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Mercury Speciation and Relationship Between Mercury and Selenium in Liver of *Galeus melastomus* from the Mediterranean Sea

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Among the toxic trace metals, mercury is one of the most hazardous environmental pollutants in the marine environment. The majority of the mercury released into the marine environment is inorganic but it can be converted to the methylmercury form, by bacteria living in the sediment. The toxicology and environmental behaviour of mercury is quite complex, since the toxicity, mobility, and bioaccumulation of mercury depend on its chemical form (D'Itri, 1990). As organometallic compound it passes easily across cell membranes and the high affinity for sulfhydryl groups of proteins causes its rapid absorption in living organisms. Marine organisms have been shown to exhibit different proportions of these two forms of mercury, depending on such factors as trophic status, size, adaptive abilities to biotransform organic mercury into inorganic mercury, as well as the tissue analysed (Thompson et al. 1990). Liver is recognized as the organ where uptaken contaminants tend to concentrate and undergo biotransformation. It transforms harmful compounds into metabolites, which are excreted directly into bile for continued detoxification. Once a compound is excreted into bile and enters the small intestine, it is either reabsorbed in the gut or eliminated in feces. Methylmercury, particularly, is reabsorbed in a process called enterohepatic recirculation (Gordon and Skett, 1986), which yields in a retention of mercury by the organisms, for long periods.

It has been shown that marine mammals, having the highest mercury burdens in the marine biota, are able to detoxify methylmercury by a specific chemical mechanism involving selenium (Koeman et al., 1973, 1975). The presence of HgSe in marine mammal liver was identified by transmission electron microscopy, X-ray microprobe analysis and X-ray diffraction. Although there is a wealth of information on the selenium:mercury relationship in liver of marine mammals (Augier et al., 1993; Palmisano et al., 1995; Storelli et al., 1998), there are comparatively few data on this topic in teleostes (Joiris et al., 1999; Dietz et al., 2000), and to our knowledge no data is available for shark liver. The objectives of the present study are to provide data about total and methylmercury concentrations in the liver of *Galeus melastomus*, to examine the relationship between specimen weight and mercury and methylmercury concentrations and, finally, to assess whether selenium is in a equal molar ratio to mercury and in this way detoxifies mercury and reduces the exposure of species to the toxicity of this

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metal.

MATERIALS AND METHODS

From June to September 1999, 450 specimens of *Galeus melastomus* were caught in the eastern Mediterranean Sea. Table 1 shows, the number of specimens, their range of length and weight and the number of pools each constituted by specimens of similar size. Liver was taken from the samples and preserved at -25 °C until their analysis. For the analyses of total Hg, and Se homogenized samples of the tissue (about 1 g) 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 (about 1 g) 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+1) 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 cromatography (Carlo Erba model HRGC-5300) equipped with a ^{63}Ni electron capture detector (ECD-400), and splitless injection tecnique 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). The values found (Hg: 0.32 ± 0.02 mg/kg d.w.; MeHg: 0.123 ± 0.014 mg/kg d.w.; Se: 6.37 ± 0.18 mg/kg d.w.) agreed with the certified values (Hg: 0.33 ± 0.06 mg/kg d.w.; MeHg: 0.128 ± 0.014 mg/kg d.w.; Se: 6.88± 0.47 mg/kg d.w.).

RESULTS AND DISCUSSION

Table 2 shows range and mean values of total Hg, MeHg, Se concentrations, expressed in mg/kg wet wt., percentages of MeHg to total Hg and Se:Hg* molar ratio (Hg* inorganic mercury = total mercury-methylmercury concentration) in liver of sharks from various areas of the Mediterranean Sea. Total mean mercury concentrations in the liver of specimens ranged from 0.04 to 4.09 mg/kg (av. 0.60 mg/kg). The mean values in the liver of the specimens from the Adriatic sea (Italy) and Aegean sea (Greece) were similar: 1.08 and 1.02 mg/kg respectively, while significantly lower levels were found in Adriatic Sea (Albania) and Ionian Sea specimens: 0.32 and 0.13, respectively ($p < 0.01$). Liver tissue may be a poor

Table 1. Biometric data and specimen number of *Galeus melastomus*.

Species	Location	n	n° pools	Range length (cm)	Range weight (g)
Blackmouth dogfish *(Galeus melastomus)*	Adriatic Sea (Italy)	104	11	24.9-55.3 45.6±8.6	41.6-440.3 272.4±119.5
Blackmouth dogfish *(Galeus melastomus)*	Adriatic Sea (Albania)	59	10	19.3-50.5 39.2±9.8	33.5-319.5 179±92.2
Blackmouth dogfish *(Galeus melastomus)*	Ionian Sea	165	18	12.6-52.1 33.3±12.7	6.0-395.7 140±117.2
Blackmouth dogfish *(Galeus melastomus)*	Aegean sea	122	18	18.8-63.0 39.8±12.1	16.2-547.3 178.5±149.1

n = number of individual sharks.

Table 2. Total mercury (T-Hg), methylmercury (MeHg), selenium (Se) concentrations (mg/kg w.w.), % MeHg and Se:Hg* molar ratio in liver samples of sharks.

Species	Provenience	T-Hg	MeHg	%MeHg	Se	Se:Hg*
G. melastomus	Adriatic Sea (Italy)	0.16-3.57 1.08±1.14	0.13-1.71 0.56±0.47	25-100 61±27.5	0.53-2.13 1.21±0.51	1.14-19.8 5.6±5.71
G. melastomus	Adriatic Sea (Albania)	0.12-1.00 0.32±0.22	0.06-0.42 0.15±0.12	21-67 49±13.9	0.76-1.61 1.04±0.25	4.05-53.9 26.6±16.6
G. melastomus	Ionian Sea	0.04-0.26 0.13±0.08	0.02-0.11 0.04±0.02	72-100 91.5±8.3	0.20-1.34 0.80±0.30	2.66-51.2 23.5±14.2
G. melastomus	Aegean Sea	0.17-4.09 1.02±1.22	0.07-1.89 0.40±0.61	4-46 29±15.8	0.41-1.05 0.77±0.16	0.67-12.9 4.8±4.5
Minimum-Maximum Mean ± S. D.		0.04-4.09 0.60±0.95	0.02-1.89 0.28±0.42	4-100 44±23.0	0.20-2.13 0.92±0.36	0.67-53.9 16.3±15.2

Hg*= inorganic mercury

choice for determining spatial or temporal trends of pollutants because of the continuous metabolic activity of this organ. However, the significant differences in total Hg concentrations among various zone can be partly explained by a spatial variation of the level of this element in the environment. On the other hand, in a earlier study carried on the muscle tissue of the same organisms (Storelli et al., in press), an identical geographical trend of total mercury was observed, reflecting a different environmental contamination degree among various areas. A positive correlation between weight and total mercury concentration in the liver of the specimens was observed ($r = 0.61$; $p<0.0001$) (Fig. 1), confirming results reported for other marine organisms (Mallory Boush and Thieleke, 1983; Law et al., 1991; Marcovecchio et al., 1991; Hornung et al., 1993; Joiris et al., 1999).

As regards methylmercury the values ranged from 0.02 to 1.89 mg/kg (av. 0.28 mg/kg), and the accumulation pattern for organisms of different areas was the

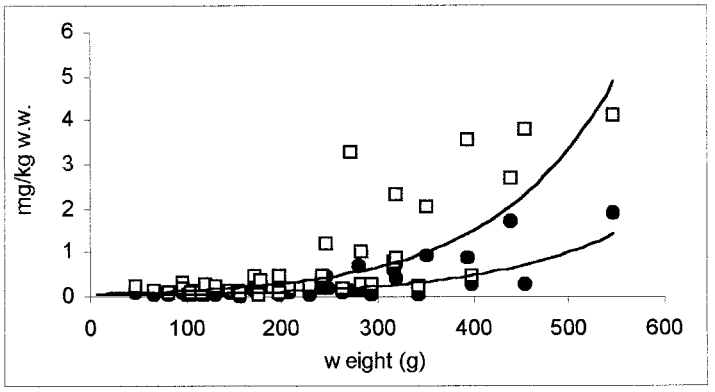

Figure 1 - Correlation between total mercury (●) and methylmercury (□) concentrations (mg/kg w.w.) and weight (g) in liver of sharks.

same observed for total mercury. Regression slope of MeHg in liver on weight was positive ($r = 0.53$; $p<0.0001$) (Fig.1) reflecting, either a increasing uptake of MeHg with age, or a decreasing elimination rate of MeHg, possibly because of a decreasing demethylating efficiency with age (Wagemann et al., 1998). Liver is the main site of methylmercury biotransformation in animals, and once a compound is excreted into bile, the principal pathway of methylmercury's elimination is through the feces. (Boening, 2000). A study carried on the hepatic excretory process of methylmercury in two elasmobranchs (*Raja erinacea* and *Squalus acanthias*), indicated that these species excrete methylmercury into bile slowly (Ballatori and Boyer, 1986). Since biliary excretion is the primary pathway contributing to fecal methylmercury elimination, a slow biliary excretory process would retard whole-body elimination of the metal and favour thus, its accumulation. The percentages of MeHg to total mercury, however, were low between 29 to 61% indicating that mercury in liver was mainly in the inorganic form. This data is consistent with previous findings on liver speciation of different marine organisms, such as marine mammals (Palmisano et al., 1995; Storelli et al., 1998; Wagemann et al., 1998), and teleostes (Dietz et al., 1990; Joiris et al., 1999), in which mercury is found as methylated form in low percentages. This trend would reflect the existence of a demethylation process in liver.

Se concentration in liver ranged from 0.20-2.13 mg/kg (av. 0.92 mg/kg). This metal, in addition to its role as micro nutrient, exerts an antidotal action on the toxic effects of mercury (Parizek et al., 1971; Ganther et al., 1974; Pelletier, 1985), through the formation of highly insoluble complexes, consisting of mercuric selenide HgSe (Martoja and Berry, 1980; Pelletier, 1985; Hansen et al., 1989). The interactions between selenium and mercury, demonstrated by a molar

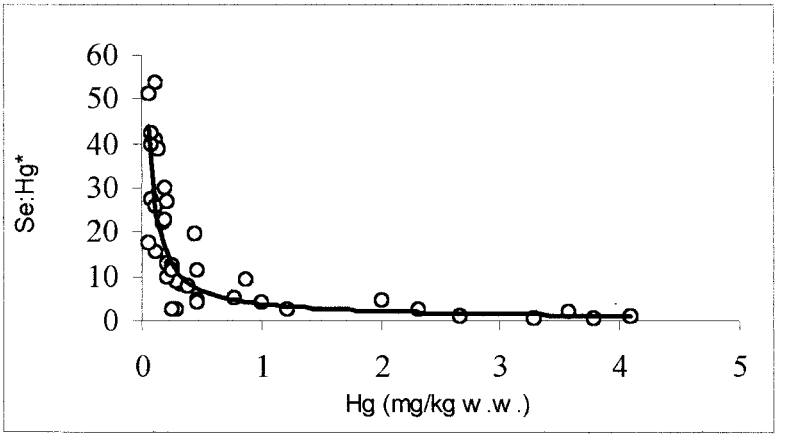

Figure 2 – Molar ratio Se:Hg* versus total Hg concentration in liver of the sharks.

ratio close to 1:1, were found numerous times for liver tissue in different marine mammals species, while for other organisms divergent results were reported in the literature. Dietz et al., (2000) in fish and in seabirds liver reported a substantial excess of selenium, being 198 and 26.3 times higher than the mercury concentration on a molar basis. As well as, a surplus of selenium in seabird liver was documented by Koeman et al. (1975). However, in marine mammals, a molar ratio close to 1:1 was observed mostly in animals with a high total mercury concentration in liver (Koeman et al., 1973; Nielsen and Dietz, 1990; Skaare et al., 1994; Storelli et al., 1998). In fact, baleen whales, toothed whales and dolphins with a low mercury concentration range showed high Se:Hg molar ratios in liver: 11.8, 42.5, and above 10, respectively (Storelli et al., 1998; Dietz et al., 2000). These findings seem to indicate that after demethylation, mercury undergoes a detoxification process involving selenium, which occurs only from a certain mercury threshold level on. Koeman et al., (1975) documented a 1:1 ratio in marine mammals with mercury concentrations up to 1000 mg/kg. Dietz et al., (1990) reported that seals, toothed whales and polar bear with mercury concentrations above 2 mg/kg wet weight showed a Se:Hg molar ratio close to 1:1. Still, Storelli et al., (1998) found a 1:1 ratio in dolphins when mercury levels were above a threshold value of about 100 mg/kg. In our case the high molar ratio Se:Hg*=16.3 would attest that selenium have not a protective action against mercury toxicity. Nevertheless, a closer examination of the data showed that at low mercury concentrations correspond high values of Se:Hg* ratio, while such ratio tends to 1:1 in specimens with total mercury in excess of 1 mg/kg wet weight (fig. 2).

Our findings show that the process of demethylation of MeHg and inorganic

mercury transformation by reaction with selenium to form mercuric selenide would be an effective mechanism for counteracting the potentially damaging action of mercury also in this animal group. The needs of additional studies of this type is obvious, mainly to establish whether also for other fish, either elasmobranchs or teleosts, will be possible to found a stoichiometric Se:Hg ratio close to 1:1 as observed in this paper and in other investigations concerning marine mammal liver. On this basis these results provide a baseline for future works.

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