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# Distribution of inorganic arsenic and methylated arsenic in marine organisms

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**Inorganic arsenic and methylated arsenic compounds in 60 specimens of marine organisms were investigated by hydride generation derivatization and cold-trap gas chromatography–mass spectrometry (GC MS). Chloroform–methanol extracts from seaweeds, shellfish, fish, crustaceans and other marine organisms were separated into water-soluble and lipid-soluble fractions. The arsenic compounds in each fraction were identified and analysed as arsine, methylarsine, dimethylarsine and trimethylarsine.**

**Trimethylarsenic compounds were distributed mainly in the water-soluble fraction of muscle of carnivorous gastropods, crustaceans and fish. The amounts of dimethylated arsenic compounds were found to be larger than that of trimethylated arsenic in the lipid-soluble fraction of fish viscera. Dimethylated arsenic compounds were distributed in the water-soluble fraction of Phaeophyceae.**

**Keywords: Arsenic, arsine, methylarsine, dimethylarsine, trimethylarsine, arsenobetaine, trimethylarsine oxide, marine organisms, gas chromatography mass spectrometry (GC MS)**

## INTRODUCTION

High levels of arsenicals are observed in marine organisms as the water-soluble and the lipid-soluble organoarsenic compounds.

Arsenobetaine [$(CH_3)_3As^+CH_2COO^-$], which is one of the water-soluble organoarsenicals, has been found widely distributed in marine animals and was considered to be the final metabolite in arsenic circulation in marine ecosystems. Recently, these arsenic metabolites have been studied in relation to the dietary habits of marine animals.[1]

It is important to elucidate the chemical structure of organoarsenic compounds in marine organisms in order to investigate the circulation of arsenic in marine ecosystems. But in most cases, it does require complicated techniques and laborious purification for the identification of these organoarsenicals in marine organisms.

Several arsenic compounds are converted to the corresponding arsines by reduction with sodium borohydride.[2] It was known that dimethylarsine and trimethylarsine were released from dimethylated and trimethylated arsenic compounds, respectively, in marine animals and seaweeds by borohydride reduction after alkaline digestion.[3] We have established a method for the separation and identification of inorganic and methylated arsines using a gas-chromatography mass spectrometer equipped with an arsine generation system.[4]

In this paper we report the distribution of inorganic and methylated arsenicals and total arsenic in 60 specimens of marine organisms, i.e. plankton, seaweeds, gastropods (plankton-feeding herbivorous and carnivorous), crustaceans (carnivorous), fish (plankton-feeding, herbivorous and carnivorous), and other marine animals.

The fractions were separated into the muscle and viscera parts in marine animals (fish and shellfishes), and all samples were further separated into water-soluble and lipid-soluble fractions by solvent distribution with chloroform, methanol and water systems.

The arsenical specimens in all fractions were determined as arsine and methylated arsines.

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## MATERIALS AND METHODS

### Preparation of samples

Many specimens were collected on the coast of the Miura Peninsula in Kanagawa Prefecture and the coast of Shimonoseki in Yamaguchi Prefecture in Japan from March to October 1987, and a portion of samples was obtained from a market. Each fish and shellfish was dissected into muscle and viscera. Other biological samples were extracted by the appropriate method.

### Extraction

Each tissue (5–10 g) was weighed and extracted three times with a mixture of chloroform and methanol (2:1, 20–40 $cm^3$) using a homogenizer. After centrifugation, an aliquot of supernatant was transfered into a separatory funnel and shaken with water (one-quarter of the volume of the supernatant). The water-soluble and lipid-soluble fractions were obtained from the upper layer and lower layer, respectively.

### Alkaline digestion

Each fraction (1 $cm^3$) was transferred separately into polymethylenepentene tubes and the aqueous and organic solvent respectively were evaporated to near-dryness under a nitrogen stream. The residues were dissolved in 0.5 $cm^3$ –1 $cm^3$ of water or methanol, and 10 $cm^3$ of 2 mol $dm^{-3}$ sodium hydroxide solution was then added to each, the top of the tube was covered with Parafilm. After heating in a water bath at 85 °C for 3 h, the aqueous solution was neutralized with dilute hydrochloric acid and made up to a volume of 20 $cm^3$ with water.

### Arsenic analysis

#### Total arsenic

The complete biological sample (1 g) was digested with 10 $cm^3$ of nitric acid (61% w/w) on a hot plate at below 100 °C until the evolution of brown humes ceased. After cooling, a mixture of 5 $cm^3$ of nitric acid, 3 $cm^3$ of sulphuric acid (97% w/w) and 5 $cm^3$ of perchloric acid (60% w/w) was added and the mixture was heated until dense fumes of sulphur trioxide appeared. After cooling, solutions were diluted with water (20 $cm^3$) and neutralized with dilute ammonium hydroxide. The degraded solution was transferred into a 100 $cm^3$ volumetric flask, 2 $cm^3$ of 36% (w/w) hydrochloric acid, 4 $cm^3$ of 20% (w/w) potassium iodide and 4 $cm^3$ of 20% (w/w) ascorbic acid were added to the solution, and the solution was made up to 100 $cm^3$ with water. Arsenic was determined by reduction of arsenic to arsine with the fully automated continuous arsine generation system using sodium borohydride and an atomic absorption spectrophotometer equipped with a heated quartz tube. This procedure has been described previously.[4]

#### Inorganic arsenic and methylated arsenic

The sample solution following alkaline digestion (3 $cm^3$) was introduced to the fully automated continuous arsine generation system.

Hydrochloric acid (0.6 mol $dm^{-3}$) and sodium borohydride solution (2.0 g per 100 $cm^3$ of 0.2 mol $dm^{-3}$ aqueous sodium hydroxide) were continuously pumped through the mixing coil of the arsine generator at 6 $cm^3$ $min^{-1}$. The generated arsines were collected in a U-shaped tube and flashed into the gas chromatograph–mass spectrometer (GC MS) using selected ion monitoring (SIM) as previously reported.[4] Inorganic arsenic (Inorg.As), methylated (MA), dimethylated (DMA) and trimethylated (TMA) arsenic compounds were identified and quantified as arsine, methylarsine, dimethylarsine and trimethylarsine.

## RESULTS AND DISCUSSION

Arsenic compounds in marine organisms were divided into three groups, water-soluble, lipid-soluble and total arsenic. The water-soluble arsenicals were further classified into inorganic, methylated, dimethylated and trimethylated arsenic, and the lipid-soluble arsenicals were also classified into similar groups except inorganic arsenic. The results are summarized in Table 1.

### Total arsenic

Of 60 specimens examined, 14 contained remarkably high concentration of total arsenic (over 30 $\mu g\ g^{-1}$). Among these 14 specimens, seven were seaweeds, four were carnivorous gastropods and three were crustaceans. Attention regarding arsenic should be focused on these three types of marine organisms.

**Table 1** The distribution of arsenic in marine organisms (as As $\mu g\ g^{-1}$)[a]

| Diet | Species | Tissues | Water-soluble | | | | Lipid-soluble | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Inorg As | MA | DMA | TMA | MA | DMA | TMA | Arsenic |
| | Plankton | Whole | ND | 0.02 | 0.31 | 0.62 | ND | 0.10 | 0.20 | 1.29 |
| | | | (0) | (1.55) | (24.03) | (48.06) | (0) | (7.75) | (15.50) | |
| | Phaeophyceae | | | | | | | | | |
| | *Laminaria japonica*[c] | | ND | ND | 36.48 | 1.07 | ND | 0.14 | ND | 44.25 |
| | | | (0) | (0) | (82.44) | (2.42) | (0) | (0.32) | (0) | |
| | *Hizikia fusiforme* | | 1.47 | ND | 33.01 | 3.86 | ND | ND | 0.17 | 41.31 |
| | | | (3.56) | (0) | (79.91) | (9.34) | (0) | (0) | (0.41) | |
| | *Hizikia fusiforme* | | 8.69 | ND | 17.68 | 0.36 | ND | ND | ND | 36.07 |
| | | | (24.09) | (0) | (49.02) | (1.00) | (0) | (0) | (0) | |
| | *Ecklonia cava* | | ND | ND | 12.59 | 7.24 | ND | ND | ND | 33.05 |
| | | | (0) | (0) | (38.09) | (21.91) | (0) | (0) | (0) | |
| | *Undaria pinnatifida* | | ND | ND | 2.74 | 0.34 | ND | 4.75 | ND | 38.27 |
| | | | (0) | (0) | (7.16) | (0.89) | (0) | (12.41) | (0) | |
| | *Ishige okamurai*[b] | | ND | ND | 0.09 | ND | ND | ND | ND | 1.48 |
| | | | (0) | (0) | (0.08) | (0) | (0) | (0) | (0) | |
| | Rhodophyceae | | | | | | | | | |
| | *Porphyra tenera*[c] | | ND | ND | 47.39 | 0.83 | ND | 0.55 | ND | 69.85 |
| | | | (0) | (0) | (67.85) | (1.19) | (0) | (0.79) | (0) | |
| | *Gelidium amansii* | | ND | ND | 0.56 | 0.10 | ND | ND | ND | 1.78 |
| | | | (0) | (0) | (31.46) | (5.62) | (0) | (0) | (0) | |
| | *Gloiopeltis tenax*[c] | | ND | ND | 0.10 | ND | ND | 0.23 | ND | 35.36 |
| | | | (0) | (0) | (0.28) | (0) | (0) | (0.65) | (0) | |
| | *Hypnea charoides*[b] | | ND | ND | 0.13 | ND | ND | 0.40 | ND | 7.87 |
| | | | (0) | (0) | (1.65) | (0) | (0) | (5.08) | (0) | |
| | *Chondrus ocellatus*[b] | | ND | ND | 0.40 | ND | ND | 0.80 | ND | 15.86 |
| | | | (0) | (0) | (2.52) | (0) | (0) | (5.04) | (0) | |
| | Chlorophyceae | | | | | | | | | |
| | *Ulva* sp. | | ND | ND | 2.10 | 0.50 | ND | ND | ND | 3.65 |
| | | | (0) | (0) | (57.53) | (13.70) | (0) | (0) | (0) | |
| Plankton | Demospongia | | | | | | | | | |
| | *Halichondria okadai* | Whole | 0.10 | 0.49 | 9.58 | 3.46 | ND | ND | ND | 15.28 |
| | | | (0.65) | (3.21) | (62.70) | (22.64) | (0) | (0) | (0) | |
| | *Halichondria japonica* | Whole | ND | 0.08 | 0.10 | 0.29 | 0.05 | 0.17 | 0.04 | 0.94 |
| | | | (0) | (8.51) | (10.64) | (30.85) | (5.32) | (18.09) | (4.26) | |

Table 1 (*continued*)

| Diet | Species | Tissues | Water-soluble Inorg As | Water-soluble MA | Water-soluble DMA | Water-soluble TMA | Lipid-soluble MA | Lipid-soluble DMA | Lipid-soluble TMA | Total Arsenic |
|---|---|---|---|---|---|---|---|---|---|---|
| Plankton | Scyphozoa | | | | | | | | | |
| | *Aurelia aurita* | Whole | 0.12 | 0.01 | ND | 0.12 | ND | ND | ND | 0.46 |
| | | | (26.09) | (2.17) | (0) | (26.09) | (0) | (0) | (0) | |
| Omnivorous and herbivorous | Echinoidea | | | | | | | | | |
| | *Pseudocentrotus depressus* | Ovary | ND | ND | 0.58 | 1.83 | 0.02 | 0.34 | 0.03 | 3.12 |
| | | | (0) | (0) | (18.59) | (58.65) | (0.64) | (10.90) | (0.96) | |
| | | Viscera | 0.48 | 0.02 | 0.85 | 0.96 | 0.02 | 0.97 | 0.41 | 3.98 |
| | | | (12.06) | (0.50) | (21.36) | (24.12) | (0.50) | (24.37) | (10.30) | |
| | *Anthocidaris crassispina* | Ovary | ND | ND | 0.04 | 0.45 | ND | 0.07 | ND | 1.34 |
| | | | (0) | (0) | (2.99) | (33.58) | (0) | (5.22) | (0) | |
| | | Viscera | ND | ND | 0.08 | 1.21 | ND | 0.09 | 0.12 | 3.59 |
| | | | (0) | (0) | (2.23) | (33.70) | (0) | (2.51) | (3.34) | |
| Carnivorous | Asteroidea | | | | | | | | | |
| | *Asterina pectinifera*[b] | Ovary | ND | ND | 0.01 | 1.75 | ND | 0.01 | 0.07 | 1.88 |
| | | | (0) | (0) | (0.53) | (93.08) | (0) | (0.53) | (3.72) | |
| | | Viscera | ND | ND | 0.02 | 1.18 | ND | 0.05 | 0.02 | 1.30 |
| | | | (0) | (0) | (1.54) | (90.77) | (0) | (3.85) | (1.54) | |
| Detritus | Holothuroidea | | | | | | | | | |
| | *Stichopus japonicus* | Muscle | ND | ND | 1.22 | 13.28 | ND | ND | ND | 23.43 |
| | | | (0) | (0) | (5.21) | (56.68) | (0) | (0) | (0) | |
| Carnivorous | Cephalopoda | | | | | | | | | |
| | *Todarodes pacificus* | Muscle | ND | 0.02 | 0.02 | 4.48 | ND | ND | ND | 7.95 |
| | | | (0) | (0.25) | (0.25) | (56.35) | (0) | (0) | (0) | |
| | | Viscera | ND | ND | 0.07 | 2.31 | ND | 0.01 | 0.54 | 2.97 |
| | | | (0) | (0) | (2.36) | (77.78) | (0) | (0.34) | (18.18) | |
| Plankton | Bivalvia | | | | | | | | | |
| | *Crassostrea gigas* | Whole | ND | ND | 0.17 | 6.15 | ND | 0.17 | 0.43 | 9.95 |
| | | | (0) | (0) | (1.71) | (61.81) | (0) | (1.71) | (4.32) | |
| | *Mytilus edulis*[b] | Whole | ND | ND | 0.07 | 2.01 | ND | 0.16 | 0.02 | 4.36 |
| | | | (0) | (0) | (1.61) | (46.10) | (0) | (3.67) | (0.46) | |
| | *Patinopecten yessoensis*[c] | Muscle | ND | ND | ND | 0.79 | ND | 0.08 | 0.02 | 1.93 |
| | | | (0) | (0) | (0) | (40.93) | (0) | (4.15) | (1.04) | |
| | | Viscera | ND | ND | 0.04 | 0.11 | ND | 0.08 | 0.02 | 0.78 |
| | | | (0) | (0) | (5.13) | (14.10) | (0) | (10.26) | (2.56) | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Meretrix lusoria*[c] | Whole | ND | ND | 0.33 | 1.03 | ND | 0.18 | 0.11 | 1.91 |
| | | | (0) | (0) | (17.28) | (53.93) | (0) | (9.42) | (5.76) | |
| | *Tapes philippinarum* | Whole | ND | ND | 1.11 | 4.19 | ND | 0.31 | 0.30 | 6.82 |
| | | | (0) | (0) | (16.28) | (61.44) | (0) | (4.55) | (4.40) | |
| Herbivorous | Gastropoda | | | | | | | | | |
| | *Aplysia kurodai*[b] | Muscle | ND | 0.01 | 0.17 | 0.20 | ND | ND | ND | 1.02 |
| | | | (0) | (0.98) | (16.67) | (19.61) | (0) | (0) | (0) | |
| | *Nordotis discus* | Muscle | ND | ND | 0.10 | 0.24 | 0.04 | 0.15 | 0.01 | 1.31 |
| | | | (0) | (0) | (7.63) | (18.32) | (3.05) | (11.45) | (0.76) | |
| | | Viscera | ND | 0.04 | 1.97 | 1.90 | 0.04 | 0.20 | ND | 7.96 |
| | | | (0) | (0.50) | (24.75) | (23.87) | (0.50) | (2.51) | (0) | |
| | *Sulculus supertexta* | Muscle | ND | ND | 0.11 | 0.24 | ND | 0.13 | ND | 1.08 |
| | | | (0) | (0) | (10.19) | (22.22) | (0) | (12.04) | (0) | |
| | | Viscera | ND | ND | 0.21 | 0.36 | ND | 0.32 | 0.27 | 1.64 |
| | | | (0) | (0) | (12.80) | (21.95) | (0) | (19.51) | (16.46) | |
| | *Batillus cornutus* | Muscle | ND | 0.03 | 0.13 | 1.29 | 0.02 | 0.14 | 0.02 | 1.64 |
| | | | (0) | (1.83) | (7.93) | (78.66) | (1.22) | (8.54) | (1.22) | |
| | | Viscera | ND | 0.01 | 0.56 | 1.42 | 0.02 | 0.29 | 0.04 | 3.28 |
| | | | (0) | (0.30) | (17.07) | (43.29) | (0.61) | (8.84) | (1.22) | |
| | *Omphalius pfeifferi* | Muscle | ND | 0.01 | 0.17 | 2.75 | 0.01 | 0.07 | 0.01 | 3.53 |
| | | | (0) | (0.28) | (4.82) | (77.90) | (0.28) | (1.98) | (0.28) | |
| | | Viscera | ND | 0.02 | 0.55 | 8.49 | 0.02 | 0.20 | 0.01 | 10.08 |
| | | | (0) | (0.20) | (5.46) | (84.23) | (0.20) | (1.98) | (0.10) | |
| Carnivorous | *Charonia sauliae* | Muscle | ND | ND | 0.08 | 16.86 | ND | 0.08 | 0.02 | 17.28 |
| | | | (0) | (0) | (0.46) | (97.57) | (0) | (0.46) | (0.12) | |
| | | Viscera | ND | 0.01 | 0.15 | 11.65 | ND | 0.22 | ND | 13.46 |
| | | | (0) | (0.07) | (1.11) | (86.55) | (0) | (1.63) | (0) | |
| | *Kellettia lischkei* | Muscle | ND | 0.07 | 1.32 | 90.39 | ND | 0.09 | ND | 125.92 |
| | | | (0) | (0.06) | (1.05) | (71.78) | (0) | (0.07) | (0) | |
| | | Viscera | ND | 0.12 | 1.43 | 83.32 | 0.03 | 0.44 | 0.03 | 86.58 |
| | | | (0) | (0.14) | (1.65) | (96.23) | (0.03) | (0.51) | (0.03) | |
| | *Rapana thomasiana* | Muscle | ND | ND | 0.71 | 31.62 | 0.02 | 0.31 | 0.01 | 38.73 |
| | | | (0) | (0) | (1.83) | (81.64) | (0.05) | (0.80) | (0.03) | |
| | | Viscera | ND | ND | 0.68 | 4.16 | 0.01 | 0.06 | ND | 5.96 |
| | | | (0) | (0) | (11.41) | (69.80) | (0.17) | (1.01) | (0) | |
| | *Reishia bronni* | Muscle | ND | 0.13 | 1.98 | 109.86 | 0.03 | 0.08 | 0.03 | 123.79 |
| | | | (0) | (0.11) | (1.60) | (88.75) | (0.02) | (0.06) | (0.02) | |
| | | Viscera | ND | ND | 1.44 | 122.43 | 0.21 | 2.80 | 0.35 | 130.30 |
| | | | (0) | (0) | (1.11) | (93.96) | (0.16) | (2.15) | (0.27) | |
| | *Babylonia japonica*[c] | Muscle | ND | 0.42 | 3.99 | 51.66 | ND | 0.42 | 2.73 | 61.61 |
| | | | (0) | (0.68) | (6.48) | (83.85) | (0) | (0.68) | (4.43) | |
| | | Viscera | ND | 1.89 | 2.81 | 114.45 | ND | 1.47 | 1.68 | 153.00 |
| | | | (0) | (1.24) | (8.37) | (74.80) | (0) | (0.96) | (1.10) | |

Table 1 (*continued*)

| Diet | Species | Tissues | Water-soluble | | | | Lipid-soluble | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Inorg As | MA | DMA | TMA | MA | DMA | TMA | Arsenic |
| Carnivorous | Crustacea | | | | | | | | | |
| | *Oratosquilla oratoria* | Muscle | ND | ND | 0.05 | 11.01 | ND | 0.01 | ND | 13.60 |
| | | | (0) | (0) | (0.37) | (80.96) | (0) | (0.07) | (0) | |
| | | Viscera | 0.36 | 0.03 | 0.16 | 10.80 | ND | 0.16 | ND | 17.64 |
| | | | (2.04) | (0.17) | (0.91) | (61.22) | (0) | (0.91) | (0) | |
| | *Scyllarus cultrifer* | Muscle | ND | 0.01 | 0.16 | 23.00 | ND | 0.07 | ND | 24.11 |
| | | | (0) | (0.04) | (0.66) | (95.40) | (0) | (0.29) | (0) | |
| | *Penaeus japonicus* | Muscle | ND | ND | 0.47 | 59.87 | ND | 0.12 | ND | 65.83 |
| | | | (0) | (0) | (0.71) | (90.95) | (0) | (0.18) | (0) | |
| | *Penaeus semisulcatus* | Muscle | ND | ND | ND | 3.42 | ND | 0.03 | ND | 5.83 |
| | | | (0) | (0) | (0) | (58.66) | (0) | (0.51) | (0) | |
| | *Panulirus japonicus* | Muscle | ND | ND | 0.14 | 42.22 | ND | 0.05 | 0.10 | 48.94 |
| | | | (0) | (0) | (0.29) | (86.27) | (0) | (0.10) | (0.20) | |
| | *Portunus trituberculatus* | Muscle | ND | ND | 0.03 | 1.40 | ND | 0.03 | 0.11 | 6.66 |
| | | | (0) | (0) | (0.45) | (21.02) | (0) | (0.45) | (1.65) | |
| | *Ranina ranina* | Muscle | ND | ND | 0.32 | 21.04 | ND | 0.20 | 2.25 | 25.01 |
| | | | (0) | (0) | (1.28) | (84.13) | (0) | (0.80) | (9.00) | |
| | *Plagusia dentipes* | Muscle | ND | ND | 0.15 | 44.99 | ND | ND | ND | 46.87 |
| | | | (0) | (0) | (0.32) | (95.99) | (0) | (0) | (0) | |
| Plankton | Fish | | | | | | | | | |
| | *Engraulis japonica* | Muscle | ND | 0.05 | 0.01 | 2.01 | ND | 0.01 | ND | 2.33 |
| | | | (0) | (2.15) | (0.43) | (86.27) | (0) | (0.43) | (0) | |
| | | Viscera | ND | 0.16 | 0.16 | 2.43 | 0.01 | 0.01 | 0.01 | 2.81 |
| | | | (0) | (5.69) | (5.69) | (86.48) | (0.36) | (0.36) | (0.36) | |
| | *Etrumeus micropus* | Muscle | ND | ND | 0.02 | 0.68 | ND | ND | ND | 1.30 |
| | | | (0) | (0) | (1.54) | (52.31) | (0) | (0) | (0) | |
| | | Viscera | ND | ND | 0.15 | 2.87 | 0.07 | 0.14 | 0.09 | 3.48 |
| | | | (0) | (0) | (4.31) | (82.47) | (2.01) | (4.02) | (2.59) | |
| | *Sardinops melanosticta* | Muscle | ND | 0.01 | 0.01 | 4.07 | ND | ND | 0.04 | 4.51 |
| | | | (0) | (0.22) | (0.22) | (90.24) | (0) | (0) | (0.89) | |
| | | Viscera | ND | 0.01 | 0.03 | 0.17 | 0.01 | 0.10 | ND | 0.36 |
| | | | (0) | (2.78) | (8.33) | (47.22) | (2.78) | (27.78) | (0) | |
| Herbivorous | *Prionurus microlepidotus* | Muscle | ND | ND | ND | 0.03 | ND | ND | ND | 0.10 |
| | | | (0) | (0) | (0) | (30.00) | (0) | (0) | (0) | |
| | | Viscera | ND | ND | 0.07 | 0.13 | ND | 0.03 | ND | 1.08 |
| | | | (0) | (0) | (6.48) | (12.04) | (0) | (2.78) | (0) | |
| | *Siganus fuscescens* | Muscle | ND | ND | ND | 0.03 | ND | ND | ND | 0.47 |
| | | | (0) | (0) | (0) | (6.38) | (0) | (0) | (0) | |
| | | Viscera | ND | 0.01 | 0.36 | 0.10 | ND | 0.05 | ND | 2.82 |
| | | | (0) | (0.35) | (12.77) | (3.55) | (0) | (1.77) | (0) | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Carnivorous | *Trachurus Japonicus* | Muscle | ND | ND | 0.01 | 1.22 | 0.02 | 0.02 | 0.02 | 1.36 |
| | | | (0) | (0) | (0.74) | (89.71) | (1.47) | (1.47) | (1.47) | |
| | | Viscera | ND | 0.02 | 0.21 | 1.30 | 0.05 | 0.06 | 0.01 | 2.23 |
| | | | (0) | (0.90) | (9.42) | (58.30) | (2.24) | (2.67) | (0.45) | |
| | *Chrysophrys major* | Muscle | ND | ND | 0.01 | 0.88 | ND | 0.02 | ND | 1.21 |
| | | | (0) | (0) | (0.83) | (72.73) | (0) | (1.65) | (0) | |
| | | Viscera | ND | ND | 0.06 | 0.49 | ND | 0.20 | ND | 1.73 |
| | | | (0) | (0) | (3.47) | (28.32) | (0) | (11.56) | (0) | |
| (Omnivorous) | *Acanthogobius flavimanus* | Muscle | ND | ND | ND | 0.51 | ND | ND | ND | 0.74 |
| | | | (0) | (0) | (0) | (68.92) | (0) | (0) | (0) | |
| | | Viscera | ND | ND | 0.05 | 0.37 | ND | 0.05 | ND | 1.73 |
| | | | (0) | (0) | (2.89) | (21.39) | (0) | (2.89) | (0) | |
| | *Reinhardtius hippoglossoides*[c] | Muscle | ND | ND | 0.02 | 1.56 | ND | 0.01 | ND | 1.91 |
| | | | (0) | (0) | (1.05) | (81.68) | (0) | (0.52) | (0) | |
| | *Oplegnathus fasciatus* | Muscle | ND | 0.02 | 0.07 | 8.84 | ND | 0.05 | 0.27 | 9.38 |
| | | | (0) | (0.21) | (0.75) | (94.24) | (0) | (0.53) | (2.88) | |
| | | Viscera | ND | 0.01 | 0.05 | 2.67 | ND | 0.04 | 0.41 | 5.02 |
| | | | (0) | (0.20) | (1.00) | (53.19) | (0) | (0.80) | (8.17) | |
| | *Pleuronichthys cornutus* | Muscle | ND | ND | 0.03 | 0.91 | ND | 0.04 | 0.13 | 2.30 |
| | | | (0) | (0) | (1.30) | (39.57) | (0) | (1.74) | (5.65) | |
| | | Viscera | ND | ND | 1.00 | 6.32 | ND | 0.27 | 0.19 | 8.08 |
| | | | (0) | (0) | (12.38) | (78.22) | (0) | (3.34) | (2.35) | |
| | *Auxis tapeinosoma* | Muscle | ND | ND | 0.01 | 0.14 | ND | 0.01 | 0.05 | 0.32 |
| | | | (0) | (0) | (3.13) | (43.75) | (0) | (3.13) | (15.63) | |
| | | Viscera | ND | ND | 0.02 | 0.96 | ND | 0.04 | 0.05 | 1.38 |
| | | | (0) | (0) | (1.45) | (69.57) | (0) | (2.90) | (3.62) | |
| | *Parapristipoma trilineatum* | Muscle | ND | ND | ND | 0.50 | ND | ND | 0.06 | 0.62 |
| | | | (0) | (0) | (0) | (80.65) | (0) | (0) | (9.68) | |
| | | Viscera | ND | ND | 0.12 | 0.84 | ND | 0.30 | 0.71 | 2.16 |
| | | | (0) | (0) | (5.56) | (38.89) | (0) | (13.89) | (32.87) | |
| | *Sphyraena schlegeli* | Muscle | ND | ND | 0.01 | 0.70 | ND | 0.06 | 0.04 | 0.88 |
| | | | (0) | (0) | (1.14) | (79.55) | (0) | (6.82) | (4.55) | |
| | | Viscera | ND | ND | 0.05 | 0.92 | ND | 0.03 | 0.07 | 1.31 |
| | | | (0) | (0) | (3.82) | (70.23) | (0) | (2.29) | (5.34) | |
| | *Neoditrema ransonneti* | Muscle | ND | ND | 0.05 | 0.32 | ND | 0.04 | 0.08 | 0.72 |
| | | | (0) | (0) | (6.94) | (44.44) | (0) | (5.56) | (11.11) | |
| | | Viscera | ND | ND | 0.15 | 0.75 | ND | 0.09 | 0.28 | 1.54 |
| | | | (0) | (0) | (9.74) | (48.70) | (0) | (5.84) | (18.18) | |
| (Omnivorous) | *Stephanolepis cirrhifer* | Muscle | ND | ND | 0.04 | 2.81 | ND | ND | 0.20 | 4.35 |
| | | | (0) | (0) | (0.92) | (64.60) | (0) | (0) | (4.60) | |
| | | Viscera | ND | ND | 0.20 | 6.87 | ND | 0.16 | 0.46 | 9.28 |
| | | | (0) | (0) | (2.16) | (74.03) | (0) | (1.72) | (4.96) | |

Abbreviations: Inorg As, inorganic arsenic; MA, methylated arsenic; DMA, dimethylated arsenic; TMA, trimethylated arsenic; ND = not detectable.
[a]The ratio (%) of each form of arsenic to total As is given in parentheses. [b]Collected at Shimonoseki. [c]Commercially available. Other samples were caught at the Miura Peninsula.

Most of these representative edible seaweeds accumulated arsenic, and Phaeophyceae especially contained high levels. Of six Phaeophyceae, five contained over 30 $\mu g\ g^{-1}$ of total arsenic.

The arsenic content of carnivorous gastropods was outstandingly high, e.g. 125.9, 123.8 and 61.6 $\mu g\ g^{-1}$ of total arsenic were observed from muscle tissue of *Kellettia lischkei*, *Reishia bronni* and *Babylonia japonica* respectively.

The arsenic content in plankton-feeding bivalves and herbivorous gastropods, which feed on marine algae containing high amounts of arsenic, was fairly low compared with that in carnivorous gastropods. This tendency is quite in agreement with the report of Shiomi *et al.*[5] They estimated that this distinction was due to metabolic differences between the species and to the differences in excretion speed between the carnivorous gastropods and the other shellfish.

## Water- and lipid-soluble arsenic

Most of the arsenic in the marine organisms was in the water-soluble fraction and in methylated form. Lipid-soluble arsenic was found ubiquitously, but its content was fairly low compared with water-soluble arsenic. Maher[1] reported that lipid-soluble arsenic was significantly higher in plankton feeders than that in herbivorous and carnivorous species, but it was difficult to obtain the same conclusion in this work. The water-soluble trimethylated arsenic was widely spread in marine animals, and was the main component of water-soluble arsenic. It was thought this trimethylated arsenic was likely to be arsenobetaine or trimethylarsine oxide [$(CH_3)_3AsO$], since both compounds released trimethylarsine by the alkaline digestion and subsequent reduction with sodium borohydride, but the other trimethylated organic arsenical, i.e. arsenocholine [$(CH_3)_3As^+CH_2CH_2OH$] does not give trimethylarsine by the procedure.[4]

We have reported that the toxicities of arsenobetaine and trimethylarsine oxide was very low, their $LD_{50}$ values in mice being more than 10 g $kg^{-1}$ and 10.8 g $kg^{-1}$, respectively.[6,7] On the other hand, water-soluble trimethylated arsenic in the edible tissues of 42 species of marine animals eaten as daily food varied between 6.38 % and 97.57 % (mean 64.97 ± 24.31 SD %) of the total arsenic. It must be significant to consider the safety of arsenic-containing marine animals as foods.

The contents of water-soluble dimethylated arsenic were specifically high in seaweeds. It was thought this dimethylated arsenic was a degradation product of the alkaline digestion of arseno-sugars[8] having a $(CH_3)_2AsO$— moiety.

The lipid-soluble dimethylated arsenic was a major component of lipid-soluble arsenic in all marine organisms. The chemical form of this dimethylated arsenic still remains to be identified.

The water-soluble inorganic arsenic was almost at the undetectable level in most marine organisms except *Hizikia fusiforme* (Phaeophycea).

We also report, in another paper that, the $LD_{50}$ value of inorganic arsenic against mice is about 50-fold lower than that of methylarsonic [$CH_3AsO(OH)_2$] acid and dimethylarsinic [$(CH_3)_2AsOOH$] acid and about 300-fold lower than that of trimethylarsine oxide.[7] The low content of inorganic arsenic in all marine organisms examined is desirable from the viewpoint of food hygiene.

Fish are in higher trophic levels, and may feed on arsenic-rich seaweeds, gastropods and so on. Despite that, the arsenic content of fish was generally low (under 10 $\mu g\ g^{-1}$). Judging from our work, which showed that arsenic in *H. fusiforme* ingested into the human body was rapidly excreted in urine as the methylated forms,[9] fish are likely to have a metabolic pathway to excrete arsenic rapidly.

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