Research Note

Total, Organic, and Inorganic Arsenic in Some Commercial Species of Crustaceans from the Mediterranean Sea (Italy)

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ABSTRACT

Total, organic, and inorganic arsenic were measured in the flesh of different crustacean species: Parapenaeus longirostris, Aristeus antennatus, Plesionika martia, and Nephrops norvegicus, species extensively commercialized and widely consumed as food. Among the different species of crustaceans, the highest mean value of total arsenic was detected in N. norvegicus (45.00 µg g⁻¹ dry wt), followed by P. martia (40.76 µg g⁻¹ dry wt), P. longirostris (34.00 µg g⁻¹ dry wt), and A. antennatus (15.45 µg g⁻¹ dry wt). In all the species examined, most of this element was present as organic compounds, while inorganic arsenic was only a very modest percentage (3.5 to 5.7%) of the total arsenic present. There is a substantial epidemiological evidence that inorganic arsenic may cause lung and skin cancer. For this reason, the Joint Expert Committee of the FAO-WHO recommended a maximum acceptable daily intake of inorganic arsenic for humans of 2 µg/kg of body weight. The estimated daily intake was below this limit; therefore, no direct hazard for human health due to the consumption of this seafood was found.

Arsenic is a ubiquitous element, present in small amounts in soils and waters throughout the world, as well as in foods. Seafood, an important constituent of the human diet, represents the main source of dietary intake of arsenic (9, 15, 34). It is known that, among the marine organisms, crustaceans contain naturally high arsenic levels. To the general public, the words “arsenic” and “poison” are synonymous (19). The toxicity of arsenic is related to its oxidation states and molecular forms, with the inorganic arsenical compounds more toxic than the organic ones. The 50% lethal dose (LD₅₀) for arsenic trioxide in rats is 20 mg/kg (1), while for arsenobetaine, no signs of toxicity were observed in mice after an oral dose of 10 g/kg (5). In humans, organoarsenic compounds are almost completely absorbed from the gastrointestinal tract, excreted in urine with little or no change in their chemical structure within a few days (23, 38), and they are not considered toxic or carcinogenic (8). On the contrary, arsenic in its inorganic forms presents a significant toxicity hazard to humans. There is substantial epidemiological evidence that inorganic arsenic may cause lung and skin cancer (16, 17). For this reason, the Joint Expert Committee of the FAO-WHO (12) recommended a maximum acceptable daily intake of inorganic arsenic of 2 µg/kg for humans per body weight. On this basis, knowledge of the chemical forms in which the element occurs in food is relevant to evaluate the toxicological implication for the humans. Information on the dietary intake of this contaminant, particularly in the inorganic form, is of special health interest since, by comparison with acceptable intake levels, a determination can be made as to whether or not consumers are likely to be at risk. However, most dietary studies have reported only total arsenic concentrations (9, 15, 34), while direct measurements of inorganic arsenic in foods are scanty (30–32). In light of the above concern, levels of total, organic, and inorganic arsenic in the flesh of different crustaceans (P. longirostris, A. antennatus, P. martia, and N. norvegicus), species of great commercial value and wide food consumption, were determined.

MATERIALS AND METHODS

Specimens of different crustacean species (Parapenaeus longirostris: 826 specimens; Aristeus antennatus: 387 specimens; Plesionika martia: 456 specimens; and Nephrops norvegicus: 270 specimens) were caught in the Southern Adriatic Sea. They were divided into pools according to size (P. longirostris: 10 pools; A. antennatus: 8 pools; P. martia: 7 pools; and N. norvegicus: 5 pools). From the organisms of each pool, the flesh was removed, and the dry weight was determined from samples kept at 60°C, until constant weight. Pulverization and homogenization were achieved by grinding the tissue in an agata mortar. Determination of total arsenic was carried out as follows. Samples of about 0.6 g of dry tissue were digested, under reflux, with 10 ml of the mixture HNO₃-H₂SO₄-HClO₄ (5:1:1) for 6 h. After cooling, the acid digest was diluted to a final volume of 100 ml with deionized water (22).

Sample digestion for the determination of inorganic and organic arsenic followed the protocol of Flanjak (13). For the determination of inorganic arsenic, samples of about 1 g of dry 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 con-
nected to a modified Bethge apparatus (13), 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 original 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 were added to the distillate and made up to 50 ml in a volumetric flask.

For the determination of organic arsenic, 30 ml of a mixture of nitric and perchloric acids (5 + 1) and 1 ml of sulfuric acid were added to the residue in the original distillation flask. The samples were slowly evaporated to fumes of sulfuric 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 an MHS-10 Perkin-Elmer hydride generator system connected to a quartz cell with open ends (i.d. 1.2 cm, length 16.7 cm; Perkin-Elmer, Norwalk, Conn.) 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. To obtain the calibration curve of total arsenic (slope: 0.00137; correlation coefficient: 0.99641), aliquots of 0.2, 0.4, 0.6, and 0.8 ml (concentration range: 20 to 80 µg/liter) were taken from an arsenic solution standard at a concentration of 100 µg/liter, prepared by dilution from a solution standard (1,000 mg/liter, Perkin Elmer Pure).

The precision of the methods was estimated from 10 replicate determinations of total arsenic for the reference material DORM-1 (Dogfish muscle) obtained from the National Research Council of Canada. The values found (As: 17.2 ± 1.3 mg/kg dry wt) agreed with the certified values (As: 17.7 ± 2.1 mg/kg dry wt). All reagents were of the Suprapur type (Merck, Darmstadt, 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 3 h in an electric oven at 100°C prior to use at room temperature. The measurements for each pool were made in triplicate, and all data were expressed in µg g⁻¹ dry weight.

RESULTS

Results for total, organic, and inorganic arsenic (mean ± SD) in the muscle of different species, 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 plus inorganic, no substantial differences have been observed. Hence, under “Results,” total arsenic values will be those of the sum. Among the different species of crustaceans, the highest mean values of total arsenic were detected in N. norvegicus (45.00 µg g⁻¹), followed by P. martia (40.76 µg g⁻¹), L. longirostris (34.00 µg g⁻¹), and A. antennatus (15.45 µg g⁻¹). The accumulation of arsenic in marine organisms varies in relation to many factors, such as species, temperature, and salinity of the water, as well as size, diet, and habitat (28). Literature values for total arsenic in crustaceans are generally much higher than in other marine organisms and show a great variability in function to species. For prawns, values range from 9 to 19 µg g⁻¹; for shrimp, values range from <1 to 40 µg g⁻¹; and for crabs, values range from <1 to 70 µg g⁻¹ (28).

The marine organisms acquire their arsenic burden through the food chain. For example, fish feeding on organisms in a food chain based on macroalgae usually contain high levels of total arsenic because algae contain very high concentrations of arsenic (11, 24). Also, marine organisms that live on or close to the seabed have a consistently higher level of arsenic than other species (27, 31). Sediments are always higher in arsenic than the waters with which they are associated, and bottom waters usually contain higher concentrations than surface waters (33). The four crustacean species chosen for this study are organisms belonging to the deep-sea benthic fauna, and the differences in accumulation among the different crustacean species may be a result of specific diets and of a specific physiological metabolism, characteristic of each species.

Another factor that may affect the accumulation of this element is the size of the marine organisms. Published studies on the relationship between total arsenic levels in marine organisms and weight are contrasting. For some species—e.g., Anarchichas minor and Reinhardtius hippoglossoides (6), Boreogadus saida (7), Sillago maculata (11), Thunnus thynnus, and Thunnus togge (3)—a positive correlation was observed, while for other species—e.g., Mugil cephalus (25), Chlamys islandica (19), and Squilla mantis (32)—no dependency of total arsenic concentrations on weight was found. In the present study, total arsenic concentrations were found not to be related to the weight of crustaceans. This result may suggest that arsenic levels in these crustacean species are probably regulated and that excretion of arsenic is occurring.
For organic arsenic, too, the highest concentrations were found in *N. norvegicus* (44.3 μg g⁻¹), followed by *P. martia* (39.36 μg g⁻¹), *P. longirostris* (32.45 μg g⁻¹), and *A. antennatus* (14.61 μg g⁻¹). The analytical data indicate that a large proportion of total arsenic is mainly in the organic form with percentages above 90%, in agreement with those reported in the literature for marine organisms (19, 25, 32). Many studies have been done on the precise nature of the organoaarsenic compounds found in marine biota (8, 19). 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, convert seawater inorganic arsenic, via several biosynthetic steps, to the water-soluble arsensogalactose and fat-soluble arsensophospholipids (10, 29). These compounds undergo a series of transformations (methylation and oxidation) that lead to the formation of the biosynthetic end product arsename. Arsenolate, the dominant organoarsenic compound in marine organisms (8, 14), is thought to be nontoxic to humans, and, after ingestion, it is rapidly excreted in the urine, unchanged (14). Arsenosugars are also regarded as nontoxic to humans; however, following ingestion, they are metabolized by humans to other arsensicals, such as dimethylarsinic acid, which is the principal metabolite in the urine, along with other arsensicals at lower concentrations (21). Although no effect has been observed in humans, there is evidence that dimethylarsinic acid may be a tumor promoter. Recent studies indicate that oral administration of dimethylarsic acid to mice (37) and rats (35) promotes chemical carcinogen-initiated tumorigenesis in the lung, kidney, liver, and thyroid of these animals.

With regard to inorganic arsenic, the highest levels were found in *N. norvegicus* (2.00 μg g⁻¹); concentrations of the same order of magnitude were observed in *P. longirostris* (1.55 μg g⁻¹) and *P. martia* (1.40 μg g⁻¹), while *A. antennatus* (0.83 μg g⁻¹) showed the lowest levels. Consistent with other studies (20, 32), concentrations of inorganic arsenic were negligible, contributing to the total arsenic burden with percentages below 10%. Inorganic arsenic is found in environmental media as oxides of arsenic or salts of the correspondent acids (As²⁺O₃, arsenite; AsVO₄, arsenate). Arsenite is more toxic than arsenate, and the mechanism of toxicity differs with the valence state of arsenic. The key to the toxicity of arsenite is its electrophilic nature because of the arsenite binds to electron-rich sulfhydryl groups on proteins with subsequent inhibition of a large number of enzymes (2). In particular, arsenite is known to inhibit enzymes of the mitochondrial citric acid cycle, with a resulting decrease in cellular ATP production (5). Arsenate’s structural similarity to phosphate allows it to substitute for phosphate in energy-producing reactions within the cell; consequently, ATP-producing reactions do not occur, and no useful store of energy is generated (2). In addition, arsenate may also exert its toxicity effects indirectly, via its reductive metabolism to arsenite (2).

The current rules in force in Italy do not specify allowable maximum limits for the amount of total arsenic in marine products, in contrast to other countries, where this limit is established (Hong Kong: 6 μg g⁻¹ wet wt for finfish and 10 μg g⁻¹ wet wt for shellfish; Australia: 1.14 μg g⁻¹ wet wt; Poland: 1 μg g⁻¹ wet wt; Cuba: 5 μg g⁻¹ wet wt; and Saudi Arabia: 0.5 μg g⁻¹ wet wt) (4). To compare the values found in the analyzed species, expressed in dry weight, with the maximum permissible limit adopted by Hong Kong (10 μg g⁻¹ wet wt) specific for shellfish, the concentrations were converted to wet weight values, using a dry weight/wet weight ratio of about 4:1. The results indicated that 71.4% of the *P. martia* samples, 60% of the *N. norvegicus* samples, and 43% of the *P. longirostris* samples presented arsenic levels higher than the limit of 10 μg g⁻¹ wet weight, while no samples of *A. antennatus* exceeded this limit (Fig. 1). To assess the potential health impact, the 2-μg d⁻¹ maximum intake limit for inorganic arsenic recommended by the Joint Expert Committee of the FAO-WHO (12) for a 70-kg person was used as a guideline. Considering the mean concentrations of inorganic arsenic (wt wt) in the flesh of the different crustacean species and according to a daily average consumption of seafood of 60 g per capita in Italy (18), the estimated daily intake varied from 0.18 to 0.43 μg/kg body weight. Other quantities of consumption of crustaceans (100, 150, 200, and 250 g d⁻¹) were considered to establish whether the daily intake approaches or exceeds the value fixed by the FAO-WHO (12), because these levels of consumption may be common among some consumer groups (e.g., fishermen). As shown in Figure 2, the estimated daily intakes for ingestion of different amounts of *A. antennatus*, *P. martia*, and *P. longirostris* flesh were far below the limit established by the FAO-WHO (12). Particularly high exposure was associated with the consumption of 150 g d⁻¹ or more of *N. norvegicus*, which yielded estimated daily intakes approaching the maximum limit (150 g d⁻¹ = 1.07 μg/kg body wt; 200 g d⁻¹ = 1.43 μg/kg body wt, and 250 g d⁻¹ = 1.79 μg/kg body wt).

The results showed that in all of the species analyzed, arsenic was present prevalently in the organic form, while inorganic arsenic made a negligible percentage contribution toward the total arsenic burden. From a public health aspect, many consider that the high concentration of arsenic in marine food does not represent a serious problem, since only a small percentage is present in the toxic inorganic...
form, and the larger part exists in a highly stable and physiologically inactive organic form. In the present case, although the amount of inorganic arsenic ingested through consumption of these seafood did not exceed the maximum intake limit established by the Joint Expert Committee of the FAO-WHO (12), it must be borne in mind that the population is also subjected to inorganic arsenic contamination via other sources. A recent report indicates that between 25 and 100% of total arsenic in terrestrial foods may be inorganic arsenic (30). Another important source is the drinking water (36). Internationally, at least 1 million people are drinking arsenic-contaminated water above the WHO-recommended value of 0.01 μg liter⁻¹, and many people show skin lesions characteristic of arsenic poisoning (26). Therefore, low levels of inorganic arsenic in the diet from crustaceans can result in a higher daily dietary intake. On this basis, further investigations are needed that discern between the different forms of arsenic in the diet in order to evaluate accurately arsenic exposure. Furthermore, it will be of great concern to fix, in Italy, too, a maximum limit for total arsenic and, most of all, for inorganic arsenic in seafood.

REFERENCES