

Occurrence and Transformation of Arsenic in the Marine Environment

by G. Lunde*

Among the trace elements, arsenic appears to possess a unique position in the environment. When comparing terrestrial and marine organisms there seems to be a significant difference between both level and chemical forms of arsenic. The level of arsenic in terrestrial organisms is seldom above 1 ppm (dry material) whereas the corresponding values for marine organisms vary from several parts per million up to more than 100 ppm.

Furthermore, results so far show that marine organisms are able to convert inorganic arsenic into organic arsenic compounds. No evidence points to a corresponding ability in the terrestrial ones. In the aquatic organisms the arsenic is present as both lipid soluble and water soluble compounds. The water-soluble organic arsenic compounds have a basic character and are very stable to chemical and metabolic breakdown.

The first determination of arsenic in fish and other marine organisms was published around 1900 by Thiergardt (1) and by Gautier and Clausman (2). Since that time there has been a series of investigations in which arsenic has been analyzed, both in plants and animals in the marine environment.

Results from these studies both with regard to fish, shellfish, and other marine organisms show approximately the same levels of arsenic that are also found in more recent investigations. Some of the results are shown in Table 1, starting with some values for water and sediments. For comparison also some results from the terrestrial environment are presented (Table 2). The most pronounced characteristic of these data is the great difference in the level of arsenic in marine and in terrestrial organisms, with much higher levels of arsenic in marine plants and animals (Tables 3 and 4).

Table 1. Arsenic in water and sediments.

Sample	As, ppm	References
Fresh water	0.4×10^{-3}	(2)
Sea water	3×10^{-3}	(3)
Rain water	1×10^{-3}	(4)
Sediment (Pacific Ocean) ^a	12	(4)
Sediment (Atlantic Ocean)	9	(4)
Sediment (North Sea)	10	(4)

^aClay.

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Table 2. Arsenic in terrestrial animals and plants.

Sample	As, ppm	References
Plants	≈0.2	(3)
Animals	≈0.2	(3)
Vegetables and cereal	≈0.4	(5)
Pig and sheep (muscle)	0.1	(6)
Chicken (muscle)	0.4	(7)
Human (muscle)	0.06	(8)
Human (muscle)	0.5	(8)

Table 3. Arsenic in marine fishes (dried material).

Sample	As, ppm	References
Cod	5.0-16.5	(9, 10)
Mackerel	2.0-6.6	(6, 11)
Herring	3.3-7.8	(9, 12)
Halibut	2.5-10.0	(13, 14)
Shark	1.9-5.9+	(15)
Tunny	3.0-7.0	(14)
Plaice	0.78-25+	(16)

+ Calculated on wet material.

Table 4. Arsenic in invertebrates (wet material) and seaweed.

Sample	As, ppm	References
Shrimp	2-40	(17-20)
Lobster	3-26	(17, 21)
Crab	2-80	(15, 17, 21, 22)
Mussels	1.3-70	(17, 21, 23)
Oyster	1.8-40	(17, 21, 23)
<i>Ascophyllum nodosum</i>	22-44	(24)
<i>Laminaria digitata</i>	95-109	(24, 25)

Around 1930, arsenic was shown to be present also in marine oils (Table 5). Among important studies in this period, that of Sadolin (26) should be mentioned. In 1928 he attempted to enrich the arsenic compounds in cod liver oil. The starting material which he used, contained about 3 ppm of arsenic. By various distillation procedures he achieved an extract which contained about 0.1% arsenic.

Chapman (17) showed in 1926 that shrimps and other organisms of this family have a higher arsenic content than most other marine organisms investigated up to that time. He also showed that the arsenic could be extracted from a dried preparation of shrimps with ethanol. It was found from analysis of samples of urine from humans who had eaten shrimps, that detectable arsenic was present mainly as an organic compound which could be degraded to inorganic arsenic only by complete breakdown of the organic material as with a mixture of sulphuric and nitric acid.

The well known studies by Coulson in 1935 should be mentioned (19). He compared arsenic from shrimps with inorganic arsenic in a series of feeding experiments with rats. While as much as 18% of the inorganic arsenic was retained by the rats 3 months after the feeding, only about 0.7% of the arsenic present in shrimps was retained by the rats. The term "shrimp arsenic" was used for this form of arsenic.

Along with the investigations which mainly showed arsenic to be present in marine organisms in concentrations far higher than those found in the terrestrial ones, there was a concern if the arsenic could be methylated in bottom sediments by microorganisms. A model for the transformation of arsenite-arsenate to methyl arsines in aquatic sediments has been proposed by Wood (31). So far, few papers have appeared that could demonstrate the role of the methylarsenic compounds on the level and transformation of arsenic in the marine environment. Some results obtained by Braman and Foreback should, however, be mentioned (32). They describe an analytical method based on atomic absorption, making it possible to determine different forms of arsenic in the sample. The sensitivity achieved is better than 1 ppb. In addition to arsenite and arsenate, methylarsenic acid and dimethylarsenic acid were found in a number of water samples. They found the total level of arsenic in most of the samples to be in the area 1–2 ppb. In a more recent investigation, dimethylarsenic acid is determined also in marine organisms such as fish and shrimps. The level of dimethylarsenic acid is, however, even here on a rather low level of about 0.01 to 0.1 ppm calculated on wet weight and rep-

resents only a small fraction of the arsenic usually formed in these organisms.

Both the concern about possible environmental effects caused by the increased release of arsenic from industrial and other sources as burning of fossil fuel and also new advanced methods of analysis resulted in renewed interest in the arsenic problem starting 10–20 years ago. The object of these investigations was to seek more information about levels in which arsenic occurs, and how it is distributed in the marine trophic levels. Especially the high levels so far demonstrated, pointed to differences in metabolic pathways, in the marine and terrestrial environment. In this connection further characterization of the organic arsenic compounds in the marine organisms was of particular interest.

As mentioned previously, it was established by Sadolin that arsenic could be present as a lipid-soluble organic arsenic compound in marine oils (Table 5). Lunde showed in 1968 (33) that the lipid-

Table 5. Arsenic in marine oils.

Sample	As, ppm	References
Cod liver	3.0–4.5	(26)
Cod liver	1.4–5.1	(27)
Cod liver (Lofoten)	2.2–3.9	(28)
Freshwater fish	1.1–60	(29)
Herring	3.6–14.3	(28)
Mackerel	4.2–7.3	(28)
Molluscs	4.6–46	(30)
Snails	30–46	(30)
Mussel	14–39	(30)
Shellfish	7.3–24	(30)

soluble organic arsenic compounds in the marine oils are present as polar compounds, and that they could be separated from the neutral lipids by chromatographic means. By further fractionation of the polar fraction, at least two different lipid-soluble compounds were detected (Fig. 1).

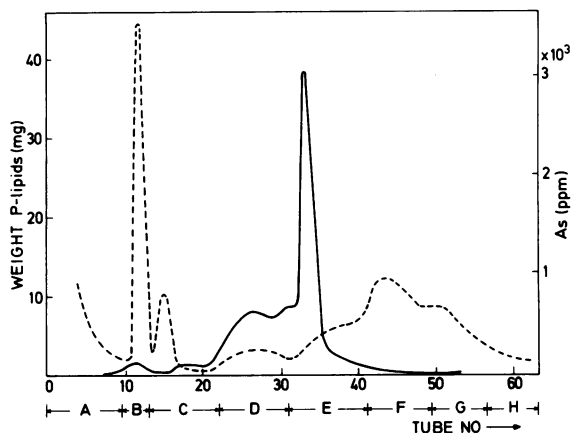


FIGURE 1. Chromatographic separation of phospholipids and arsenic (herring oil): (---) phospholipids; (—), arsenic. Data of Lunde (33).

Vaskovsky et al. (30) in this period studied the lipids prepared from mussels, shrimps, and snails of marine origin. The lipids were fractionated by means of thin layer chromatography and several different lipid-soluble organic arsenic compounds were shown to be present.

Among the results of these studies it should be mentioned that the lipid-soluble organic arsenic compounds did not appear to be bound to cell membranes in the same way as phospholipids (14). In other experiments it was shown that when the marine oil was boiled with acid, for instance with hydrochloric acid, the lipid-soluble organic arsenic compounds were transformed to water-soluble ones (34). This treatment, however, did not transform the arsenic further into an inorganic form.

The results obtained so far, show the arsenic to be present in the form of several lipid-soluble organic compounds in marine oils, and that the amounts present may vary within wide limits from about 1 ppm to about 50 ppm and in some cases even higher. The results available to about 1960 did not permit any further conclusions to be drawn with regard to the chemical properties of the water-soluble (or ethanol-soluble) organic arsenic compounds that had been previously detected. As the lipid-soluble organic arsenic compounds roughly make up 10–30% of the total, there was still 70–90% of the arsenic left, of which very little was known.

Indication that arsenic in aqueous extracts from fish was not present as inorganic arsenic or as a derivative of an arsenic acid came when it was shown that arsenic in the aqueous extract after boiling fish (the so-called glue water) was not absorbed to an anion exchange resin (36). Practically all of the arsenic present in the glue water is recovered in the eluate after such elution (36). Later investigations have shown that the organic arsenic compounds are absorbed to a cation resin, and that it is eluted only when pH is increased to about 10 (35, 37). The water-soluble organic arsenic compound must therefore be characterized as a base. When the glue water from fish is fractionated by means of gel filtration, i.e., divided into fractions with decreasing molecular weights, these arsenic compounds are eluted before the amino acids (23). This indicates that these compounds have a molecular weight that is somewhat higher than that of the amino acids, or somewhat more than 300–400. An example of such fractionation is given in Figure 2. At this time it was not clear if the arsenic was present as one or more compounds. Later results show that there is one main arsenic compound present in the aqueous phase. It is interesting to note that arsenic is not detected in the fractions in which the high molecular weight material is eluted, and con-

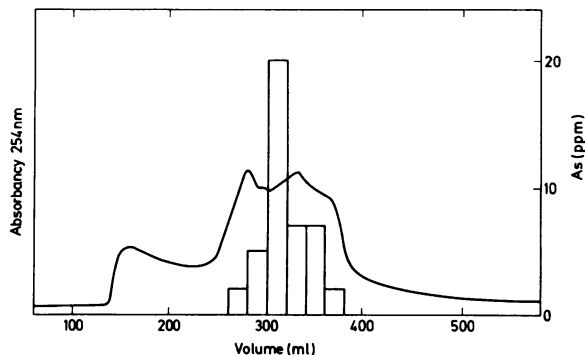


FIGURE 2. Molecular gel filtration of glue water (cod): (—) absorption at 254 nm; (□) concentration of arsenic. Data of Lunde (23).

sequently no part of the arsenic is bound to proteins or high-molecular weight peptides. If the arsenic compounds originally are bound to proteins, the binding is disrupted during this type of treatment.

An important question in these studies of arsenic in the marine environment is whether the organic arsenic compound observed in glue water from fish, is fishmeal, fish liver, and other organs is the same compound, and if this compound is identical to the compounds that are found in shellfish and in other marine organisms. In a recent study the organic arsenic compounds in different marine organisms was compared. Water-soluble extracts were prepared from different marine samples as well from aqueous extracts of marine oils boiled with hydrochloric acid. Following an enrichment procedure, the various samples were compared in four different thin-layer chromatographic systems (34). The results show that the arsenic compound present in fresh and boiled fish, in glue water from fish, in fish liver, and in various extracts from shrimp, lobster, and squid to have identical R_f values in all four TLC systems. The organic arsenic compounds in seaweed oil as well as in fish oil after treatment with hydrochloric acid appear also to be identical, but different from the first group (Fig. 3). It is also interesting to note that when treating the lipid-soluble arsenic compounds with hydrochloric acid, only one single water-soluble organic arsenic compound is formed, while the oils always contain more than one arsenolipid.

It was shown by Chapman as early as in 1926 that in order to analyze the arsenic present in the urine from people eating shrimp, the sample had to be digested with sulfuric and nitric acids (17). These results have recently been confirmed (38). A continuation of these studies showed that it was possible to fractionate the arsenic in marine raw materials into one part that could be distilled off with constant-boiling 6.6N hydrochloric acid and one part that was not volatilized under these conditions

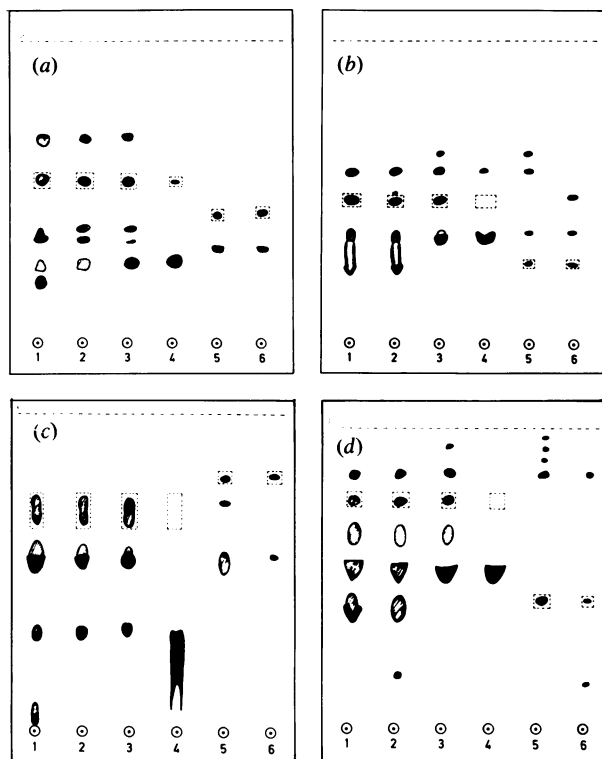


FIGURE 3. Thin-layer chromatograms of extracts enriched in arsenic and developed with ninhydrin reagent: in four different systems: (a) developing solvent, *n*-butanol–acetone–formic acid–water, 10:10:2:5; (b) developing solvent, *n*-butanol–acetone–ammonia–water, 10:10:2:5; (c) developing solvent methanol–pyridine–water, 20:1:5; (d) developing solvent, chloroform–methanol–ammonia, 2:2:1. Extracts used: (1) shrimp, N liquor; (2) shrimp, enzyme hydrolyzed meal; (3) cod liver, enzyme hydrolyzed meal; (4) haddock, N liquor; (5) seaweed, HCl-treated oil; (6) fish oil, HCl-treated oil (□) denotes arsenic. Data of Lunde (34).

(20) (Table 6). The results show that only a few parts per million of the total amount of arsenic in marine material is present or may be converted to inorganic arsenic. The major part of the arsenic is not destroyed by the treatment with hydrochloric acid described here. It should, however, be noted that Crezelius (39) has shown that when treating samples containing the organic arsenic compound with a base, for instance sodium hydroxide, the compound is converted to dimethylarsenic acid (cacodylic acid).

Table 6. Inorganic and organic bound arsenic in marine samples.

Sample	Locality	As, ppm dried material	
		Inorganic	Organic
Prawn	Oslofjord	1.8	36.5
Mussel	Western Norway	1.5	12.4
Mackerel	Southern Norway	1.1	8.9
Haddock	Western Norway	0.9	12.0
Cod	Western Norway	1.3	23.0
Tunny	Western Norway	1.2	8.4
Coalfish	Western Norway	1.0	7.8
Herring	Western Norway	1.0	5.2

By means of an isolation procedure (35), the main water-soluble organic arsenic compound has been enriched almost to purity, using techniques based on extraction, ion exchange, molecular gel filtration, and thin-layer chromatography. A few experiments have been carried out towards a further characterization of the compound. Thus the compound has an ultraviolet absorption at 260 nm, has infrared absorption indicating amino and hydroxyl functions and also hydroxyl groups of a phenolic aromatic character. Trimethylsilyl derivatives may be prepared by derivatization with bistrimethylsilyl trifluoroacetamide (35). The compound gives also a positive reaction (blue color) with ninhydrin reagents, indicating the presence of amines. This reaction is, however, not observed when an Al_2O_3 column is used for the fractionation and the enrichment of the organic arsenic compound (40).

Only a few studies on how the arsenic is taken up in marine organisms and how it is transferred through the different trophic levels have been reported. Some investigations concerning the assimilation of arsenic in algae and in fish should, however, be mentioned. In one study the algae were

cultured with addition of radioactive inorganic arsenic in the medium. It was shown that all the algae tested (strains of green algae, diatoms, and blue-green algae) were able to synthesize both lipid- and water-soluble organic arsenic compounds. Furthermore, the degree of enrichment was found for both the water-soluble and the lipid-soluble organic arsenic compounds to be between 200 and 3000 as compared to the level of arsenic in the growth medium (41).

Feeding experiments of fish have also been carried out by Penrose (37) and Lunde (42). In the first case, radioactive arsenic was administered orally and by intramuscular injection; in the second, the radioactive arsenic was mixed into the fish feed. In both studies a transformation of inorganic arsenic to the organic form was demonstrated. The yield of the organic form of arsenic differed however. When giving the arsenic mixed into the feed, the yield was rather low, indicating that inorganic arsenic only to a small degree is responsible for the rather high levels of arsenic found in fish. This was shown indirectly also by increasing the level of organic bound arsenic in the feed in one group of fish and reducing it in another. This resulted in a distinct increase respectively reduction, of the content of arsenic in the fish. These results are shown in Figure 4. A higher yield of organic arsenic was observed in the other experiments where arsenic was given orally or intramuscularly.

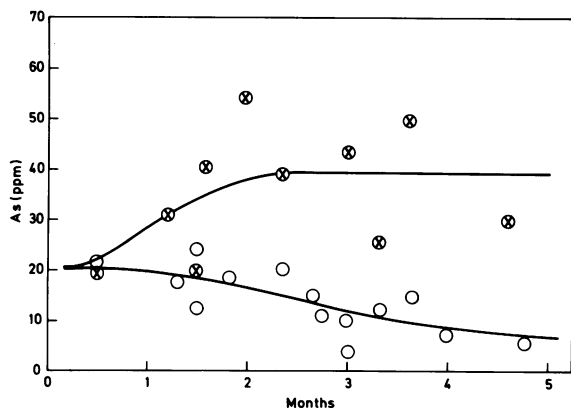


FIGURE 4. Arsenic in glue water produced from fish fed on (o) marine and (x) terrestrial feed. Data of Lunde (42).

Further studies showed that the organic arsenic compounds formed in the fish quite evidently concentrated in those organs of the fish that are most exposed to attack from microorganisms. This may point to a function of the organic arsenic compounds as a protection against attack by microorganisms (Fig. 5).

In some of the feeding experiments with fish, radioactive inorganic arsenic was added to the water in which the fish was kept. In this case it was not possible to show any direct synthesis of organic arsenic compounds by the methods used here. Also the amount of inorganic arsenic absorbed appeared to be rather low.

In conclusion the following should be stated. Based on the results obtained so far, arsenic is transformed in marine organisms, probably mainly in the lower trophic levels to both lipid-soluble and water-soluble organic arsenic compounds. The main compound among the water-soluble ones appears to be rather stable. No evidence points to any significant contribution by the methylarsenic compounds in the marine food web.

Among the most important problems to be solved in the future is: (1) elucidation of the structure of the water-soluble organic arsenic compound; (2) clarification of the physiological significance of the organic arsenic compounds in the marine organisms; (3) determination of the biological effect of the organic arsenic compound on terrestrial animals including man.



FIGURE 5. Autoradiograph of a section cut perpendicular on the back bone and through the eyes of a fish fed radioactive arsenic. The fish was sacrificed 12 days after the termination of adding radioactive arsenic to the feed. Data of Lunde (42).

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