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Role of complex organic arsenicals in food in aggregate exposure to arsenic☆

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ABSTRACT

For much of the world's population, food is the major source of exposure to arsenic. Exposure to this non-essential metalloid at relatively low levels may be linked to a wide range of adverse health effects. Thus, evaluating foods as sources of exposure to arsenic is important in assessing risk and developing strategies that protect public health. Although most emphasis has been placed on inorganic arsenic as human carcinogen and toxicant, an array of arsenic-containing species are found in plants and animals used as foods. Here, we 2evaluate the contribution of complex organic arsenicals (arsenosugars, arsenolipids, and trimethylarsonium compounds) that are found in foods and consider their origins, metabolism, and potential toxicity. Commonalities in the metabolism of arsenosugars and arsenolipids lead to the production of di-methylated arsenicals which are known to exert many toxic effects. Evaluating foods as sources of exposure to these complex organic arsenicals and understanding the formation of reactive metabolites may be critical in assessing their contribution to aggregate exposure to arsenic.

Contents

☆ This manuscript honors Dr. William Cullen for his extraordinary contributions to the field of arsenic chemistry. His advice and encouragement supported the work of many scientists and his generosity in sharing his armamentarium of arsenic compounds spurred many research efforts. His willingness to share ideas is an example of the collaborative spirit of science.

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Introduction

The biogeochemical cycling of arsenic involves chemical, physical, and biological processes that result in substantial fluxes of arsenic through the environment (Zhu et al., 2014). Some of this arsenic is incorporated into food and water sources, providing routes for human exposure to this toxic metalloid. In terms of risk to humans, most emphasis has been placed on the role of inorganic arsenic in drinking water as a source of exposure (Smith and Steinmaus, 2009; Bloom et al., 2014; Shankar et al., 2014; Tsuji et al., 2014; Karagas et al., 2015). However, absent a water supply contaminated with inorganic arsenic, the major source of exposure to arsenic for most individuals is through consumption of foods that contain the metalloid (Kurzius-Spencer et al., 2014; Wilson, 2015). Inorganic arsenic is classified as a Group 1 carcinogen in humans (IARC, 2004, 2012). Consumption of foods containing inorganic arsenic probably contributes to the global cancer burden (Oberoi et al., 2014). Thus, altered patterns of food use, particularly in vulnerable subpopulations such as infants, have been recommended as a strategy to reduce exposure to this metalloid (Gundert-Remy et al., 2015). Although emphasis has usually focused on exposure to inorganic arsenic from consumption of contaminated foodstuffs, widely consumed foods such as rice that can contain both inorganic and di-methylated arsenicals may be significant sources of exposure (Zhao et al., 2013; Wang et al., 2015b). The presence of inorganic and di-methylated arsenic in rice is an important public health issue. Rice is the staple food for over one-half of the world's population (Muthayya et al., 2014). In the U.S., rice consumption makes a significant contribution to arsenic exposure in children (Davis et al., 2012), raising special concerns about exposure in an age group that may be especially vulnerable to adverse health effects induced by inorganic arsenic or its metabolites.

Besides inorganic and methylated arsenicals present in foods, three additional classes of arsenicals that are present at high concentrations in foods may make significant contributions to aggregate exposure to arsenic. These classes are arsenosugars, arsenolipids, and tri-methylated arsonium compounds of which arsenobetaine is most abundant. Here, we follow the nomenclature used in an earlier study (Borak and Hosgood, 2007) and refer to these compounds as complex organic arsenicals. Representative structures of some of the complex organic arsenicals are shown in Fig. 1. Complex organic arsenicals are characterized by the presence of a di- or tri-methylated arsenic-containing moiety in aliphatic or aromatic molecule. As described below, methylated arsenic moieties in complex organic arsenicals are derived from enzymatically catalyzed reactions that convert inorganic arsenic to methylated products (Thomas et al., 2007; Thomas, 2015). Thus, there is a critical linkage between the methylation pathway that produces metabolites in which toxic potencies are determined by the oxidation state of arsenic (Styblo et al., 2000) and a series of reactions that incorporate methylated arsenicals into larger biomolecular structures.

Research over the last four decades has identified complex organic arsenicals in many foods. Studies of their fate after ingestion suggest that some of these compounds can be transformed into metabolites which may have biological effects. In recent years, improved analytical methods have made possible the characterization and quantitation of these molecules and their metabolites, creating opportunities to understand their roles in aggregate exposure to arsenic. In the following paragraphs, we first summarize current knowledge of the origin and fate of these complex organic arsenicals and then suggest future directions for research to understand their role in aggregate exposure to arsenic from dietary sources.

1. Arsenosugars

1.1. *Origin and occurrence*

Arsenosugars are a class of arsenic-containing carbohydrates in which a di- or tri-methylated arsenical is incorporated into a ribofuranoside which contains glycerol, phosphate, sulfate or a sulfonate. Originally these compounds were identified as water-soluble components of seaweeds (Edmonds and Francesconi, 1981). The pathway for the formation of arsenosugars has not been fully elucidated but the formation of arsenosugars has been linked to metabolic processes that transform inorganic arsenic into methylated species. The marine brown macroalga *Fucus serratus* was shown to convert arsenate to arsenosugars (Geiszinger et al., 2001). Freshwater unicellular green alga *Chlamydomonas reinhardtii* which was exposed to arsenate produced mono- and di-methylated

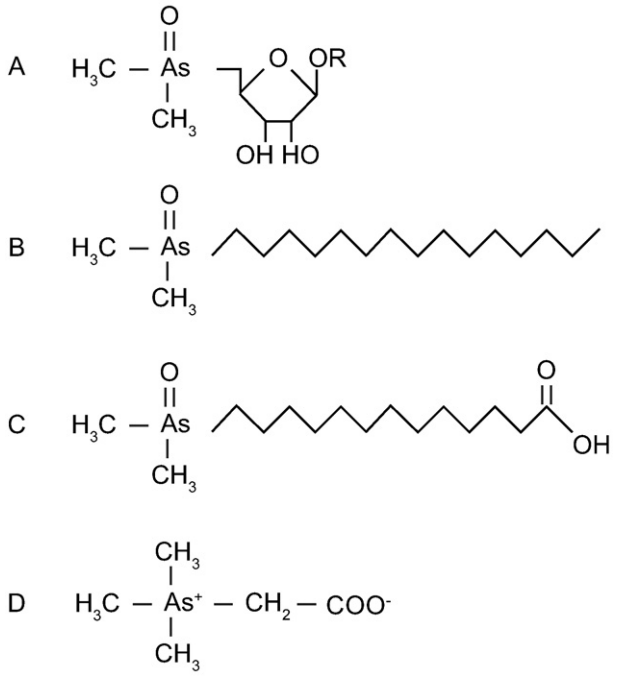

Fig. 1 – Structures of representative complex organic arsenicals – A). arsenosugar, B). arsenohydrocarbon, C). arsenofatty acid, D). arsenobetaine.

arsenicals and arsenosugars (Miyashita et al., 2011). Reactions that methylate arsenic in algae are likely catalyzed by members of the arsenic methyltransferase gene family that is widely distributed in all domains of life (Ye et al., 2012; Thomas and Rosen, 2013; Zhu et al., 2014). Marine unicellular microalgae, the primary producers in marine ecosystems, produce arsenosugars which are transferred to organisms at higher trophic levels (Duncan et al., 2015). The concentration of arsenic in the lake water has been shown to affect the relative contribution of arsenosugars to the total arsenic burden of plankton; high levels of arsenic reduced the proportion of arsenic present as an arsenosugar (Caumette et al., 2011). It has been suggested that the formation of arsenosugars by algae relied on interactions between macroalgae and commensal microbial species (Caumette et al., 2012). Studies with the brown macroalga *Fucus gardneri* exposed to arsenate found that arsenosugar production was diminished under axenic conditions (Granchinho et al., 2001), although the commensal microbe involved in arsenosugar synthesis was not identified (Granchinho et al., 2002). Seaweed-microbe interactions play critical roles in seaweed growth (Singh and Reddy, 2014) and an intimate commensal microbe might contribute to the formation of arsenosugars by seaweeds.

1.2. Metabolism in humans

The results of several studies in humans that examined the metabolite fate of ingested arsenosugars using foods or synthetic compounds as sources are shown in Table 1. An early study compared patterns of arsenicals in urine of volunteers after ingestion of seaweed (nori), shrimp, or crab (Le et al., 1994). After shrimp or crab ingestion, arsenobetaine was the predominant arsenical in urine. In contrast, after ingestion of nori there was considerable variation among volunteers in patterns of arsenicals in urine with di-methylated arsenic as the predominant urinary metabolite. Additional studies affirmed that di-methylated arsenic was the major metabolite in urine after seaweed ingestion and found that arsenosugars are also excreted in urine (Ma and Le, 1998). Volunteers who ingested *Porphyria* seaweed also excreted large amounts of di-methylated arsenic in urine (Wei et al., 2003). Bioaccessibilities of arsenosugars in seafoods have been shown to be high, suggesting that exposure through ingestion could be substantial (Almela et al., 2005). A wide range of metabolites have been identified in urine of volunteers who ingested a synthetic arsenosugar (Francesconi et al., 2002; Raml et al., 2005, 2009). These include oxo- or thio-dimethylarsinoylethanol, oxo- or thio-dimethylarsinoylacetate, and trimethylarsine oxide. Among the volunteers there was wide variation in capacity to metabolize arsenosugars (Raml et al., 2009). The percentage of arsenic ingested as an arsenosugar that was recovered in urine of these volunteers ranged from 4% to 95%. There was evidence that the magnitude of clearance of ingested arsenic correlated with the complexity of profile of arsenic-containing metabolites in urine. That is, volunteers who excreted lowest percentages of the ingested dose in urine had the lowest levels of metabolites and highest levels of the parent compound and its thio-arsenosugar analog in urine. Notably, di-methylated arsenic has also been identified as the predominant urinary metabolite in Orkney Island sheep that consumed arsenosugars-rich seaweed as a main food source (Hansen et al., 2003).

1.3. Toxicity

The toxicity of arsenosugars and arsenicals produced by their catabolism has been examined. In *in vitro* assays that measured nicking of plasmid DNA, an arsenosugar-containing trivalent arsenic was more potent than its homolog that contained pentavalent arsenic, although both compounds were less toxic than methylarsonous acid and dimethylarsinous acid (Andrewes et al., 2004). In most cases, arsenosugars that have been evaluated for toxicity have contained pentavalent arsenic. Cytotoxicities of two arsenosugars and of some of the metabolites excreted in urine after ingestion of arsenosugars have been compared (Leffers et al., 2013a). Here, neither arsenosugar (a glycerol-sugar and a sulfate-sugar) nor the metabolites, oxo- or thio-dimethylarsenoacetic acid and oxo- or thio-dimethylarsenoethanol, were cytotoxic or genotoxic to human urinary bladder cells at concentrations up to 500 μmol/L. In contrast, oxo- and thio-dimethylarsinic acids were potently cytotoxic, reducing cell numbers by 30% at media concentrations of 2.3 and 205 μmol/L, respectively. Across a range of endpoints reflecting cell viability, thio-dimethylarsinic acid was more potent than arsenite. Additional studies found the metabolite thio-dimethylarsinic acid was more potent than arsenite as an inhibitor of the ribosylation of poly-adenosine diphosphate that is induced by DNA damage (Ebert et al., 2014). Notably, both thio-dimethylarsinic acid and thio-dimethylarsenoethanol readily crossed the cell barrier in a CaCo-2 cell model of the human gastrointestinal barrier, suggesting that metabolic degradation of arsenosugars mediated by the microbiota of the gastrointestinal tract produced these di-methylated species (Leffers et al., 2013b). Intracellular conversion of thioarsenical species to their oxo-analogs

Table 1 – Metabolic fate of ingested arsenosugars and arsenolipids.

	Test compound	Test species	Urinary metabolites	Reference
Arsenosugars	Seaweed containing arsenosugars	Human	Dimethylarsinic acid and unknown arsenicals	Le et al. (1994), Ma and Le (1998)
	2, 3′-Dihydroxypropyl 5-deoxy-5 dimethylarsinoyl-β-D-riboside		Dimethylarsinic acid, dimethylarsinoylethanol, trimethylarsine oxide	Francesconi et al. (2002)
	Seaweed containing arsenosugars		Dimethylarsinoylethanol, dimethylarsinic acid, methylarsonic acid	Wei et al. (2003)
	2, 3′-Dihydroxypropyl 5-deoxy-5 dimethylarsinoyl-β-D-riboside		Dimethylthioarsenoacetate, dimethylthioarsenoethanol, thio-arsenosugar, dimethyloxoarsenoacetate	Raml et al. (2009)
Arsenolipids	Cod liver (predominantly arsenolipids with arsenobetaine)	Human	Dimethylarsinic acid Arsenofatty acids — oxo- and thio-dimethyloxoarsenopropanoic acid, oxo- and thio-dimethyloxoarsenobutanoic acid Arsenobetaine	Schmeisser et al. (2005, 2006)
	Cod liver oil (arsenolipids)			
	Phosphotidylarsenocholine (synthetic)	Mouse	Arsenobetaine Dimethylarsinic acid	Fukuda et al., 2011

combined with facile transport of thio-arsenicals across cell membranes may be a factor that potentiates the toxicity of these compounds (Naranmandura et al., 2009; Suzuki et al., 2010). Because anaerobic microbiota of mouse cecum readily convert oxo-arsenicals to thio-arsenicals (Pinyayev et al., 2011), thiolated metabolites derived from arsenosugars in the gastrointestinal tract may play important roles in exposure to arsenic after ingestion of arsenosugars. In particular, thio-dimethylarsinic acid which is readily formed and transported and is toxic should be fully characterized as a potential risk to human health (Ebert et al., 2014).

2. Arsenolipids

2.1. Origin and occurrence

Lipid-soluble arsenicals have long been known to be present in lipid extracts of algae and fish (Sele et al., 2012). Early work found that oils of marine organisms contained 1 to 50 μg of arsenic per g and that lipid-soluble arsenicals accounted for up to 30% of the arsenic in these organisms (Lunde, 1977). Exposure of the marine diatom *Chaetoceros concavicornis* to arsenate was associated with the formation of an arsenophospholipid (Cooney et al., 1978), suggesting a pathway for conversion of inorganic arsenic into this organic species. Arsenic-containing fatty acids and arsenohydrocarbons were subsequently identified in fish oils (Rumpler et al., 2008; Taleshi et al., 2008). Arsenolipids can be a major class of arsenicals in some tissues (*e.g.*, liver, fat) of marine invertebrates, fish, or mammals (Sele et al., 2012). In the marine alga *Undaria pinnatifida*, arsenolipids account for about one-quarter of all arsenic (Morita and Shibata, 1990). The brown macroalgae *U.pinnatifida* and *Hizikia fusiformis* were found to contain a variety of arsenosugar-containing phospholipids and arsenic-containing hydrocarbons (Morita and Shibata, 1988; Garcia-Salgado et al., 2012), suggesting a possible link between metabolic processes that produce arsenosugars and arsenolipids. Some algal species (*Saccharina latissima*) simultaneously contain arsenosugar-containing phospholipids, arsenic-containing hydrocarbons, and arsenic-containing fatty acids (Raab et al., 2012). Arsenolipids formed in phytoplankton may be transferred to higher trophic levels and account for arsenolipids in predator species (Wrench et al., 1979; Devalla and Feldmann, 2003).

The pathway for biosynthesis of arsenolipids remains uncertain. Production of arsenic-containing fatty acids has been described as the result of "biosynthetic infidelity" in which a di-methylated arsenical species is incorporated during fatty acid synthesis (Rumpler et al., 2008). The identity of the di-methylated arsenical used in fatty acid synthesis is not known. Dimethylarsinic acid is unlikely to be used for this process because its incorporation into a growing fatty acid would produce only arsenic-containing fatty acids with odd numbered chain lengths. Synthesis of arsenic-containing fatty acids with even numbered chain length could use dimethylarsenopropionic acid as a substrate. A pathway for synthesis of dimethylarsenopropionic acid by arsenoylation of an oxo-acid by dimethylarsinous acid has been proposed (Edmonds, 2000). Dimethylarsenopropionic acid and other aliphatic dimethylarsenicals have been identified in a number of marine organisms, including algae (Sloth et al., 2005). Trophic transfer of these compounds to predator species could provide substrate for synthesis of arsenic-containing fatty acids. By comparison, little is known about pathways that result in the formation of arsenic-containing hydrocarbons or arsenophospholipids.

2.2. Metabolism in humans and mice

The metabolic fate of arsenolipids has been examined in volunteers who ingested arsenolipid-rich foods, cod liver and cod liver oil (Table 1). Peak concentrations of arsenic in urine occurred within 15 hr of treatment with more than 85% of the ingested arsenic excreted in urine within two days of ingestion (Schmeisser et al., 2005). Arsenobetaine present in cod liver was excreted unchanged. Arsenolipids present in cod liver and cod liver oil were converted to dimethylarsinic acid and to oxo- and thio-di-methylated arsenic-containing fatty acids (Schmeisser et al., 2006). These studies in humans have been complemented by a study of the fate of phosphatidylarsenocholine in adult male mice (Fukuda et al., 2011). Over seven days after a single oral dose, 87% of the administered arsenic was recovered in

urine and 6% was recovered in feces. About 50% of the dose was recovered in urine during the first 72 hr after treatment. The major arsenical in urine of phosphatidylarsenocholine-treated mice was arsenobetaine. Dimethylarsinic was also minor metabolite in urine. Notably, the biological half-life for arsenic in phosphatidylarsenocholine-treated mice was relatively long. By comparison, the biological half-life for arsenic in mice treated with arsenobetaine is less than 24 hr (Vahter et al., 1983). The prolonged clearance time for arsenic after phosphatidylarsenocholine treatment could reflect its transformation to a lysophospholipid before absorption across the gastrointestinal barrier and its subsequent conversion into a glycerophospholipid before its incorporation into a chylomicron.

Recent studies of the metabolism and transport of arsenic-containing hydrocarbons and arsenic-containing fatty acids used an *in vitro* system in which differentiated Caco-2 intestinal cells mimic the human gastrointestinal barrier (Meyer et al., 2015). In this system, arsenic-containing hydrocarbons were more cytotoxic to Caco-2 cells than were arsenic-containing fatty acids; the EC_{70s} for arsenic-containing hydrocarbons were in the 50–100 μmol/L range or higher. From 40% to 50% of the applied arsenic-containing hydrocarbons was transferred to the basolateral compartment without significant metabolism. In contrast, arsenic-containing fatty acids were transferred to the basolateral compartment at lower rates (<40% of applied material). In both the apical and basolateral compartments, arsenic-containing fatty acids were largely transformed to di-methylated arsenic. Thus, both pharmacokinetic and pharmacodynamic factors differentiated the transfer of these classes of arsenolipids across the gastrointestinal barrier.

2.3. Toxicity of arsenolipids

The cytotoxicities of three arsenic-containing hydrocarbons and arsenite have been compared in UROtsa cells, an immortalized human uroepithelial cell line (Meyer et al., 2014a). Arsenic-containing hydrocarbons and arsenite were about equipotent as cytotoxins when evaluated by effects on cell number, lysosomal integrity, and dehydrogenase release. Unlike arsenite, treatment with arsenic-containing hydrocarbons lowered cellular adenosine triphosphate (ATP) concentrations. Disruption of ATP metabolism is commonly seen in cells exposed to relatively high levels of arsenate (Delnomdedieu et al., 1994). Notably, cellular levels of arsenic were markedly higher after exposure to arsenic-containing hydrocarbons than after exposure to arsenite, reflecting, in part, accumulation of arsenic in the membrane fraction of cells after arsenic-containing hydrocarbon exposure. The metabolic fate of arsenic in cells exposed to these compounds has not been determined. The developmental toxicity of arsenic-containing hydrocarbons has been examined in the fruit fly *Drosophila melanogaster* (Meyer et al., 2014b). Here, arsenic-containing hydrocarbons and arsenite were roughly equipotent as inhibitors of pupal development but arsenic-containing hydrocarbons were more potent as inhibitors of eclosion, suggesting that events occurring late in development were particularly sensitive to arsenolipids. As seen in cellular systems, arsenic was readily accumulated in flies after exposure to arsenic-containing hydrocarbons.

3. Arsenobetaine

3.1. Origin and occurrence

Arsenobetaine (2-trimethylarsoniumylacetate, 2-(trimethylarsaniumyl)acetate) is an arsenic-containing analog of trimethylglycine (glycine betaine). It was originally identified as the major arsenical species in a variety of marine organisms, including fish and shellfish (Edmonds et al., 1977; Maher, 1985; Hanaoka et al., 1987; Francesconi and Edmonds, 1987). Arsenobetaine also occurs in non-marine organisms as diverse as mushrooms, earthworms, and terrestrial birds (Smith et al., 2007; Button et al., 2011; Koch et al., 2005). Like other betaines, arsenobetaine can serve as an osmolyte. Correlation of concentrations of arsenobetaine and glycine betaine, the major cellular osmolyte, in livers of seals, seabirds, and sea turtles has been reported (Fujihara et al., 2003). In shellfish and fish sensing of environmental salinity regulated accumulation of arsenobetaine (Amlund and Berntssen, 2004; Clowes and Francesconi, 2004).

The pathway for arsenobetaine production in marine or freshwater ecosystems is not fully understood. In marine systems, arsenosugars are found in unicellular microalgae and phytoplankton; arsenobetaine initially appears in herbivorous zooplankton and becomes the predominant arsenical in carnivorous zooplankton (Caumette et al., 2012). Studies in a bacterial system provided evidence of a pathway that linked arsenosugars' degradation to arsenobetaine synthesis (Ritchie et al., 2004). As shown in Fig. 2, the arsenosugar is catabolized to dimethylarsinoylethanol. This intermediate is oxidized to dimethylarsinoylacetic acid; reduction and methylation of this compound yields arsenobetaine. Anaerobic decomposition of arsenosugars in *Ecklonia radiata* has been shown to release dimethylarsinoylethanol that could be the starting material for arsenobetaine synthesis (Edmonds et al., 1982). The sequence of reactions in this synthetic pathway is not well characterized and it is unclear whether the reactions are catalyzed by the host alga or by a commensal microbe.

3.2. Exposure and metabolism in humans

Consumption of foods containing arsenobetaine is an important contributor to aggregate exposure to arsenic. In Japanese diets which typically contain a variety of arsenic-rich foods, average daily intake of arsenic ranges from 148 to 273 μg and arsenobetaine accounts for 48% to 75% of the arsenic consumed (Mohri et al., 1990; Yamauchi et al., 1992). Analysis of arsenicals in urine from participants in the 2003–2006 National Health and Nutrition Examination Survey found that recent consumption (within 24 hr) of seafood is associated with increased median concentrations of arsenobetaine, dimethylarsenic, and total arsenic in urine (Navas-Acien et al., 2011). Here, increased urinary concentration of arsenobetaine after seafood ingestion accounted for about 40% of the increase in total arsenic concentration. *In vitro* measures show that arsenobetaine in seafoods in highly bioaccessible, suggesting that ingested arsenobetaine readily crosses the gastrointestinal barrier in humans (Laparra et al., 2007).

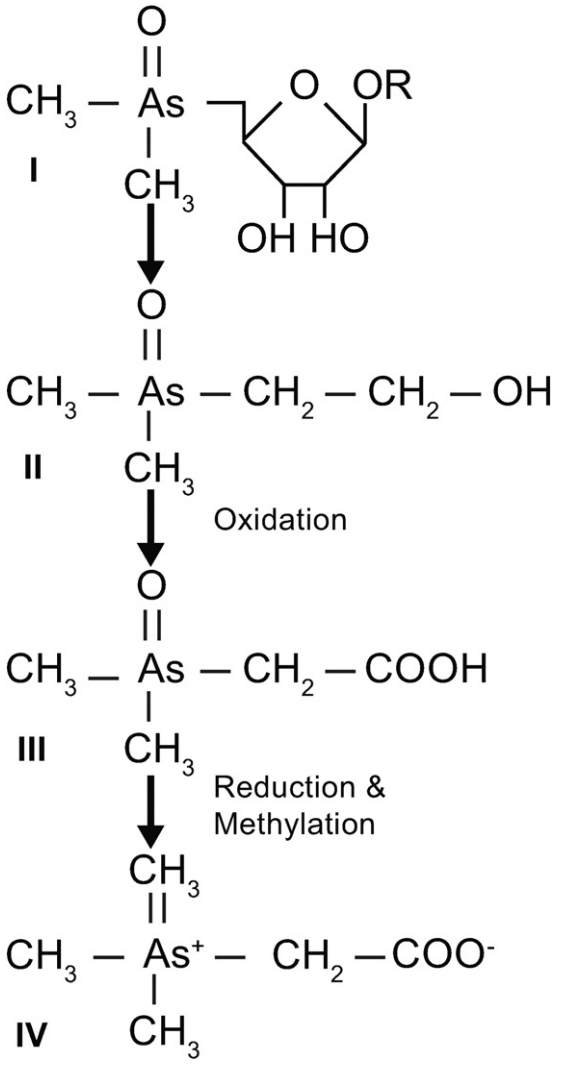

Fig. 2 – Possible scheme for the production of arsenobetaine from the degradation of an arsenosugar. Here, an arsenosugar is metabolized to yield dimethylarsinoylethanol. This compound is oxidized to yield dimethylarsinoylacetic acid which is reduced and methylated to yield arsenobetaine.

In humans, whole body retention of arsenic after ingestion of arsenobetaine (Brown et al., 1990) can be compared with whole body retention after ingestion of arsenate (Pomroy et al., 1980). Notably, whole body clearance of arsenic was faster after ingestion of arsenobetaine than after ingestion of arsenate. Cumulative urinary excretion of arsenic can also be compared for volunteers who ate arsenobetaine-rich flounder (Freeman et al., 1979; Tam et al., 1982) or who ingested 500 μg of arsenic as monomethylarsonic acid or dimethylarsinic acid (Buchet et al., 1981). Over 96 hr after exposure, about 80% of the dose of either monomethylarsonic acid or dimethylarsinic acid and about 65% of arsenic ingested as arsenobetaine was excreted in urine. These comparative data on whole body retention and urinary clearance of arsenic suggest that there is little potential for tissue accumulation of arsenic following ingestion of arsenobetaine.

Most research suggests that ingested arsenobetaine does not undergo metabolic transformation before excretion. Urine of mice, rats, and rabbits (Vahter et al., 1983) or hamsters (Yamauchi et al., 1986) exposed orally or intravenously to arsenobetaine contained only the administered compound. Similarly, analysis of urine from volunteers who ingested arsenobetaine did not find any evidence of metabolism of this compound (Yamauchi and Yamamura, 1984; Brown et al., 1990). Given evidence of low acute toxicity of arsenobetaine in animals (Kaise et al., 1985) and lack of toxicity in cellular systems (Jongen et al., 1985; Sabbioni et al., 1991), ingestion of arsenobetaine likely poses little hazard to human health.

3.3. Potential production of arsenobetaine in humans

Studies of patterns and levels of arsenobetaine in urine after occupational exposure to arsine, ingestion of seafood, or a low-arsenobetaine diet suggested that, under some circumstances, the amount of arsenobetaine excreted in urine exceeded the amount that could be accounted by recent ingestion of this compound (Apostoli et al., 1997; Molin et al., 2012; Newcombe et al., 2010). These findings were tentatively interpreted as evidence of *in vivo* synthesis of arsenobetaine in humans. However, evidence of *de novo* synthesis of arsenobetaine was not confirmed by analysis of urinary arsenobetaine excretion by rats exposed to arsine (Buchet et al., 1998). A more recent study of arsenic metabolism in volunteers who consumed a meal of arsenic-rich blue mussels (*Mytilus edulis*) found arsenobetaine and di-methylated arsenic to be the major urinary metabolites (Lai et al., 2004). Notably, one volunteer had high levels of arsenobetaine in urine before ingestion of blue mussels when dietary restrictions would be expected to limit intake of this compound and other arsenicals. This finding could reflect interindividual variation in endogenous production of arsenobetaine, a diminished rate of whole body clearance of arsenobetaine, or an unrecognized source of exposure to arsenobetaine.

4. Summary

4.1. Linkages in the production of complex organic arsenicals

Production of complex organic arsenicals occurs at the lowest trophic levels of aquatic food chains. Trophic transfer of these compounds coupled with additional metabolism results in a variety of complex organic arsenicals present in foods consumed by humans. One important feature in the production of each of the classes of compounds considered here is the central role of a di-methylated arsenical in the pathways for their synthesis. Fig. 3 illustrates the linkage between the uptake of inorganic arsenic into organisms, the enzymatically catalyzed production of di-methylated arsenic, and the use of this metabolite in the synthesis of complex organic arsenicals. In this scheme, production of di-methylated arsenic provides the precursor needed to form each type of the complex organic arsenic. In a variety of organisms, conversion of inorganic arsenic to di-methylated arsenic is closely linked to production of arsenosugars. For arsenolipids, dimethylarsenopropionic acid and other aliphatic dimethylarsenicals could be used

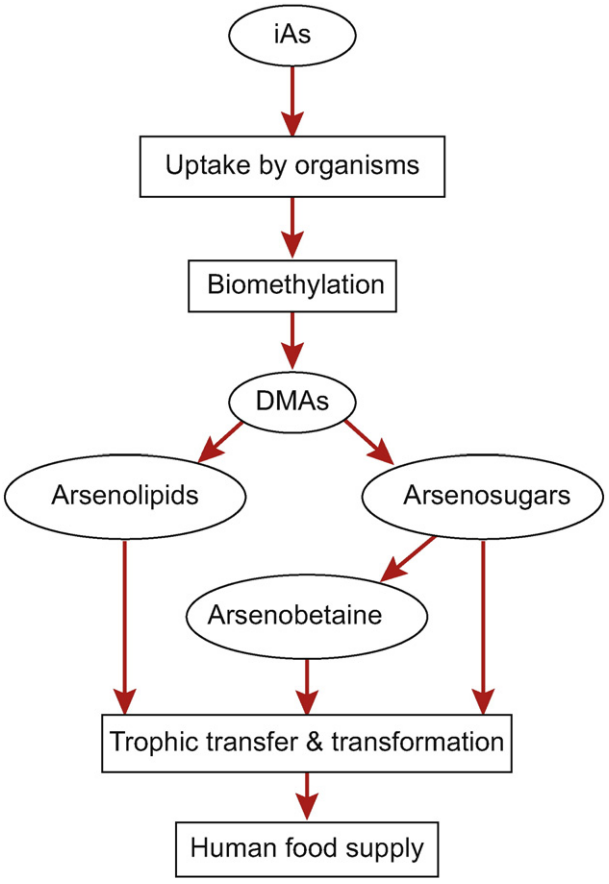

Fig. 3 – Pathway for common origins of complex organic arsenicals in food chains.

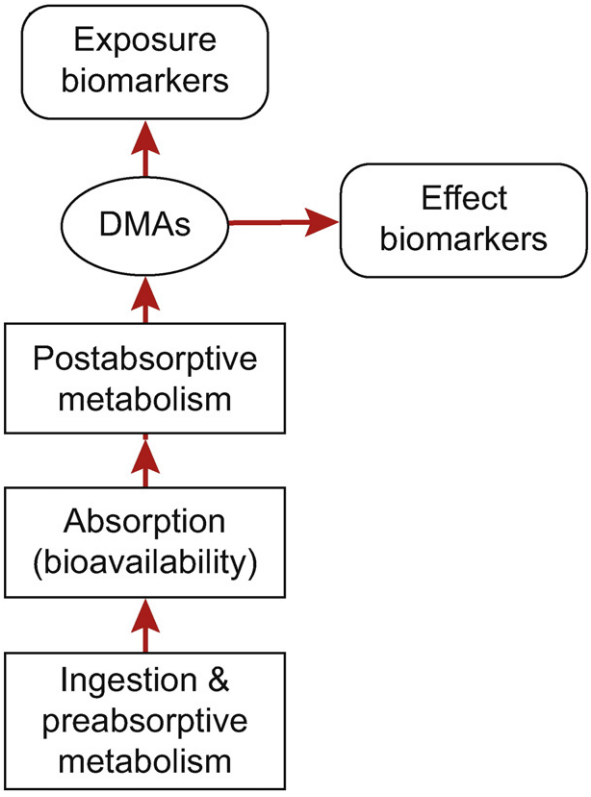

Fig. 4 – Pathway for evaluation of human exposure and metabolism of complex organic arsenicals.

as the precursor for synthesis. In the case of arsenobetaine synthesis, evidence suggests that the degradation of an arsenosugar could provide dimethylarsinoylethanol that can be incorporated into this arsonium compound.

4.2. *Common factors in metabolism of complex organic arsenicals*

As described in the preceding paragraphs, ingested arsenosugars or arsenolipids are degraded to yield di-methylated arsenicals as metabolites. These metabolites include both oxo- and thio-arsenicals that contain arsenic in the less reactive pentavalent oxidation state. Thus, although ingested complex organic arsenicals may have fairly low toxicity *per se*, the more significant issue may be the potential toxicity of these metabolites. The oxo-arsenical species dimethylarsinic acid is a carcinogen. Treatment of adult rats with dimethylarsinic acid induces urinary bladder cancer (Arnold et al., 2006; Wei et al., 1999) and is a multi-site tumor promoter in a variety of animal models (Yamamoto et al., 1995; Tokar et al., 2012). Thio-di-methylated arsenicals have also been identified as metabolites formed and excreted after ingestion of arsenosugars. Thio-di-methylated arsenicals display distinct patterns of tissue distribution and are more cytotoxic than oxo-di-methylated arsenic (Wang et al., 2015a). Preabsorptive metabolism of complex organic arsenicals likely includes generation of both oxo- and thio-di-methylated metabolites.

Factors affecting the extent of exposure to di-methylated arsenicals produced as metabolites of ingested arsenosugars or arsenolipids are summarized in Fig. 4. Following ingestion of foods containing arsenosugars or arsenolipids, preabsorptive metabolism mediated by members of the gut microbiome probably produces a range of metabolites. For example, it has been shown that an oxo-arsenosugar was quickly converted to its thio-homolog by the anaerobic microbiota of mouse cecum (Conklin et al., 2006). After preabsorptive metabolism, the parent compound and its metabolites must cross the gastrointestinal barrier to undergo systemic circulation and metabolism. The extent to which compounds cross the gastrointestinal barrier is often termed bioavailability; in the case of complex organic arsenicals, bioavailability refers to the absorption of the parent compound and the metabolites formed by preabsorptive metabolism. For purposes of this evaluation, postabsorption metabolism of complex organic arsenicals and their metabolites is assumed to result in the formation of di-methylated arsenicals. Levels of di-methylated metabolites could be assessed in tissues and urine as biomarkers of exposure and adverse health effects could be assessed using biomarkers of effect.

4.3. *Assessing consequences of ingestion of complex organic arsenicals*

Although risk associated with exposure to arsenobetaine can probably be discounted, consumption of seafood can be an important source of exposure to other complex organic

arsenicals. Currently about 3 billion people worldwide use seafoods as a key source of high quality animal protein (Tveteras et al., 2012) with demand for fish as a food source projected to rise (Sumaila et al., 2014). Exposure to arsenolipids may occur through consumption of fish or fish oil. The use of fishmeal or fish oil as foodstock for aquaculture may introduce arsenolipids into the food supply (Tacon and Metian, 2008). Growing use of fish oil supplements as sources of cardioprotective omega-3 polyunsaturated fatty acids in the U.S. population (Clarke et al., 2015) may make this dietary supplement a source of exposure to arsenolipids. Active carbon treatment or steam deodorization that was used to reduce levels of persistent organic pollutants in fish oil has been shown to lower arsenic levels (Sele et al., 2013). The use of seaweed as alternate source of omega-3 and omega-6 polyunsaturated fatty acids could increase exposure to arsenolipids present in these materials (Misurcova et al., 2011). Similarly, extracts of brown algae that contain high levels of antioxidant phlorotannins could be the source of exposure to co-extracted arsenolipids (Wang et al., 2012).

Similarly, commercial use of algae is likely to grow in the future as algal biomass is exploited as a source of renewable fuels and as a source of high quality protein and carbohydrates (Graziani et al., 2013; Trentacoste et al., 2015). Algae are also likely to be increasingly used as the source of functional foods, pharmaceuticals, and nutraceuticals (Fan et al., 2014; Cardoso et al., 2015; Abdul et al., 2016). Depending on processing, arsenosugars present in algae might be incorporated into products that use algae as starting material for a food product, pharmaceutical, or nutritional supplement. The use of algal oils as a source of cardioprotective omega-3 polyunsaturated fatty acids (Lenihan-Geels et al., 2013) may also result in exposure to arsenolipids present in these extracts. Evaluation of procedures for processing of algae will be required to determine strategies that minimize retention of arsenolipids and arsenosugars in products.

5. Future research

Additional research is needed to characterize the occurrence of complex organic arsenicals in the human food supply. This will require the systematic survey of foods using standardized analytical methods that will allow detection and quantitation of all species of interest. The pioneering work on the metabolism of arsenosugars and arsenolipids in humans should be extended with more studies of the fate of complex organic arsenicals, including studies that examine interindividual variability in metabolism and bioavailabilities of these compounds in different foods. Evaluation of patterns of consumption of foods containing complex organic arsenicals will provide a better estimate of the contribution of these compounds to aggregate exposure to arsenic, particularly in individuals whose patterns of food use increases their intake of food rich in complex organic arsenicals. Although the evidence is limited, arsenic toxicity in humans has been associated with consumption of foods that contain high concentrations of complex organic arsenicals. In one case, the affected individual who used an herbal kelp powder supplement that contained 8.5 ppm of arsenic displayed signs and symptoms compatible with arsenic toxicosis (Amster et al., 2007). The use of dietary supplements including fish oils and herbal supplements has been linked to arsenic toxicity in two other case studies (Kim et al., 2012; Barton and McLean, 2013). It should be noted that none of these studies provided measurements of levels of complex organic arsenicals in the suspected source material. Attribution of toxic effects to exposure to these compounds must be considered provisional. However, these studies are significant as they demonstrate a plausible route of exposure to complex organic arsenicals and suggest that further studies in individuals using these supplements could be useful. In addition, there is a need for parallel studies in animal models and cellular systems to evaluate pathways for metabolism of complex organic arsenicals and to identify modes of action for these compounds as toxins.

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