53.28 mg/kg (aver, 17.4±14.1). As for MeHg, values in the liver ranged from 0.58 to 29.69 mg/kg (aver.14.82±8.7), while in the muscle tissue the concentrations varied from 0.56 to 45.66 mg/kg (aver. 14.52±11.0). Se values in the liver ranged from 0.89 to 423.98 mg/kg (aver. 141.65±104), while in the muscle tissue they were lower, ranging between 1.09-7.75mg/kg (aver.4.52±2.15).

The ratio of MeHg/total Hg, expressed as a percentage, was higher in the muscle tissue, than in the liver. In the muscle tissue, the mean percentage was about 88%,

while in the liver it was of 24% approximately. In muscle tissue, MeHg is firmly bound through carbon-mercury and sulphydryl linkages, which could account for the high ratio of organic mercury in this tissue (Gaskin 1982) while in the liver, MeHg only accounts for a fraction of total Hg present. Several authors (Kari and Kauranen 1978; Smith and Armstrong 1978) support the hypothesis of a detoxifying activity taking place in this organ, whereby MeHg is converted to the less toxic inorganic form (Koeman et al. 1975). The results found indicated that in the liver at the lowest total Hg concentrations corresponded the highest percentages of MeHg (fig. 1); moreover it appeared that above a concentration of approximately 100 mg total Hg/kg, the proportion of MeHg became relatively independent of the total Hg amount (fig. 1) in agreement with those found by Palmisano (1995) in a recent study on the mercury speciation in *S. coeruleolba.* In fact, fig 2(a) shows for total Hg concentrations ranging from 0.58 to 110 mg/kg, aver. 29.07±35.95 mg/kg, a percentage of MeHg varying from 100 to 27.7% aver. 70%±20.21 while, in fig 2(b), as total Hg concentrations increase from 154.81 to 966.31 mg/kg, aver. 383.82±218.31 mg/kg, the percentage of MeHg keeps quite constant in a low range of values between 1.74 - 10.52% aver. 4.67%±2.42.

Total Hg levels in the turtle's liver were in the range 0.37-1.10 mg/kg (aver. 0.70 ±0.32). In the muscle tissue total Hg concentrations ranged between 0.07 and 0.43 mg/kg (aver. 0.21±0.13) and were much lower than in the liver. For MeHg, values in the liver varied from 0.24 to 0.33 mg/kg (aver. 0.28±0.03). In 43% of the examined muscle tissue samples MeHg concentrations were below the instrumental detection limit, while the remaining 57% were in the range 0.16-0.41 mg/kg (aver. 0.23±0.12). Se concentrations in the liver were in the range 4.00-6.11 mg/kg (aver. 4.86±0.85), while in the muscle tissue they varied from 1.19 to 3.24 mg/kg (aver. 2.33±0.67). The ratio of MeHg/total Hg, expressed as a percentage, was higher in the muscle tissue (aver.79.75%±17.61) than in the liver (aver. 46.45%±17.20). In the liver as total Hg concentrations increase from 0.37 to 1.10 mg/kg the percentages of MeHg decrease from 65 to 27 (fig.3).

On the basis of the analytical results discussed above it seems clear that in the organisms examined the highest total Hg, Se and MeHg concentrations were in the liver. The highest levels of these pollutants were observed in *S. coeruleoalba*. In the muscle tissue of *S. coeruleoalba* Hg was present mainly in the methylated form, in agreement with data reported in literature for cetaceans (Itano et al. 1984; Honda et al. 1983). In *C. caretta* too, Hg in the muscle tissues was present mainly in the methylated form although no literature data are available. In the muscle tissue of both organisms the molar ratio Se/Hg* found was high, particularly in C. *caretta*. It must be pointed out that most of the Se in the muscle tissue is water soluble and does not form stable complexes with mercury which is mainly in the organic form. Bismethylmercury selenide, the complex formed between selenide and methylmercury (Magos et al. 1979), is not stable (Masukova et al. 1982) in contrast to mercury selenide, thus the formation of bismethylmercury selenide cannot be a good detoxification mechanism (Barghigiani et al. 1991).
In the liver of both examined species, Hg in the methylated form was present in low percentages. In *S. coeruleoalba,* the results obtained show the presence of a

two stage mechanism for the accumulation of MeHg already observed by other authors (Palmisano et al. 1995). In the first stage mercury is stored in the liver as MeHg. Above a threshold concentration a demethylation process takes place. This process is not completely clear. When percentages of MeHg are higher than 10%, the molar ratio Se/Hg* is approximately 15; where the percentages are lower than 10%, the molar ratio Se/Hg* is approximately 1. This may suggest that when Hg* concentrations are above a threshold value (about 100 mg/kg), Hg* forms with Se a less toxic insoluble compound such as HgSe, already identified in the liver of some dolphins by Martoja and Berry (1980). In young specimens (see specimens 1-9), the detoxification process, involving Se, seems not to be active because Hg* concentration is not above the threshold value.

The same trend found in *S. coeruleoalba* was observed in the liver of *C. caretta* specimens though in a less remarkable way. In fact, for total Hg concentrations lower than 1 mg/kg, MeHg percentage is in a close range of 57-65 % and then decreases considerably when total Hg concentrations are at about 1 mg/kg. The decreasing of methylmercury percentages in the liver shows, that a detoxification process exists which involves the demethylation of MeHg. For other marine organisms efforts to identify the mechanism of the demethylation process did not yield definite results and it is as yet not clear whether the process is of enzymatic, bacteriological or chemical nature. The high molar ratio Se/Hg* = 48 found in the liver turtles, seems to indicate that selenium does not have a protective action against mercury toxicity. Nevertheless it can be observed that in the liver turtle samples where MeHg percentages are higher than 50%, the molar ratio Se/Hg* is approximately 74; when the percentages are lower than 30%, the molar ratio Se/Hg* decreases markedly to 15. This may suggest, consequently, that when Hg* concentrations increase, though in a small proportion, Se might be involved in the detoxification mechanism.

Table 1. Total Hg, MeHg (as Hg) and Se concentrations in reference material (TORT-1), coefficient of variation (CV), recovery and detection limit (D.L.).

	Hg	MeHg	Se
TORT-1 (mg/kg)	0.33±0.06	0.128±0.014	6.88±0.47
found values(mg/kg)	0.32±0.02	0.123±0.14	6.37±0.18
	n = 10	n = 10	n = 10
CV %	13.62	11.94	2.82
Recovery %	97	92	93
D.L. (ng/g)	50	20	50

Table 2. Total Hg, MeHg, Hg*, Se mean values (mg/kg w. w.), %MeHg and Se/Hg* in muscle tissue samples of *S. coeruleoalba* (A) (n=30) and *C. caretta* (B) (n=7).

	Total Hg	MeHg	Hg*	% MeHg	Se	Se/Hg*
A	0.56-53.28	0.56-45.66	0-17.62	61.85-100	1.09-7.75	0.43-56.44
	17.4±14.1	14.5±11.0	2.96±4.08	88.2±10.2	4.52±2.15	11.36±10.19
B	0.07-0.43	ND-0.41	0.02-0.15	55.00-95.00	1.19-3.24	21.60-59.56
	0.21±0.13	0.23±0.12	0.06±0.05	79.75±17.61	2.33±0.67	38.83±22.13

Table 3. Total Hg, MeHg, Hg*, Se concentrations (mg/kg w. w.), % MeHg and Se/Hg* in liver samples of *S. coeruleoalba* (A) (n=30) and C. *caretta* (B) (n=7).

A	Total Hg	MeHg	Hg*	% MeHg	Se	Se/Hg*
1	0.58	0.58	---	100.00	0.89	---
2	1.36	1.03	0.33	75.74	1.94	14.93
3	1.40	1.13	0.27	80,71	2.31	21.73
4	3.00	2.40	0.60	80.00	2.57	10.88
5	23.88	18.50	5.38	77.47	46.61	22.01
6	25.37	18.00	7.37	70.95	50.84	17.52
7	46.81	27.71	19.10	59.20	74.28	9.88
8	49.26	28.15	21.11	57.15	178.03	21.42
9	110.00	30.44	18.44	27.67	104.92	14.52
10	154.81	14.17	140.64	9.15	91.18	1.65
11	182.21	8.62	173.59	4.73	101.79	1.49
12	183.68	10.45	173.23	5.69	108.13	1.59
13	185.58	11.90	173.68	6.41	118.62	1.73
14	219.39	10.45	208.94	4.76	132.51	1.61
15	250.00	26.29	223.71	10.52	181.82	2.06
16	257.43	19.15	238.28	7.44	204.86	2.18
17	285.18	9.14	276.04	3.20	179.09	1.65
18	300.92	11.24	289.68	3.74	157.73	1.38
19	344.74	15.23	329.51	4.42	6.83	0.05
20	358.18	12.01	346.17	3.35	214.74	1.58
21	366.81	29.69	337.12	8.09	147.66	1.11
22	367.76	13.95	353.81	3.79	212.31	1.52
23	371.38	11.04	360.34	2.97	165.80	1.17
24	371.79	17.74	354.05	4.77	166.67	1.20
25	428.55	14.62	413.93	3.41	229.36	1.41
26	455.78	8.03	447.75	1.76	153.78	0.87
27	495.92	15.71	480.21	3.17	276.95	1.46
28	579.78	10.08	569.70	1.74	117.23	0.52
29	934.06	17.38	916.68	1.86	396.29	1.10
30	966.31	29.69	952.60	3.07	423.98	1.13
	0.58-966.31	0.58-29.69	0.00-952.60	1.74-100	0.89-423.98	0.05-22.01
	277.40±246	14.82±8.7	261.1±247	24.23±32.26	141.65±104	5.38±7.30

B	Total Hg	MeHg	Hg*	%MeHg	Se	Se/Hg*
1	0.37	0.24	0.13	64.86	4.00	78.15
2	0.42	0.25	0.17	59.52	5.78	86.36
3	0.49	0.28	0.21	57.14	6.11	73.90
4	0.56	0.33	0.23	58.93	5.17	57.09
5	0.91	0.27	0.64	29.67	4.03	15.99
6	1.08	0.30	0.78	27.78	4.21	13.71
7	1.10	0.30	0.80	27.27	4.69	14.89
	0.37-1.10	0.24-0.33	0.13-0.80	27.27-64.86	4.00-6.11	13.71-86.36
	0.70±0.32	0.28±0.03	0.42±0.30	46.45±17.20	4.86±0.85	48.58±32.73

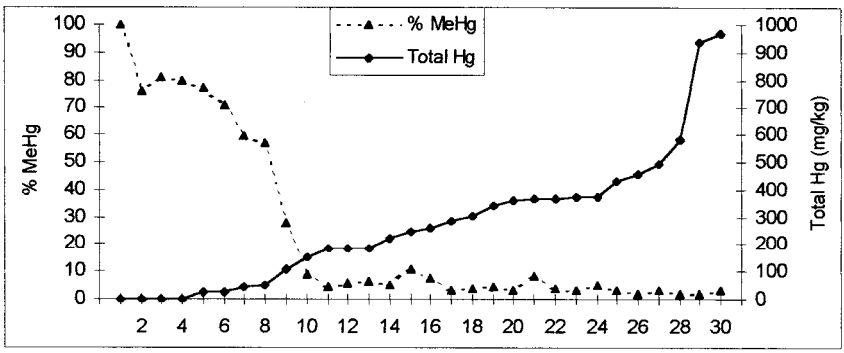

Figure 1. Total Hg concentrations and MeHg expressed as % of the total Hg in 30 dolphin liver. Specimens are numbered in order of increasing length.

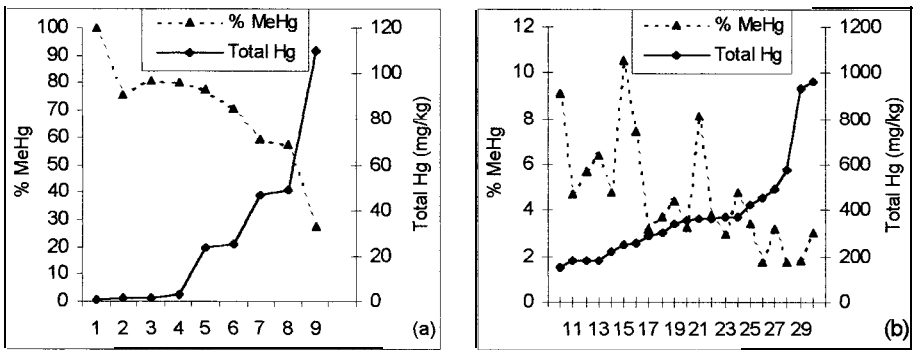

Figure 2. Total Hg concentrations and MeHg expressed as % of the total Hg in 9 dolphin liver (a) and in 21 dolphin liver (b).

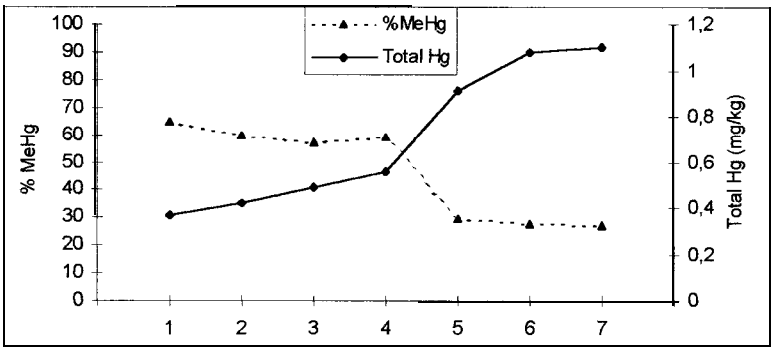

Figure 3. Total Hg concentrations and MeHg expressed as % of the total Hg in 7 turtle liver. Specimens are numbered in order of increasing weight.

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