Bull. Environ. Contam. Toxicol. (2000) 65:732–739
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DOI: 10.1007/s001280000184

Bulletin of Environmental Contamination and Toxicology

Total Organic and Inorganic Arsenic from Marine Turtles (*Caretta caretta*) Beached Along the Italian Coast (South Adriatic Sea)

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Received: 6 April 2000/Accepted: 1 August 2000

Arsenic is widely distributed in the biosphere. Millions of tons enter the environment annually (Ricci et al., 1981); Fitzgerald (1983) has estimated the world production of arsenic to be about 31,700-34,500 t in 1980. Unpolluted seawater contains 2-3 ng/l (Hunt & Howard, 1994), the earth's crust has an average concentration of 2 mg/kg (Thomas & Sniatecki, 1995) and the concentration of arsenic in marine organisms varies from less than 1 ng/g to more than 30 ng/g (Ballin et al., 1994). The toxicity of arsenic depends very much on its oxidation states and molecular form. For example, As (III) is more toxic than As (V), while arsenobetaine and arsenocholine, arseno-organic compounds, are relatively non- toxic. The LD_{50} for arsenic trioxide in rats is 20 mg/kg (Andreae, 1986), while for arsenobetaine no signs of toxicity were observed in mice after an oral dose of 10 g/kg (Ballin et al., 1994). Marine organisms accumulate widely varying concentrations of arsenic. The knowledge of the chemical forms in which the element occurs in tissues is relevant to evaluate the toxicological implications as well as to elucidate the biogeochemical cycle of the same element in the marine environment. A large number of investigations have been carried out on arsenic compounds in crustaceans (Cooney & Benson, 1980; Kurosawa et al., 1980) and fish (Lunde, 1973; Bohn, 1975; Lunde, 1977; Brooke & Evans, 1981; Falconer et al., 1983; Shinagawa et al., 1983; Lawrence et al., 1986, Leah et al., 1992) but there is no information on the chemical form of these compounds in marine turtles, except a paper dealing with a sample of *Dermochelys coriacea* beached in Australia (Edmonds et al., 1994). In this work are reported the results of a survey on the content of total, inorganic and organic arsenic in muscle tissue and liver of marine turtles (*Caretta caretta*) beached along the Italian coast (South Adriatic Sea).

MATERIALS AND METHODS

Seven marine turtles (species *Caretta caretta*), weighting 1.8-90 kg were found beached along the Apulian (South Adriatic sea) coast in different localities in June-September 1998. Following necropsy, liver and muscle tissue were removed and transferred into labelled polythene bags, frozen at –70 °C and stored at –20 °C. After thawing and subsequent homogenisation, aliquots of samples were taken for the analysis. The determination of total arsenic was carried out as follows.

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Samples of about 3 g of wet tissue were digested, under reflux, with 10 ml of the mixture H_2SO_4–HNO_3 - $HClO_4$ for 6 h. After cooling the acid digest was diluted to a final volume of 100 ml with bidistilled water (Leah *et al.*, 1992).

Sample digestion for the determination of inorganic and organic arsenic followed the protocol of Flanjak (1982). For the determination of inorganic arsenic, samples of about 5 g of wet tissue were accurately weighed into flat-bottom 100 ml distillation flasks. To each was added 1 ml of hydrobromic acid (48%) and 25 ml of 6.6 M hydrochloric acid. The distillation flask was connected to a modified Bethge apparatus (Flanjak, 1982) and the sample was refluxed for about 15 min after which about 20 ml of distillate was collected. A further 20 ml of 6.6 M hydrochloric acid was added to the flask and distillate again and collected to make a total volume of about 40 ml. After cooling, the condenser and receiver were washed with distilled water and the washings added to the distillate and made up to 50 ml in a volumetric flask. For the determination of organic arsenic to the residue in the distillation flask were added 30 ml of a mixture of nitric and perchloric acids (5+1) and 1 ml of sulphuric acid. The samples were slowly evaporated to fumes of sulphuric acid, cooled, and made up to a volume of 10 ml in a volumetric flask with distilled water. All atomic absorption measurements were carried out using a Perkin-Elmer model 5000 spectrophotometer equipped with a MHS-10 Perkin-Elmer hydride generator system connected to a quartz cell with open ends (i.d. 1.2 cm, length 16.7 cm) after arsine generation by addition of sodium borohydride (4% in 0.05 M sodium hydroxide). The spectral source was a Perkin-Elmer hollow cathode lamp operated at 8 w. The spectrometer was set at 193.7 nm with a slit width of 0.7 nm. The detection limit was 50 ng/g (CV = 1.25%). The precision of the method was estimated from ten replicate determinations of total arsenic for the reference material TORT-1 (Lobster Hepatopancreas) obtained from the National Research Council of Canada. The values found (24.4±0.3 mg/kg d.w.) agreed with the certified values (24.6± 2.2 mg/kg d.w.). All the measurements were made in triplicate. All reagents were of the Suprapur type (Merch, Germany). These were checked for any probable arsenic contamination at the detection level prior to use. All glassware used for the processing of the samples and for the preparation of standards was thoroughly washed with detergent solution and later with distilled water. It was then soaked in 5% nitric acid, rinsed with distilled water and kept for 3h in electric oven at 100 °C prior to use at room temperature.

RESULTS AND DISCUSSION

Results for total, organic and inorganic arsenic (mean ± SD) in muscle tissue and liver of marine turtles, together with the percentage of organic and inorganic arsenic with respect to their sum are given in Table 1. Between the total arsenic concentrations and the values obtained from the sum of organic + inorganic, no substantial difference have been observed. Hence, in the results section total arsenic values will be those of the sum. Total arsenic levels were higher in muscle tissue with values ranging between 2.64-31.21 mg/kg w.w. (mean 15.38 mg/kg w.w.) than in liver that showed lower concentrations varying between 2.53-13.76

Table 1. Values of total arsenic, organic and inorganic (mg/Kg w.w.) and % of organic and inorganic arsenic in muscle tissue and liver of *Caretta caretta* specimens (n = 7).

	Total As	Organic As	inorganic As	Sum org. + inorg.	% org. As	% inorg. As
C. caretta 1 M. tissue	5.16±0.18	4.51±0.20	0.32±0.03	4.83	93.4	6.6
C. caretta 1 Liver	2.68±0.40	2.17±0.12	0.36±0.06	2.53	85.8	14.2
C. caretta 2 M. tissue	2.64±0.23	2.38±0.31	0.26±0.15	2.64	90.2	9.8
C. caretta 2 Liver	3.44±0.35	2.54±0.06	0.58±0.13	3.12	81.4	18.6
C. caretta 3 M. tissue	12.00±0.38	12.10±0.12	0.15±0.02	12.25	98.8	1.2
C. caretta 3 Liver	6.03±0.40	6.39±0.48	0.47±0.04	6.86	93.1	6.9
C. caretta 4 M. tissue	10.78±0.30	11.05±0.22	0.26±0.13	11.31	97.7	2.30
C. caretta 4 Liver	13.07±0.14	12.18±0.19	1.22±0.40	13.40	90.9	9.1
C. caretta 5 M. tissue	13.82±0.23	14.13±0.42	0.08±0.05	14.21	99.4	0.6
C. caretta 5 Liver	2.98±0.14	2.24±0.12	0.30±0.07	2.54	88.2	11.8
C. caretta 6 M. tissue	39.11±0.45	31.11±0.20	0.08±0.06	31.19	99.7	0.3
C. caretta 6 Liver	5.79±0.17	5.69±0.29	0.35±0.03	6.04	94.2	5.8
C. caretta 7 M. tissue	32.39±0.35	31.10±0.65	0.11±0.10	31.21	99.6	0.4
C. caretta 7 Liver	12.92±0.18	12.66±0.18	1.10±0.46	13.76	92.0	8.0
Total M. tissue	2.64-32.39 15.47±11.91	2.38-31.11 15.20±11.64	0.08-0.32 0.18±0.10	2.64-31.21 15.38±11.56	90.2-99.7 97.0±3.7	0.3-9.8 3.0±3.7
Total Liver	2.68-13.07 6.70±4.49	2.17-12.66 6.27±4.53	0.30-1.22 0.63±0.38	2.53-13.76 6.89±4.87	81.4-94.2 89.4±4.5	5.8-18.6 10.5±4.5

mg/kg w.w. (mean 6.89 mg/kg w.w.). It is known that, generally, metals accumulate preferentially in liver rather than in muscle tissue, but higher values in muscle tissue, are reported in literature for total arsenic in other marine turtle individuals of the same species (Storelli et al. 1998) and in *D. coriaceea* (Edmonds et al. 1994). The accumulation of arsenic in marine organisms varies in relation to many factors such as species, arsenic concentration, temperature, salinity of the water as well as weight of the organism (Norin et al., 1985). In fact, as regards this latter parameter, the values found solely for muscle tissue were correlated with the specimen's weight (R=0.67, P<0.01) (Fig. 1).

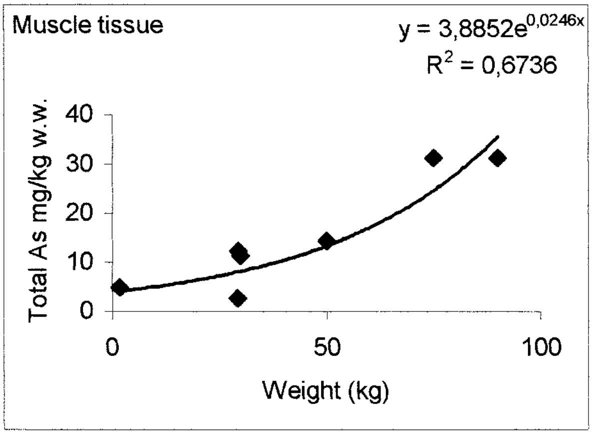

Figure 1 Relationship between total arsenic (sum organic + inorganic) level in muscle tissue and weight.

More, the marine organisms acquire their arsenic burden through the food chain (Norin et al., 1985). In fact, the high concentrations found may be principally due to the fact that marine turtles feed mostly on small crustaceans that usually show high levels of total arsenic. Literature values for total arsenic in crustaceans are generally very high. For prawns, arsenic values range from 9 to 19 mg/kg; shrimps, from < 1 to 40 mg/kg, and crabs from < 1 to 70 mg/kg (Norin et al., 1985). The higher arsenic content in tissues of these bottom dwellers can be attributed to their habitat being closer to the sediment. Sediments are always higher in arsenic than waters with which they are associated, and bottom waters usually contain higher concentrations than surface waters (Byrd, 1988; Trembley & Gobeil, 1990).

The concentrations of organic arsenic in muscle tissue ranging between 2.38–31.11 mg/kg w. w. (mean 15.20 mg/kg w.w.), while in liver the levels varying from 2.17 to 12.66 mg/kg w.w. (mean 6.27 mg/kg w.w.). In both tissues the arsenic was present prevalently in the organic form with percentages varying 90.2-99.7% (mean 97.0%) in muscle tissue and 81.4-94.2% (mean 89.4%) in liver. The percentages detected in muscle are in accordance with what found by Edmonds et al.(1994), while in liver Edmonds et al. (1994) reports a percentage (65%) lower than that encountered by us. Many works have been carried out over the precise nature of the organoarsenic compounds found in marine biota (Cullen & Reimer, 1989) and over the fact that they vary according to species (Lawrence et al., 1986). The results suggest, that above all, primary producers such as microorganisms, phytoplankton and zooplankton, as well as algae and other organisms of the lower trophic stage, detoxicate arsenate by developing methylated substances, water-soluble arseno-sugars and fat-soluble arseno-phospholipids (Edmonds & Francesconi, 1978; Phillips & Depledge, 1985). The transformation of arseno-sugars to arsenobetaine does not occur in the organisms

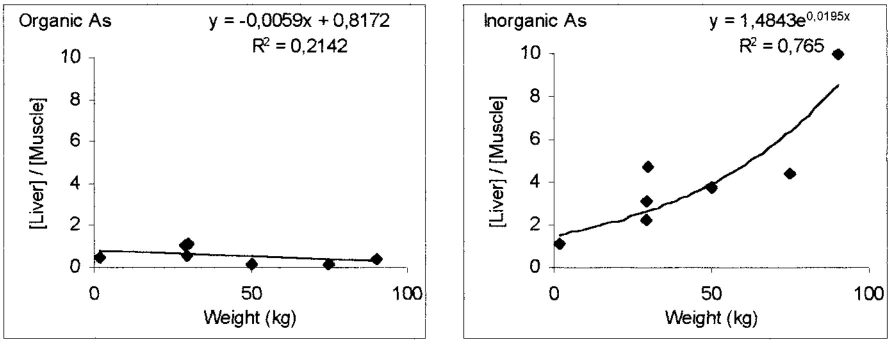

Figure 2. Ratio [liver]/[muscle] of organic and inorganic arsenic with respect to weight.

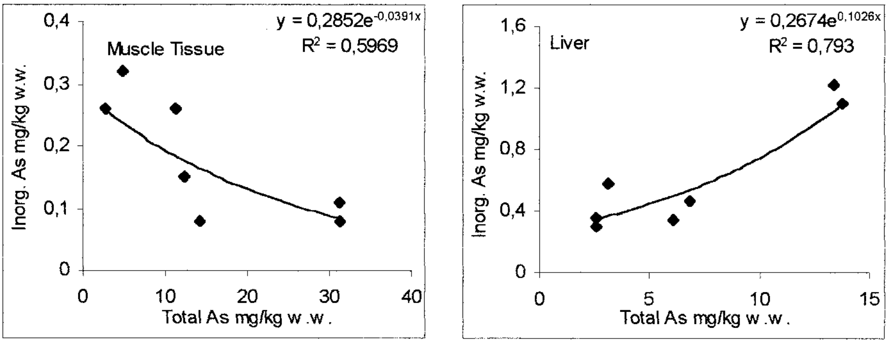

Figure 3. Concentration of inorganic arsenic in muscle tissue and in liver with respect to total arsenic.

at the higher trophic level, such as fish and crustaceans, but in the sediments by formation of dimethylarsenoylethanol. From it, by methylation can be obtained arsenocholine, which assimilated by higher organisms get then oxidised to arsenobetaine. In fact no arseno-sugars could be identified in fish and crustaceans, unlike arsenocholine which has been found in some species of shrimps (Lawrence et al., 1986; Norin et al., 1987).

A different trend was observed for inorganic arsenic that showed the higher concentrations in liver with values in the range of 0.30-1.22 mg/kg w.w. (mean 0.63 mg/kg w.w.) than in muscle tissue whose values were in the range 0.08-0.32 mg/kg w.w. (mean 0.18 mg/kg w.w.), with differences of concentrations significant ($p<0.01$). The percentages of inorganic arsenic, potentially toxic form, varying 0.3-9.8% (mean 3.0%) in muscle tissue and 5.8-18.6% (mean 10.5%) in liver.

The results of this investigation lead to the following consideration:

1.- Organic arsenic concentrations ratio liver/muscle tissue in function of the specimens' weight resulted on an average of about 1 (Fig. 2). Inorganic arsenic concentrations ratio liver/muscle tissue increased as the specimens' weight rose ($R = 0.76$, $p<0.02$) (Fig. 2).

2.- The fraction of inorganic arsenic decreased significantly ($p<0.04$) with the increasing of total arsenic concentration in muscle tissue, while in liver an opposite trend, equally significant, was observed (Fig. 3) ($p<0.01$).

The different accumulation of inorganic arsenic between two tissues, might depend on different chemical structures and/or chemical interactions of the accumulated arsenic in the various tissues. Feasible hypotheses of the greater inorganic arsenic accumulation in liver than in muscle tissue fixed by Edmonds et al. (1994) could be a stage in the degradation of arsenobetaine accumulated from food, or it could be absorbed from imbibed sea-water (sea-water contains 2-3$\mu g\ l^{-1}$ arsenic as arsenate, Andreae,1979).

Marine turtle sensitivity to the inorganic arsenic toxicity is unknown. Nevertheless, considering that inorganic arsenic concentrations in liver were present in a non negligible amount, about 3 times higher than in the muscle tissue, it may be reasonable to suppose that because inorganic arsenic carries out its toxic action at the level of the sulfhydrylic groups of some enzymes, many metabolic processes of vital importance, which occur in liver, might be altered.

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