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Seafood arsenic: Implications for human risk assessment

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Abstract

Concerns about the adverse effects of chronic arsenic exposure have focused on contaminated drinking water and airborne workplace exposures; the risks of naturally occurring arsenic in foods have received less attention. About 90% of the arsenic in US diets comes from seafood, of which only a small proportion occurs in inorganic forms; the great majority consists of complex organic compounds that generally have been regarded as non-toxic. However, recent studies of seafood have documented formation of metabolites carcinogenic in some rodents. To calculate the risks of ingested seafood arsenic, therefore, it is necessary to identify the nature and quantity of arsenic species present and the metabolites formed by expected metabolic activities. We review the nature and quantities of the various arsenical compounds found in dietary seafood and discuss their metabolic processing and fate. Based on conservative dose estimates and the likelihood that arsenic's carcinogenic mechanisms follow sub-linear dose–response curves, we estimate a margin of exposure of at least 10^3–10^4 between carcinogenic doses used in rodent studies and those expected after human consumption of large quantities of seafood.

Keywords: Arsenic; Organic arsenic; Seafood arsenic; Seafood; Arsenosugar; Risk assessment

1. Introduction

The adverse effects of chronic arsenic exposure in humans have been the subject of long standing concerns. Historically such concerns focused primarily on environmental exposures from contaminated drinking water and airborne workplace exposures (WHO, 1981; American Conference of Governmental Industrial Hygienists, 2002; Mead, 2005). Recent regulatory efforts have mainly addressed those two important exposure routes (National Research Council, 2001; US Environmental Protection Agency, 2001; American Conference of Governmental Industrial Hygienists, 2002; IARC Working Group, 2004). By contrast, the toxicological significance of arsenic occurring naturally in foods has received less attention, but has generally been assumed to be insignificant. Researchers have only recently documented the individual levels of various organic and inorganic arsenical compounds, rather than just total arsenic, in different food groups (Yost et al., 1998; Schoof et al., 1999a,b) and in daily diets by means of duplicate portion sampling (Robberecht et al., 2002). These studies indicate that arsenic is found routinely in most diets. For example, based on the Food and Drug Administration's 1982–1997 Total Diet Study (Adams et al., 1994; National Research Council, 1999), the estimated average daily dietary intake of arsenic by US adults is 25–75 μg/day.

The largest quantity of dietary arsenic, about 90% of the arsenic in US diets, comes from saltwater finfish and seafood (Adams et al., 1994). Only a small proportion of seafood-derived arsenic occurs in inorganic forms; the great majority of seafood arsenic consists of complex organic arsenical compounds. Thus, consumption of fish and seafood provides a relatively small share of dietary inorganic arsenic. By contrast, the most important dietary sources of inorganic arsenic include uncooked rice, grains and flour (Schoof et al., 1999a,b). In addition, significant amounts of inorganic arsenic are absorbed when rice, grains and other vegetables are cooked in arsenic-contaminated water.

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Accordingly, dietary arsenic can be categorized by arsenic type (e.g., organic vs. inorganic) and by source (e.g., seafood vs. rice and grains).

Despite high levels of total arsenic found in seafood, seafood ingestion has not been linked to arsenic toxicity in humans or other mammals (Edmonds and Francesconi, 1993). Such apparent inconsistency has led to considerable research aimed at understanding the biochemistry and metabolism of the arsenic compounds found in marine species. In pursuing those goals, some researchers have characterized the arsenical compounds found in marine species (Cullen and Reimer, 1989; Kaise and Fukui, 1992; Yamauchi et al., 1992; Ballin et al., 1994; Francesconi and Edmonds, 1998), while others have studied the forms and relative quantities of arsenical compounds excreted by seafood eaters in order to understand the metabolic consequences of seafood ingestion (Yamauchi et al., 1992; Le et al., 1994; Buchet et al., 1996; Heinrich-Ramm et al., 2001, 2002).

2. The origin of seafood arsenic

Arsenic is ubiquitous in open ocean seawater, where typical levels are 1–2 μg As/L (Francesconi and Edmonds, 1998; WHO, 2001). Levels of arsenic are most constant in deep ocean waters, while levels in surface waters show seasonal variation (Cullen and Reimer, 1989). It is found mostly as inorganic arsenate (As[V]), particularly in deeper waters. Reduction and methylation by microorganisms occur in the more superficial photic zone, the ocean layer into which sufficient sunlight penetrates to support photosynthesis; levels of methylation correlate with photosynthetic activity (Andreae, 1979, 2005). Accordingly, in addition to As[V], surface waters contain small amounts of inorganic arsenite (As[III]), methyl arsonate (MA) and dimethyl arsinate (DMA). Deep-sea sediments may contain high concentrations of arsenic (Francesconi and Edmonds, 1994; Andreae, 2005), but such sediment-bound arsenic is "generally regarded as unavailable" to marine organisms (Francesconi and Edmonds, 1998).

Very little inorganic arsenic is taken up from sea water by most marine animals. To the contrary, marine animals accumulate arsenic primarily from their food, an accumulative process that reflects the sequence of metabolic transformations occurring as arsenic passes up the food chain (Sanders et al., 1989; Francesconi and Edmonds, 1994). That metabolic sequence, beginning with inorganic arsenate, leads to the accumulation by higher animal species of complex methylated compounds that they cannot directly synthesize from inorganic arsenic (Cullen and Reimer, 1989; Francesconi and Edmonds, 1994, 1998).

The sequence starts with phytoplankton (freely floating microscopic plants or algae) which readily take up arsenate from sea water via trans-membrane transport systems normally dedicated to the uptake of essential phosphate anions (Francesconi and Edmonds, 1998; Meharg and Hartley-Whitaker, 2002; Ullrich-Eberius et al., 2005). Following uptake, algae rapidly detoxify arsenate by reduction and methylation, resulting in the formation of arsenic-containing sugars as well as minor amounts of DMA and other methylated arsenical compounds (Phillips, 1990; Tamaki and Frankenberger, 1992). Levels of arsenic are generally about 1000- to 10,000-fold greater in algae than in seawater, with levels differing across specific algal strains.

In marine animals, the predominant form of arsenic is arsenobetaine, a tri-methylated pentavalent (As[V]) compound first identified in 1977 (Edmonds et al., 1997). It is now recognized as nearly ubiquitous in the marine environment, particularly in finfish and seafood consumed by humans (Edmonds and Francesconi, 1988; Hanaoka et al., 1988; Cullen and Reimer, 1989) and accounts for virtually all of the water-soluble arsenic in such animals. There is sufficient evidence that higher marine animals do not synthesize arsenobetaine from arsenate, but the full details of its synthesis remain uncertain (Francesconi and Edmonds, 1994). It is likely that arsenosugars, released into ocean waters and sediment by the death and decay of algae, are transformed by microbial species to yield arsenobetaine or its precursors which are then ingested by marine animals (Edmonds and Francesconi, 1988; Francesconi and Edmonds, 1998; McSheehy et al., 2002). Experimental findings support the view that microbial species in seawater can transform MA and DMA, but not arsenate, into arsenobetaine (Cullen and Nelson, 1993; Cullen and Pergantis, 1993). It has also been proposed that some marine animals transform small amounts of arsenosugars into arsenobetaine (Edmonds and Francesconi, 1988; McSheehy et al., 2002).

In addition to arsenobetaine, other organic and inorganic arsenic compounds have been found in fish and marine animals that are common components of the human diet. The proportion of inorganic arsenic in those foods is generally low, less than 1–4% of total arsenic (Edmonds and Francesconi, 1993; National Research Council, 1999). Other organic arsenicals found in seafood include simple methylated compounds, particularly MA, DMA and trimethyl arsine oxide, and more complex organic compounds such as arsenocholine and arsenosugars. Their presence probably results from ingestion of algae containing such compounds as a consequence of algal transformation of arsenate. Tri-methylated arsenicals (e.g., trimethyl arsine oxide) are also formed via methylation of ingested arsenate by the microbial intestinal flora of fish and by post-mortem bacterial breakdown of arsenobetaine (Norin et al., 1985; Edmonds and Francesconi, 1988; Hanaoka et al., 1988; Hanaoka et al., 1992; Francesconi and Edmonds, 1994; Hanaoka et al., 1995). Arsenocholine, a key precursor of arsenobetaine (Christakopoulos et al., 1988), has only rarely been found at greater than trace levels in aquatic animals (Cullen and Reimer, 1989; Edmonds and Francesconi, 1993; Francesconi and Edmonds, 1994), suggesting that it exists mainly as a metabolic intermediary.

Arsenical compounds are not uniformly distributed throughout the tissues of marine animals. To the contrary, tissue levels of total arsenic and specific types of arsenic

(e.g., organic vs. inorganic compounds; MA vs. DMA) are variable. In finfish, for example, inorganic compounds and DMA were found almost exclusively in the viscera (stomach; intestines; liver; heart; gills), while the arsenic content of muscle was nearly all arsenobetaine (Kirby and Maher, 2002). Differences in tissue distribution between muscle and viscera have been documented in a variety of fish, crustaceans and carnivorous gastropods (Kaise et al., 1988; Edmonds and Francesconi, 1993). These findings indicate that the total arsenic content and the types of arsenic compounds in seafood consumed by humans will differ according to which particular tissues are consumed. As discussed below, how the seafood is prepared and cooked can also affect the types of arsenic compounds consumed.

3. Nature and metabolism of seafood arsenic

Following ingestion and uptake, inorganic arsenic is transformed by a series of well-described metabolic steps involving sequential reduction of pentavalent arsenic species to their corresponding trivalent forms followed by oxidative addition of a methyl group. The monomethyl, dimethyl, and trimethyl compounds thus formed are generally less acutely reactive with tissue components and more readily excreted in urine than are the original inorganic species (Buchet et al., 1981; Marafante et al., 1987; Hughes and Kenyon, 1998). For such reasons, the metabolic sequence was historically viewed as a detoxification mechanism (Vahter, 1999, 2002).

That view, however, now seems overly simplistic. Some methylated arsenic compounds may be retained, at least in the erythrocytes of rats (Vahter et al., 1984), and some show characteristic toxicity patterns. In addition, the sequential metabolism of arsenic yields reactive intermediates, including oxygen free-radicals, that can initiate cascades of toxic effects (Nesnow et al., 2002; Aposhian et al., 2003). To calculate the risks of ingested seafood arsenic, therefore, it is necessary to identify the nature and quantity of arsenic species present in those foods and the metabolites formed as a consequence of expected metabolic activities (National Research Council, 1999). As described below, some studies have determined the types of arsenic compounds found in various categories of seafood (e.g., finfish, crustaceans, mollusks, and sea weed) while others have categorized the arsenic species excreted in the urine following consumption of such seafood.

3.1. Arsenic compounds found in seafood

The arsenic compounds most often found in seafood or excreted by seafood eaters are discussed in the following section. It should be noted that the data cited below were derived by a variety of analytical methods used in numerous laboratories over a period of decades; caution must be used when comparing such results across studies.

3.1.1. Inorganic arsenic (Fig. 1)

Inorganic arsenic, found mainly as arsenate (As[V]) and to a much lesser extent as arsenite (As[III]), is the predominant form of arsenic in seawater (Cullen and Reimer, 1989; Tamaki and Frankenberger, 1992; Francesconi and Edmonds, 1998), but inorganic compounds comprise only a small proportion of total seafood arsenic. An analysis of five types of ocean finfish and also shrimp found that inorganic arsenic was less than 0.1% of total arsenic in all samples (Schoof et al., 1999a,b). Others have reported that inorganic arsenic is rarely more than 3% of the total arsenic in fish and crustaceans (Donohue and Abernathy, 1999) and more recent surveys report levels of less than 1% (Borum and Abernathy, 1994; Shiomi, 1994; Le et al., 1999; Sloth et al., 2005). For example, a review of data from 96 finfish and shellfish samples found that only six samples contained inorganic arsenic at levels greater than 3–4% of total arsenic levels (Donohue and Abernathy, 1999). Statistical reanalysis of data from 10 primary studies found that the proportion of inorganic arsenic was about 1% of total arsenic at "very low total arsenic concentrations", and about 0.5% at total arsenic levels $\geqslant 20$ mg/kg (Edmonds and Francesconi, 1993).

3.1.2. MA and DMA (Fig. 1)

Generally little or no MA or DMA is found in seafood; detectable levels have been reported in mainly fatty types of fish. For example, "trace" levels of MA were detected by means of high-performance liquid chromatography

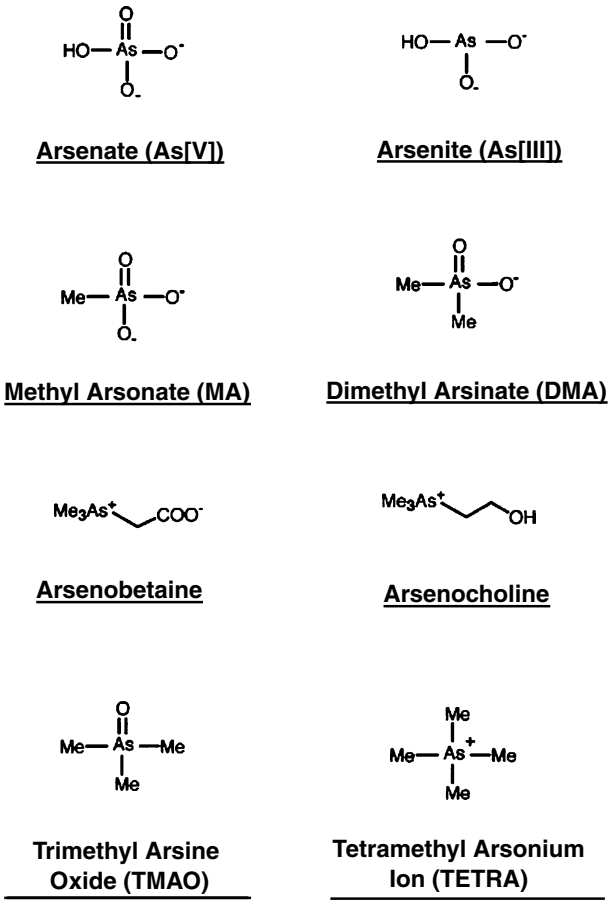

Fig. 1. Some arsenic compounds found in seafood.

coupled to atomic absorption spectrometry (HPLC-AAS) in mackerel and herring, but not in tuna (Arbouine and Wilson, 1992). It has also not been found in crab (Arbouine and Wilson, 1992). MA was not detectable in a more recent HPLC-AAS analysis of white herring (Heinrich-Ramm et al., 2002). DMA has been detected at low levels (i.e., μg As/kg) in mackerel and herring by means of HPLC with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and in prawns by means of arsine-generator flameless AAS, but it was not detectable in cod, dab, haddock, sole, plaice, tuna or whiting analyzed by HPLC-ICP-MS or HPLC-AAS (Yamauchi and Yamamura, 1984; Arbouine and Wilson, 1992; Branch et al., 1994; Heinrich-Ramm et al., 2002). When detectable, tissue levels of DMA were "toxicologically insignificant" (i.e., <0.5 mg As/kg dry weight) (Branch et al., 1994).

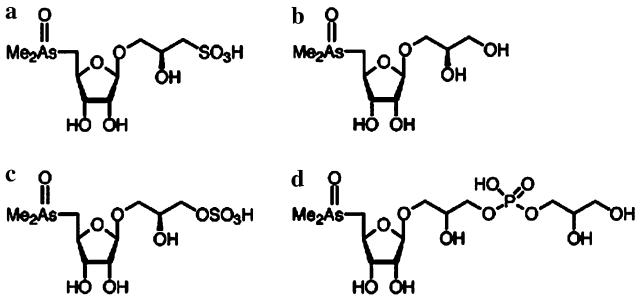

Fig. 2. Molecular structures of four principal arsenosugars. These sugars have been identified by variable nomenclature. Francesconi and Edmonds (1998) identify them as: (a) arsenosugar 1; (b) arsenosugar 2; (c) arsenosugar 3; (d) arsenosugar 4. By contrast, Le et al. (1999) and the National Research Council (1999) refer to them as: (a) arsenosugar XII; (b) arsenosugar X; (c) arsenosugar XIII; (d) arsenosugar XI.

3.1.3. Arsenobetaine (Fig. 1)

In contrast to the small amounts of inorganic arsenic in most finfish and shellfish, arsenobetaine represents the vast majority of total arsenic in marine animals (Edmonds and Francesconi, 1988; Hanaoka et al., 1988; Ballin et al., 1994; Francesconi and Edmonds, 1998). It has been proposed that arsenobetaine represents an "end point of the arsenic cycle in the marine ecosystem" (Cullen and Reimer, 1989) because it is largely inert, non-toxic and rapidly excreted. Sedimentary microorganisms can degrade arsenobetaine, but it is generally not transformed in humans and other mammals, in whom it is excreted essentially unchanged (Tam et al., 1982; Cullen and Reimer, 1989; Brown et al., 1990). With respect to potential toxicity, arsenobetaine was not mutagenic in *in vitro* tests (Jongen et al., 1985), was not cytotoxic and had no transforming activity in mammalian cells (Sabbioni et al., 1991; Sakurai and Fujiwara, 2001) and was not immunotoxic (Sakurai et al., 2004). It was also not embryotoxic in rats (Irvin and Irgolic, 1988). Rare clastogenic effects were noted in cultured human fibroblasts, but only at levels sufficiently high to significantly alter the osmotic pressure of the culture medium (Oya-Ohta et al., 1996). The acute oral LD_{50} of arsenobetaine in mice was greater than 10 g As/kg (Kaise et al., 1985).

3.1.4. Arsenocholine (Fig. 1)

Arsenocholine is a metabolic precursor of arsenobetaine in marine animals (Marafante et al., 1984; Christakopoulos et al., 1988; Francesconi and Edmonds, 1994). Following administration of labeled arsenocholine, it is rapidly absorbed and transformed to arsenobetaine with little or no degradation to inorganic arsenic, MA or DMA (Marafante et al., 1984; Kaise et al., 1992). Early reports described significant levels in shrimp (Norin et al., 1983; Lawrence et al., 1986), but those findings have been questioned and have not been confirmed; arsenocholine has otherwise been reported at only "trivial" levels (Cullen and Reimer, 1989; Edmonds and Francesconi, 1993; Francesconi and Edmonds, 1994). Although studies of arsenocholine toxicity are limited, it is regarded as "essentially non-toxic" (Agency for Toxic Substances and Disease Registry, 2000; Sakurai, 2002). It was not embryotoxic in rats (Irvin and Irgolic, 1988) and, like arsenobetaine, it caused rare clastogenic effects but only at very high levels (Oya-Ohta et al., 1996). The acute oral LD_{50} in mice was 6.5 g As/kg, while the acute intravenous LD_{50} was 187 mg As/kg (Kaise et al., 1992).

3.1.5. Arsenosugars (Fig. 2)

At least 15 different arsenosugars have been identified in marine algae, virtually all containing a pentavalent (As[V]) arsenic bound to two methyl groups (Edmonds and Francesconi, 1993). Of these, there are four principal arsenosugars (Fig. 2): the specific patterns with which those sugars are found in particular algal strains differ in relatively characteristic ways (Phillips, 1990; Francesconi and Edmonds, 1994, 1998; Le et al., 1999). Their principal dietary source is seaweed, a common component of the Japanese diet (Yamauchi et al., 1992). Other sources include herbivorous marine mollusks such as oysters, mussels, and clams (Le et al., 1999). There have been only limited studies of arsenosugar toxicity. In mammalian cells, a synthesized arsenosugar was not cytotoxic at micromolar levels; paradoxically, enhanced viability was seen in some cell types at much higher levels (Sakurai et al., 1997). A more recent study compared the *in vitro* toxicity of two synthetic arsenosugars, one trivalent and the other pentavalent (Andrewes et al., 2004). The trivalent sugar was positive for cytotoxicity and DNA nicking at concentrations of about 500–600 μM, but inactive for Salmonella mutagenicity. The pentavalent sugar was not active in any of those tests.

3.1.6. Trimethyl arsine oxide (Fig. 1)

Trimethyl arsine oxide (TMAO) has been identified in some species of marine animals as a minor arsenic species, rarely detected except at "trace" levels (Cullen and Reimer, 1989; Francesconi and Edmonds, 1994, 1998; ICES Marine Habitat Committee, 2004). Levels are higher in stored, frozen fish than in fresh fish (Norin et al., 1985; Cullen and Reimer, 1989; Hanaoka et al., 1992), probably secondary to

post-mortem breakdown, but dietary intake of TMAO is probably "extremely small" (Yamauchi and Fowler, 1994). It is "virtually non-toxic" (Shiomi, 1994), with an acute oral LD_{50} in mice of 10.6 g As/kg (Kaise et al., 1989). In *in vitro* studies, TMAO caused no cell growth inhibition, did not induce sister chromatid exchange, and caused only rare clastogenic effects at concentrations up to 10 mg/cm^3 ($\sim 10^{-2}$ M) (Oya-Ohta et al., 1996; Kaise et al., 1998).

3.1.7. Tetramethyl arsonium ion (Fig. 1)

The tetramethyl arsonium ion (TETRA) is generally a minor arsenic species in finfish, but may be a major species in some mollusks (Cullen and Reimer, 1989; Francesconi and Edmonds, 1994, 1998; ICES Marine Habitat Committee, 2004). It is below detection limits in most finfish, but TETRA was reported up to 0.17 μg/g dry weight in anchovies (Suner et al., 2002), from 0.05–1.0 μg/g dry weight in some crustaceans (Suner et al., 2002), and levels ranging from 0.2 to 16 μg/g were found in various organs of some clams (Shiomi et al., 1987; Francesconi and Edmonds, 1994). Levels of TETRA may be enhanced by freezing or dry cooking (grilling, roasting, and baking) at temperatures >160 °C, especially in charred meat, likely due to the thermal decarboxylation of arsenobetaine (Hanaoka et al., 2001; Devesa et al., 2005b). As a result, levels of TETRA >1.0 μg/g dry weight have been reported in cooked fish in which TETRA was not detectable prior to cooking.

The halide salts of TETRA have "considerable" acute toxicity; in mice, the acute oral LD_{50} of TMA-chloride was 0.89 g As/kg (Shiomi, 1994). However, such toxicity may be a property of the halogen anion, not TETRA itself; studies of synthetic TMA-hydroxide reported essentially no toxicity (Sakurai, 2002). Because of the limited amount of TETRA in consumed fish, acute poisoning by TETRA is unlikely: the highest reported levels of TETRA in grilled or roasted fish were 0.571 μg/g wet basis and 1.79 μg/g dry basis (Devesa et al., 2005a,b). In *in vitro* studies, TETRA inhibited cell growth, but only at high concentrations (IC_{50} of 8 mg/cm^3) (Kaise et al., 1998). It did not induce sister chromatid exchange, and caused only rare clastogenic effects at concentrations up to 10 mg/cm^3 ($\sim 10^{-2}$ M) (Oya-Ohta et al., 1996; Kaise et al., 1998).

3.2. Arsenic excretion after seafood meals

The metabolic fate of ingested seafood arsenic has been studied following consumption of various seafood meals. Buchet et al. (1996) studied urinary arsenic in 137 Italian soldiers categorized according to consumption patterns (regular vs. intermittent) of (a) finfish plus shellfish; (b) finfish but no shellfish; or, (c) seafood abstainers. "Regular" seafood consumers had significantly greater total urinary arsenic levels than did intermittent consumers and abstainers. Inorganic arsenic, MA and DMA levels were statistically increased only in regular consumers of fish plus shellfish, but that increase (1 μg As/g creatinine) was deemed "negligible" and "biologically not significant" (Buchet et al., 1996). Arsenic excretion was also evaluated in nine Belgian adults and in an unspecified number of laboratory staff. After consuming finfish (cod, sole or whiting), urinary levels of inorganic arsenic and its metabolites were "of the same order of magnitude" as in those who ate no seafood. By contrast, DMA levels were nearly 20-fold greater after consumption of an unspecified quantity of mussels.

Heinrich-Ramm et al. (2002) studied 13 German adults who abstained from seafood for 7 days and then consumed one finfish meal: "type and mass… chosen by each person independently". Another group of eight adults avoided seafood for seven days and then ate a standardized meal of white herring. In both groups, urine levels of DMA increased significantly over the following 48 h, but no increases were seen for inorganic arsenic or MA. DMA levels reflected the types of fish consumed. Herring was associated with substantially higher DMA levels (50–78 μg As/g creatinine), while ingestion of salmon, cod, perch or pollock resulted in lower DMA levels (9–25 μg As/g creatinine).

In a study of eight Dutch adults who ate 100 g of plaice, arsenobetaine was the only detectable arsenical in urine (Luten et al., 1982). It is possible, however, that the analytical methods in this early study were not adequately sensitive to detect expected background levels of other arsenic species. Le et al. (1994) found that arsenobetaine was the "major" urinary arsenical after eating crabmeat or shrimp; specific levels of inorganic arsenic, MA and DMA were not described. Hsueh et al. (2002) reported that dietary seafood intake was not significantly correlated with excretion of inorganic arsenic, MA or DMA in 78 Taiwanese adults evaluated before and after avoiding seafood for 3 days. In this study, however, the types and quantities of seafood consumed were not well characterized. Moreover, the subjects drank arsenic-containing tap water and had relatively high baseline arsenic excretion levels.[1] Hence the contribution specifically due to seafood may have been concealed by the elevated background levels.

In contrast to the above reports, which indicate little or no metabolism of arsenobetaine and other arsenic compounds in finfish and crustaceans, complex metabolism of arsenosugars has been documented after consumption of arsenosugar-rich seaweed and mollusks (Le et al., 1994, 1999; Ma and Le, 1998; Wei et al., 2003) and after ingestion of synthetic arsenosugars (Francesconi et al., 2002). In those studies, the major excreted arsenic compound was DMA. Wei et al. reported peak DMA urine levels of about 100 μg As/L following ingestion of 15 g of seaweed (containing about 3 mg of extractable arsenic) (Wei et al., 2003), Le et al. reported peak DMA urine levels of 90 μg As/L following consumption of 250 g of mussels (Le et al., 1999), and Franesconi et al. reported peak DMA urine levels of

[1] Throughout the paper, the authors reported that urine levels of total inorganic arsenic metabolites were in the range 50–70 mg/g creatinine (Hsueh et al., 2002). We assume that this reflects an editing error and that the units should have been reported as "μg/g creatinine".

675 μg As/L after ingestion of a synthetic arsenosugar containing 1220 μg of arsenic (Francesconi et al., 2002). Because seaweed does not contain significant amounts of DMA, such urinary findings provide strong evidence of arsenosugar metabolism. Moreover, the specific ingested arsenosugars were not found in the urine, but trace quantities (<30 ng/ml) of at least 12 other, mainly uncharacterized arsenicals have been noted in the urine of seaweed consumers (Le et al., 1999; Francesconi et al., 2002). Failure to characterize these metabolites has been mainly due to the very small quantities excreted and available for analysis. Franesconi et al. also detected small amounts of TMAO, equivalent to about ~0.5% of total excreted arsenic (Francesconi et al., 2002). Because TMAO is of generally low toxicity and has not been otherwise detected in the urine of seafood eaters, the toxicological significance of that finding is uncertain.

There is also evidence that metabolism of arsenosugars varies from person to person. Le et al. (1994) observed significantly different patterns and quantities of arsenic metabolites in the urines of nine subjects after eating the same quantity of an arsenic-rich seaweed. For example, striking differences were seen among four members of a single family who normally shared a common diet. Such findings emphasize that arsenosugar metabolism is complex and likely to be influenced by heretofore unknown genetic or environmental factors.

More recent attention has focused on the speciation and oxidation state of methylated arsenic compounds excreted in the urine of seafood eaters (Francesconi et al., 2002; Hansen et al., 2003) and those with non-seafood diets (Del Razo et al., 2001; Mandal et al., 2004). Trivalent methylated arsenic compounds have greater *in vitro* toxicity than do the corresponding pentavalent compounds (Styblo et al., 2000) and evidence suggests that the trivalent species, but not the corresponding pentavalent forms may be directly genotoxic (Mass et al., 2001). To date, however, trivalent DMA (DMA[III]) has not been documented in the urine of seafood eaters. That failure, however, may reflect inappropriate analytical protocols (Hansen et al., 2004; Francesconi and Kuehnelt, 2004) or the unstable nature of DMA[III] (Gong et al., 2001; Francesconi et al., 2002, 2004); even when frozen at −20 °C, DMA[III] in urine was completely oxidized within 17 h.

4. Implications for risk assessment

Current risk assessments for chronic exposure to arsenic (e.g., (Agency for Toxic Substances and Disease Registry, 2000; US Environmental Protection Agency, 2005)) are based principally on studies of Taiwanese exposed to high inorganic arsenic levels in drinking water drawn from artesian wells (Tseng et al., 1968, 1977). The arsenic content of those wells ranged up to 1.82 ppm; 70% of wells had levels ⩾400 ppb (Tseng, 1977). For reasons of both mode of action and exposure dose, the relevance of those risk assessments to seafood arsenic consumption is uncertain and perhaps irrelevant. The general absence of arsenic toxicity reported in humans and other mammals after consumption of large amounts of seafood (Edmonds and Francesconi, 1993) and seaweed (Andrewes et al., 2004) lends weight-of-evidence support to its lack of acute toxicity. However, because the ingestion of seafood may lead to the generation of metabolites involved in arsenic-induced carcinogenesis, it is worthwhile to consider the potential role of dietary seafood in long term cancer risk.

Various modes of action have been proposed to explain arsenic carcinogenicity including: oxidative stress; disturbed DNA methylation; altered DNA repair; chromosomal damage; alterations of gene transcription; modulation of signal transduction; and, proliferative cell regeneration (Oya-Ohta et al., 1996; National Research Council, 1999; Kitchin, 2001; Schoen et al., 2004). Although the actual mode of action has not been established, evidence suggests that most plausible mechanisms are not directly genotoxic (DNA-reactive) and would be expected to have low-dose non-linear (i.e., sub-linear) dose–response curves (National Research Council, 1999; Nesnow et al., 2002; Schoen et al., 2004; Cohen et al., 2006). By implication, one might expect that arsenic-associated cancers would be especially dose-sensitive such that high-doses effects might not be readily extrapolated to low-dose settings. Some of the possible mechanisms also follow directly from the metabolic processes by which inorganic arsenic is sequentially reduced, oxidized and methylated (Challenger, 1945; Francesconi and Edmonds, 1994; Le et al., 2000; Thomas et al., 2001). Two examples are generation of superoxide free-radicals leading to oxidative stress and depletion of cellular methyl pools leading to DNA hypomethylation. It is possible that it is the process of metabolic transformation, rather than the arsenic metabolites themselves that lead to cellular injury and cancer.

Based on consideration of anticipated dose and anticipated metabolism, it is likely that seafood arsenic does not contribute significantly to arsenic-associated carcinogenicity. The vast majority of arsenic in finfish and crustaceans is in the forms of arsenobetaine, a compound that is essentially inert, non-toxic and excreted without transformation. The quantities of TETRA formed by dry cooking of arsenobetaine-containing fish are not likely to achieve toxic levels. Likewise, the quantities of inorganic arsenic and MA found in seafood are sufficiently small to mitigate concerns about their possible adverse effects in seafood eaters (Donohue and Abernathy, 1999). On the other hand, DMA and arsenosugars in seafood pose at least theoretical risks. Of principal concern is that under some conditions, DMA has been shown to be carcinogenic and genotoxic, but not mutagenic. At issue is whether the quantity of DMA contained in ingested seafood or derived from arsenosugars in ingested seaweed and mollusks is sufficient to cause such effects. It is unlikely that other arsenical species and metabolites, such as TETRA and TMAO, would be present or formed in clinically relevant quantities cooking of arsenobetaine-containing fish.

High-dose exposures to DMA, either 40 or 200 ppm in drinking water (Wei et al., 2002) or 100 ppm in the diet (van Gemert and Eldan, 1998) caused bladder cancer in rats, but not in mice at levels up to 500 ppm (van Gemert and Eldan, 1998; Fukushima et al., 1998; Wei et al., 2002). Carcinogenic effects were not seen in other organs (Wei et al., 2002). DMA also enhanced the incidence of bladder tumors when administered after genotoxic bladder carcinogens (Cohen et al., 2002). The likely mechanism for these carcinogenic effects involves DMA-induced necrosis of the urothelium, probably mediated by reactive oxygen species, followed by sustained increased cellular proliferation (Cohen et al., 2001, 2002, 2006; Wei et al., 2005). That such effects may be species specific is suggested by the observation that urinary tract effects were not seen in hamsters exposed to 100 ppm in their diet for ten weeks (Cano et al., 2001); by contrast, similar doses caused cytotoxic effects in rats after only 6 h (Cohen et al., 2001).

It is useful to compare these exposure levels to those that might result from seafood consumption. In the animal cancer studies, drinking water DMA levels of 40 and 200 ppm yielded urinary DMA levels of 20.3 and 44.1 mg As/L, respectively (Wei et al., 2002), while dietary levels of 100 ppm DMA led to urinary DMA levels of 9 mg As/L (Cohen et al., 2002). By contrast, consumption of 250 g of mussels, equivalent to about 3 average servings (personal communication: J. Exler, USDA Nutrient Data Lab, 11/7/05) resulted in peak excretion of only 90 μg As/L (Le et al., 1999) and consumption of 15 g of seaweed, equivalent to about 3 days average consumption by the Japanese (Yamauchi et al., 1992; Francesconi et al., 2002) resulted in 100 μg As/L (Wei et al., 2003).[2] Thus, a margin of exposure of at least 10^3–10^4 exists between carcinogenic doses used in that rat studies and those expected after human consumption of large quantities of seafood.

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