



Human exposure to organic arsenic species from seafood



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HIGHLIGHTS

- Seafood is a major source of organic arsenic exposure.
- Arsenolipids and arsenosugars can be metabolized by humans.
- Some organic arsenic compounds and their metabolites can produce cytotoxic effects.
- Analytical standards for organic arsenic are a priority for advancing research.

GRAPHICAL ABSTRACT



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ABSTRACT

Seafood, including finfish, shellfish, and seaweed, is the largest contributor to arsenic (As) exposure in many human populations. In contrast to the predominance of inorganic As in water and many terrestrial foods, As in marine-derived foods is present primarily in the form of organic compounds. To date, human exposure and toxicological assessments have focused on inorganic As, while organic As has generally been considered to be non-toxic. However, the high concentrations of organic As in seafood, as well as the often complex As speciation, can lead to complications in assessing As exposure from diet.

In this report, we evaluate the presence and distribution of organic As species in seafood, and combined with consumption data, address the current capabilities and needs for determining human exposure to these compounds. The analytical approaches and shortcomings for assessing these compounds are reviewed, with a focus on the best practices for characterization and quantitation. Metabolic pathways and toxicology of two important classes of organic arsenicals, arsenolipids and arsenosugars, are examined, as well as individual variability in absorption of these compounds. Although determining health outcomes or assessing a need for regulatory policies for organic As exposure is premature, the extensive consumption of seafood globally, along with the preliminary toxicological profiles of these compounds and their confounding effect on assessing exposure to inorganic As, suggests further investigations and process-level studies on organic As are needed to fill the current gaps in knowledge.

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1. Introduction

Seafood has been identified as the major source of arsenic (As) for many human populations (WHO-IARC, 2011), with concentrations in many fish and shellfish vastly exceeding those in most terrestrial foods. In marine foods, As is usually present primarily in the form of organic compounds (Edmonds and Francesconi, 1993, Feldmann and Krupp, 2011, EFSA, 2014). Currently guidelines for As exposure only exist for inorganic As (iAs) (EFSA, 2009, WHO-IARC, 2011, FDA, 2016), which is the major form of As in drinking water, and can be present at appreciable levels in rice eg. (Williams et al., 2005, Davis et al., in this issue) and a limited number of other food sources (Koch et al., 2013). Current reference doses for iAs are based on studies of high exposure from contaminated drinking water sources, but more recent studies have found effects of iAs from lower drinking water concentrations and from food (Heck et al., 2009, Davis et al., 2015, Baris et al., 2016, Gilbert-Diamond et al., 2016). Regulators are now focusing on identifying the impacts of As at concentrations relevant to those populations without a specific contaminant source of As (NRC, 2014).

Arsenobetaine (AB), the major As species in most fish, is non-toxic and not metabolized. Other more complex organic As compounds in the form of arsenosugars and arsenolipids (AsSugars and AsLipids), are also present at significant quantities in some types of seafood, and have been shown to be taken up and metabolized in humans. These classes of As compounds produce the same urinary metabolite as iAs, namely dimethylarsinate (DMA), therefore their presence in diet could be confounding to studies of iAs using biomarkers such as urine and blood, or in studies evaluating the contribution of other dietary sources to biomarker concentrations of As. Recent findings have also shown that some forms of organic As and their intermediate metabolites display cytotoxicity in cell cultures (Leffers et al., 2013b, Meyer et al., 2014a, Meyer et al., 2015a), suggesting that further studies of toxicity and metabolic pathway are needed (Carlin et al., 2015, Molin et al., 2015). In this paper, we review the current understanding of exposure to organic As from seafood, outline the gaps in knowledge in As metabolism and shortcomings of As speciation techniques. Finally, we suggest research needs and approaches to determining the exposure, metabolic products and potential risk from these compounds.

2. Sources and distribution of organic As species in marine organisms

Concentrations of As in seawater are low and uniform (1–5 µg/L) (Phillips and Depledge, 1985, Caumette et al., 2012), with As predominantly present as As(V). In freshwater, As is also present primarily as iAs, but concentrations can be very much higher than those in seawater (Reimer et al., 2010). Despite the lower As seawater levels, much higher concentrations of As are found in marine foodwebs compared with freshwater systems. This apparent anomaly may be explained by the transformation of iAs to organic As compounds at the base of the marine foodweb, and the higher accumulation and retention of these organic compounds in marine organisms (Francesconi and Edmonds, 1997, Reimer et al., 2010).

Arsenic in seafood is primarily present as organic As, with some notable exceptions (see Section 2.4). Seaweed has some of the highest total As concentrations in the marine foodweb (EFSA, 2009); shellfish generally have higher As levels than finfish (Lorenzana et al., 2009), and demersal fish often contain more As than pelagic fish (Uneyama et al., 2007, Slejkovec et al., 2014, Wu et al., 2014), although differences between and within species can be large (Uneyama et al., 2007, EFSA, 2009).

Arsenic compounds found in seafood are divided into classes depending on their structure and properties (Fig 1). Estimated As species distributions, based on literature reports and mean total As concentrations for different seafood types (EFSA, 2009, Julshamn et al., 2012), are depicted in Fig. 2A and discussed in detail below. It is noted that

assessment of the proportions of organic As compounds in seafood is limited by the small number of studies and sample sizes which report organic As speciation, and by differences in analytical approaches between studies.

2.1. Arsenosugars

Arsenosugars are associated with marine algae, which accumulate As from seawater and store it largely in the form of AsSugar, usually at high concentrations (20–100 mg/kg dry wt), and as the major As species (>80% total As) (Tukai et al., 2002b, Feldmann and Krupp, 2011). Molluscs (Li et al., 2003, Fricke et al., 2004, Khokiattiwong et al., 2009, Whaley-Martin et al., 2012) and crustaceans (Terol et al., 2012) that are predominantly filter feeders or grazers, can also contain AsSugars from consuming algae or phytoplankton, though concentrations are generally much lower (Li et al., 2003).

Arsenosugars are ribose derivatives, which contain predominantly a dimethylarsinoyl (Me₂As(O)-) moiety bound via the C-5 of the ribose ring, with various substituents at C-1 (Edmonds and Francesconi, 1981, Edmonds and Francesconi, 1983, Francesconi and Edmonds, 1996). They can also contain a trimethyl As moiety instead of the dimethylated one, although these are far less prevalent (Shibata and Morita, 1988, de Bettencourt et al., 2011). There are so far at least 20 known AsSugar compounds, of which four (see Fig. 1 – AsSugars -Gly, -PO₄, -SO₃ & -SO₄) are by far the most widespread in marine organisms.

The concentrations and proportions of different AsSugars in seaweed vary with taxa (Lai et al., 1997, 1998, Van Hulle et al., 2002, Šlejkovec et al., 2006, Garcia-Salgado et al., 2012a). No biological function for AsSugar has so far been elucidated, just as their exact bio-synthesis is still unknown. None of the enzymes involved in the attachment of the ribose-moiety to DMA have been identified, although it is highly likely that the methyl groups and ribose moiety attached to As are provided by S-adenosylmethionine (SAM) (Edmonds and Francesconi, 1987, de Bettencourt et al., 2011). It has been shown that AsSugars are directly synthesized by phytoplankton (Edmonds et al., 1997, Foster et al., 2008, Duncan et al., 2013a, 2013b, Duncan et al., 2014) and the brown macroalgae *Fucus serratus* (Geiszinger et al., 2001). AsSugars have also been found in deep sea vent mussels (Larsen et al., 1997, Taylor et al., 2012), which suggests a bacterial source of these compounds. However, neither the *Fucus*-associated fungi *Fusarium oxysporum meloni* isolated from *Fucus gardneri* (Granchinho et al., 2002), nor the culturable bacteria associated with unicellular phytoplankton cultures (Duncan et al., 2014), were found to transform As species.

2.2. Arsenobetaine

Arsenobetaine is the major As species in most finfish and shellfish. AB is also found in zooplankton (Shibata et al., 1996, Takeuchi et al., 2005) and some algae at the base of the foodweb, but its presence in algae might possibly be due to epiphytic plankton or bacteria on the surface of marine flora (Thomson et al., 2007). In bivalve molluscs, which have complex As speciation, AB can be a significant portion of water soluble As (Francesconi and Edmonds, 1997, Moreda-Piñeiro et al., 2010, Berges-Tiznado et al., 2013a), whereas in cephalopods (Suner et al., 2002) and crustaceans, which have much simpler As speciation, AB is the dominant species (Francesconi and Edmonds, 1997, Hunter et al., 1998, Francesconi et al., 1999, Li et al., 2003). In finfish As is also predominantly AB, although AsLipids can be a significant fraction in some oily fish (Taleshi et al., 2010, Lischka et al., 2013).

Arsenobetaine is an As analog of the amino acid derivative, trimethylglycine (glycine betaine), and is highly stable to breakdown or metabolism. The source of AB in the foodweb is unclear, and there are a number of theories about the biosynthetic pathway of AB formation (Caumette et al., 2012). One possible pathway is via the oxidation of arsenocholine (AC) following breakdown of the trimethylated AsSugar species. These compounds, however, are usually present at

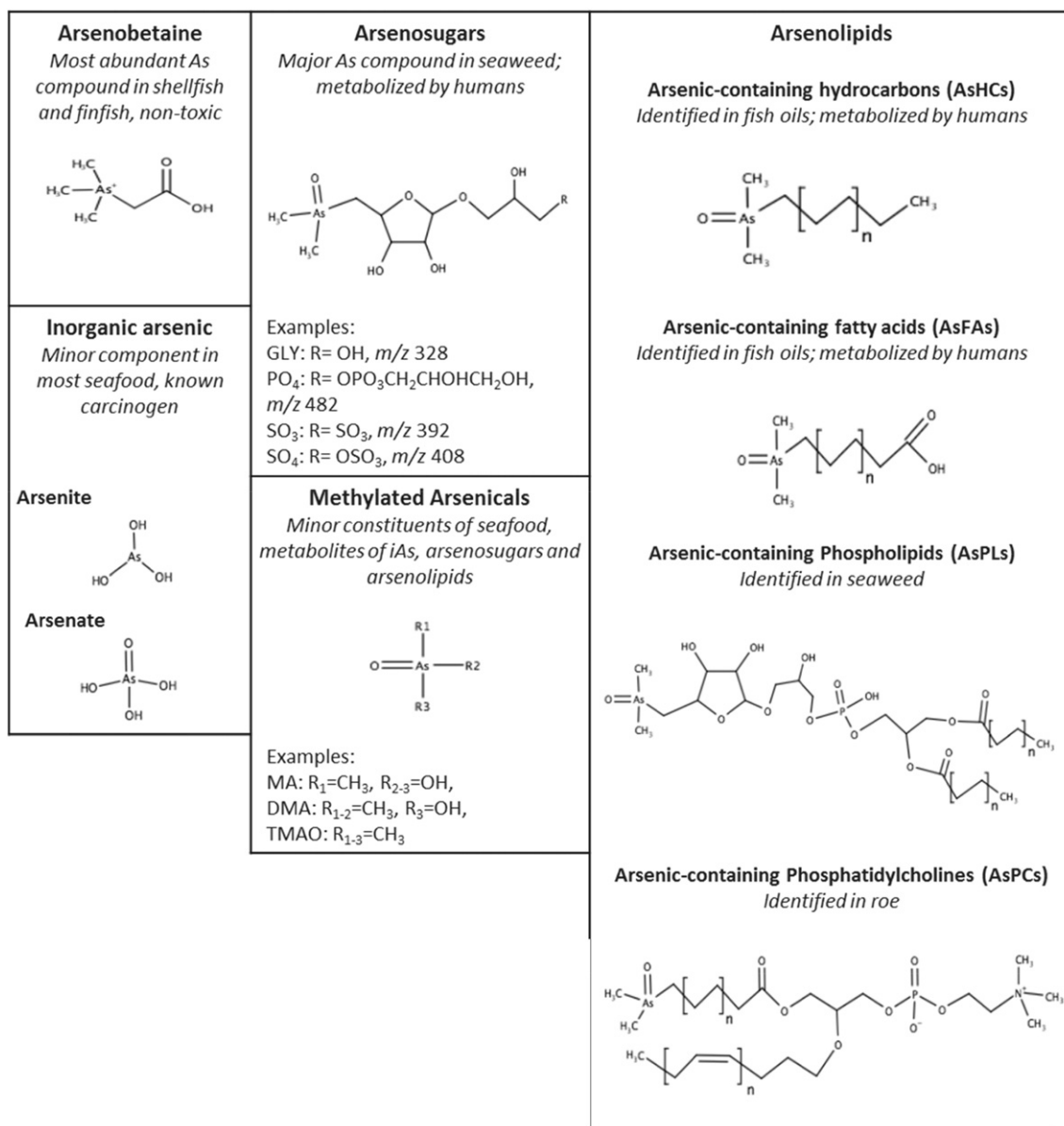


Fig. 1. As compounds found in seafood.

such low concentrations that it is difficult to perceive them as precursors to the abundant AB. The breakdown products of AsSugar, especially dimethyl arsenoethanol (DMAE) may well serve as a substrate for methyltransferase enzymes normally involved in glycine betaine synthesis in some archaea (Nyyssola et al., 2000); a problem with this pathway is that it seems to be limited to archaea and plants, whereas most microbes and all animals are not able to synthesize choline itself (Caspi et al., 2014).

A second pathway, not involving AsSugars, is *via* the reaction of DMA(III) with glycoxylate (Caumette et al., 2012). This may account for the presence of AB in terrestrial organisms such as mushrooms (Nearing et al., 2014a). Whatever the source of AB, its predominance in seafood is probably due to its similarity to the important osmolyte, glycine betaine, which translates into AB being bioaccumulated from dietary sources. Experimentally, AB has been shown to be efficiently absorbed from seawater by mussels (Francesconi et al., 1999, Saeki et al., 2000), whereas shrimp (Cai et al., 2000) and fish (Francesconi et al., 1989, Amlund and Berntssen, 2004) accumulate AB efficiently only from food. AB is not bio-synthesized by fish when fed possible AB precursors DMAE or dimethyl arsenoacetate (DMAA) (Francesconi et al.,

1989). In mussels, the retention of AB seems to depend on the salinity of their surrounding water (Clowes and Francesconi, 2004) supporting the idea that AB can mimic an osmolyte. Similarly, a trend in increasing total As with salinity was observed across three species of pelagic fish, where AB is expected to be the dominant As species, also suggesting AB uptake and retention is related to salinity (Larsen and Francesconi, 2003).

2.3. Arsenolipids

Another group of As compounds in seafood is AsLipids, which include fatty acids (AsFA), hydrocarbons (AsHC), and glycolphospholipids (AsPL). As-containing alcohols, phosphatidylcholines and phosphatidylethanolamines have also been identified, but currently the characterization of AsLipid compounds is far from complete. There is very little information on the distribution of AsLipids in seafood, but these compounds are generally associated with oily fish and fish oils. In brown algae, AsLipids, mostly as AsPL and AsHC, have been reported to constitute 1.6–6.7% of As, and proportions may vary with taxa (Garcia-Salgado et al., 2012a, 2012b, Raab et al., 2013). A single sample of squid showed

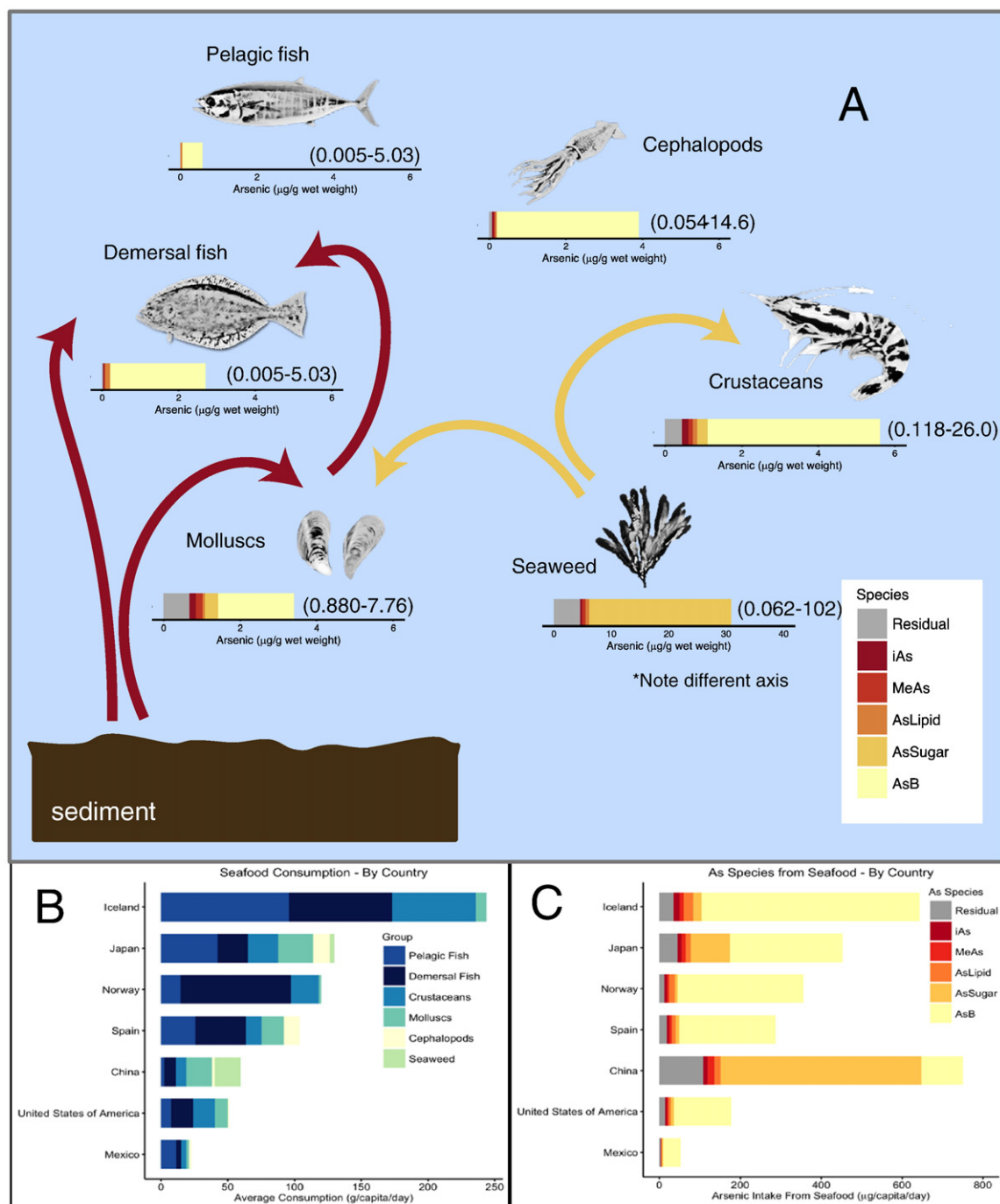


Fig. 2. Estimated intake scenarios of As species from different seafood diets. A) Estimated distributions of As species found in different seafood types. Total As concentrations are based on mean concentrations from EFSA, 2009, plotted demersal vs. pelagic fish concentrations were estimated from the EFSA mean As concentration for fish and the ratio of mean As in demersal to pelagic fish from the Norwegian fish survey (Julshamn et al., 2012); proportions of As species are estimated from literature reports (see Section 2). The range of total As concentrations (5%–95% confidence limit) from EFSA, 2009, are given in brackets. B) Seafood consumption by country based on FAOSTAT Food Consumption Database. Countries were chosen to provide a side distribution of dietary patterns, rather than to analyze a particular population. C) Hypothetical intake of As species based on estimated distributions of As compounds and seafood consumption data.

no measurable AsLipid fraction in the muscle, although lipid soluble As was detected in organs (Ninh et al., 2007). Concentrations of AsLipids are higher in pelagic than demersal fish (Lischka et al., 2013) and levels of 50–62% have been observed in filet of oily fish (Taleshi et al., 2010, Lischka et al., 2013). The relative amounts of AsHC and AsFA in fish may depend on the amount of lipophilic As present, with AsHC present in fish with higher AsLipid concentrations (Amayo et al., 2014).

Lipophilic As compounds in marine organisms were first investigated by Lunde, 1968, although their actual molecular structures remained unknown for a long time (Morita and Shibata, 1988). The first identified

AsLipid was dipalmitoylglycerophospho-2-hydroxypropyl-5-deoxy-5-(dimethylarsinoyl)-beta-ribofuranoside in the brown alga, *Unidaria pinnatifida* (Morita and Shibata, 1988). The structures of further members of this phospholipid family (AsPL) were only identified in recent years in seaweeds (García-Salgado et al., 2012, Raab et al., 2013). Research so far on several seaweeds points to AsPL958 (an As phospholipid with molecular mass 958 Da) being the dominant member of this family. These compounds are most likely esters of AsSugarPO₄ whereby they enter the phospholipid bio-synthetic pathway, but no details are yet known about their synthesis or their possible biological role.

AsHC compounds have been found in seaweed (García-Salgado et al., 2012, Raab et al., 2013) and in oily fish like capelin (Taleshi et al., 2008, Sele et al., 2013). They are likely formed by a biosynthetic pathway different from that for AsPL (Viczek et al., 2016) and may either be metabolic products or precursors of the AsFAs, which have only been identified in finfish (Rumpler et al., 2008, Arroyo-Abad et al., 2013). The members of these groups identified so far contain the dimethylarsinoyl-group and a carbon-chain of variable length and degree of saturation. A number of possible biosynthetic routes suggested for their production were summarized by Sele et al. (2012). Whether AsHC and AsFA play any biological role is not yet known.

AsLipid compounds containing AC, DMA and iAs groups have also been hypothesized based on identification of their water-soluble hydrolysis products (Shibata et al., 1996, Lai et al., 1998). So far these AsLipids have been found in lobsters, houndshark and squid (Edmonds et al., 1992, Hanaoka et al., 1999, Ninh et al., 2007). Recently five As-containing phosphatidylcholines and an As phosphatidylethanolamine compound were identified in fish roe, and are suggested to be analogs of phosphatidyl compounds found in cell membranes (Viczek et al., 2016).

2.4. Inorganic As

While iAs is not a focus of this paper, its presence is considered as a component of As exposure from seafood. Regulations for iAs seafood as a food source and for use in animal feed were recently reviewed in detail (Petursdottir et al., 2015). Concentrations of iAs are negligible in most seafoods. However, the brown algae, hijiki (*Sargassum fusiforme*), an edible seaweed used extensively in Asian cooking, is well-documented as having high amounts of total As, the majority of which is in inorganic form (Shibata et al., 1996, Almela et al., 2006, Hirata and Toshimitsu, 2007, Rose et al., 2007). Some samples of other species of brown algae have also been found to contain high proportions of iAs, suggesting more monitoring is needed (Tukai et al., 2002a, 2002b, Llorente-Mirandes et al., 2011, Maulvault et al., 2015).

Whereas taxa affects the proportion of iAs present in seaweed, elevated levels of iAs in bivalves and gastropods have been reported at some sites (Sloth and Julshamn, 2008, Whaley-Martin et al., 2012, Whaley-Martin et al., 2013), and have recently been the source of consumption guidelines in the Pacific US (OHA, 2015). Elevated iAs body burdens may correspond to augmented As in sediments and the water column, depending on the organisms' feeding mechanism, and can be caused by proximity to a point source of contamination (Lorenzana et al., 2009, Whaley-Martin et al., 2012). Conversely, levels of organic As in seafood are not affected by contaminated sites (Whaley-Martin et al., 2012). Pelagic fish, which generally have a low proportion of iAs (Julshamn et al., 2012) tend not to accumulate higher concentrations of total As from areas with elevated As, whereas benthic-feeding organisms can accrue increased concentrations of iAs (Lorenzana et al., 2009). Compared with organic As, efforts to assess iAs exposure from seafood have become more frequent, but there remains significant uncertainty in predicting iAs levels in different marine-sourced foods (EFSA, 2014, Lynch et al., 2014), again suggesting routine monitoring is needed.

2.5. Methylated As compounds

Methylated As compounds are present in marine ecosystems from enzymatic methylation of iAs to form compounds containing 1–4 methyl groups. These compounds generally occur as minor As species in seafood, with DMA being the most prominent. Molluscs can contain DMA at higher proportions (3–46%) than are typically seen in finfish or algae (Fricke et al., 2004, Cleland et al., 2009, Moreda-Piñeiro et al., 2010, Whaley-Martin et al., 2012, Berges-Tiznado et al., 2013a, 2013b). Monomethyl As (MA) is uncommon in marine environments and is generally present in trace amounts only. The trimethylated form, TMAO, another minor compound, has so far not been found in seaweeds but can occur in higher concentrations in some fish species

(Kirby and Maher, 2002, de la Calle et al., 2011). High percentages of tetramethyl arsonium (TETRA) have been found in clams (Shiomi et al., 1987) and gastropods (Francesconi et al., 1988).

The bio-synthetic pathway from iAs to TMAO has been studied extensively in fungi (Challenger, 1945) and involves reduction of the pentavalent As followed by oxidative methylation with the methyl-group being contributed by SAM (Cullen, 2014). Some of the enzymes involved have been identified also in mammals (Aposhian et al., 2004). For marine organisms, the involvement of SAM in the methylation of As has been directly shown for the alga *Polyphysa peniculus* using CD₃-labeled SAM (Cullen et al., 1994). None of the enzymes involved has been identified so far, but there is no reason to assume that the biosynthetic pathway up to DMA or TMAO is different between terrestrial fungi and marine organisms.

Other minor As compounds include AC which is rarely found in seafood, probably because it is effectively metabolized to AB (Francesconi et al., 1989), although it is a major arsenical in some sea anemones (Ninh et al., 2008) and species of jelly fish (Hanaoka et al., 2001). The methylated compounds DMAE, DMAA and dimethyl arsenopropionate (DMAPr) can be minor constituents of marine organisms (Sloth et al., 2005), but are discussed further as products of mammalian metabolism of AsSugars and AsLipids (Feldmann et al., 2000, Schmeisser et al., 2006a, Raml et al., 2005, Raml et al., 2009). Several of As compounds can also occur as thiol analogs, where sulfur replaces the oxygen atom. While these compounds have been observed in seaweeds and invertebrates (Schmeisser et al., 2004, Kahn et al., 2005, Maher et al., 2013), they will be also be discussed further as metabolites of the oxo- As compounds (Section 5).

2.6. "Residual" As

A residual, non-extractable fraction often remains following speciation analysis, and can contain a significant proportion of total As in some samples (Leufroy et al., 2012, Petursdottir et al., 2016). The form of As in this fraction remains unclear. Seaweeds can contain variable amounts of un-extractable or residual As (Lai et al., 1998, Raab et al., 2005, van Elteren et al., 2007, Petursdottir et al., 2016), which is thought to be bound to thiol-containing structural compounds (Thomson et al., 2007). Molluscs frequently have high levels of residual As (8–58%; (Fricke et al., 2004, Whaley-Martin et al., 2012, Berges-Tiznado et al., 2013a, 2013b)), and while As in crustaceans is mostly water soluble, the residual fraction can also be significant (9–17% (Li et al., 2003)). In mussels, the residual fraction was identified as predominantly As-S compounds, suggesting this to be a metallothionein-rich protein-bound fraction rather than a lipid fraction (Whaley-Martin et al., 2012). Very little is known about the metabolic fate of protein-bound As when consumed.

2.7. Effects of cooking and processing

The effects of cooking and canning, common to storage and preparation of seafood on organic As concentrations are also not well studied. Freezing and storage were observed to cause decreases in AB and total As in mussels but not finfish (Dahl et al., 2010). Loss of water during cooking can lead to an increase in concentration in total As or iAs in some seafood types (Devesa et al., 2005), whereas a loss of soluble As from seaweed has also been observed (Devesa et al., 2008). Heating has been associated with the transformation of AB to TETRA (Devesa et al., 2001, Dahl et al., 2010), but there is little information on transformation of other organic As species.

3. Intake of organic As from seafood

3.1. Consumption patterns

Globally, the highest consumers of seafood are populations from Iceland, Maldives and Japan (FAO STAT, 2012, Micha et al., 2015), while

parts of Scandinavia, the North Baltics and Southeast Asia are also high consumers (Sioen et al., 2009). Japan and Korea are high consumers of pelagic fish and shellfish (mollusks, and crustaceans), whereas pelagic and demersal fish are highest in the North Baltics, and shellfish consumption is a major source of seafood in Southeast Asia (Sioen et al., 2009). Seaweed has highly variable consumption rates between countries, and likely between ethnic sub-populations. Seaweed is a staple in Japan, Korea and China, and consumption rates in Japan have been estimated as high as ~20 g wet wt per day (Yamauchi et al., 1992), with a shift in the prominent type of seaweed consumed from kombu, which can have higher iAs concentrations (EFSA, 2014), to wakame and nori (Zava and Zava, 2011).

The USA and Western Europe are medium consumers of seafood on a global scale (FAOSTAT, 2012), yet seafood consumption is estimated to account for 90% of total As exposure in the U.S. (U.S. F.D.A., 1993). Variation between sub-populations is large, both in terms of total consumption and seafood type. Sex, age and proximity to the coast influence fish consumption, and ethnicity plays a major role in consumption rates, with the “Other” category of ethnicity, which includes Asians, Native Americans, Pacific and Caribbean Islanders and mixed races, having the highest consumption of fish and shellfish (Mahaffey et al., 2004, U.S. E.P.A., 2014).

The consumption of different classes of seafood from several countries with varying diets is shown in Fig. 2B. Hypothetical intake of As species by different consumer groups was then estimated in Fig. 2C, based on median seafood concentrations and estimates of As species distribution (Fig. 2A). Exposure is also affected by the concentrations of organic As species in seafood. Concentrations of total As varied by greater than two orders of magnitude for most of the seafood types (EFSA, 2009), and the distribution of As species can also vary. Intakes were compared using estimates of augmented iAs reported in mussels (Sloth and Julshamn, 2008), and of higher AsLipid concentrations found in oily pelagic fish (Taleshi et al., 2010) compared with median estimates (Fig. 3). The effect of these reported As species concentrations on intake suggests further evaluation of As in seafood is needed.

3.2. Evidence of exposure from biomarkers

Despite a lack of complete speciation data across seafood types for estimating exposure, indirect evidence for the presence of organic As compounds in food comes from urinary biomarkers. While AB has been shown to pass through the body rapidly and unchanged, both AsLipids and AsSugars break down to form DMA as the major metabolite in urine (Schmeisser et al., 2006a, 2006b, Raml et al., 2009) (Fig. 4). Feeding studies have found DMA to be the most abundant form of As in urine following repeated ingestion of seaweed, shellfish, and fish, although individual variability is high (Choi et al., 2010). In a series of studies where 3 types of seafood (cod, salmon and mussels) were consumed, urinary total As, AB, and DMA concentrations were elevated following consumption (Molin et al., 2012a, 2012b, Molin et al., 2014), as was MA for the mussel consumption group (Molin et al., 2014). While urinary DMA is also a product of iAs metabolism, low levels of iAs in the test meals confirmed organic As to be the major source of the DMA metabolite in these studies. Similarly, concentrations of DMA in urine were found to increase substantially following herring consumption, with total DMA excretion reaching 6 times the amount consumed, again suggesting the remainder came from breakdown of organic As (Heinrich-Ramm et al., 2002).

Several population studies have found recent seafood consumption to be a predictor of elevated urinary As (Cleland et al., 2009, Navas-Acien et al., 2011, Birgisdottir et al., 2013, Calderon et al., 2013, Davis et al., 2014, Fort et al., 2014). Elevated urinary AB was observed with seafood consumption (Caldwell et al., 2009) (Navas-Acien et al., 2011), which is expected as it is the major component of As in most seafood. Increases in urinary DMA have also been associated with seafood consumption in some studies (Buchet et al., 1996, Navas-Acien et al., 2011, Lovregio et al., 2012). Shellfish consumption was linked with increased total urinary As, as well as DMA, MA and iAs in a population of high seafood consumers (Soleo et al., 2008). In a large National Health and Nutrition Examination Survey (NHANES) study of U.S. populations, both DMA and, to a lesser extent, MA, were found to correlate with AB in urine, suggesting a seafood source of the methylated compounds

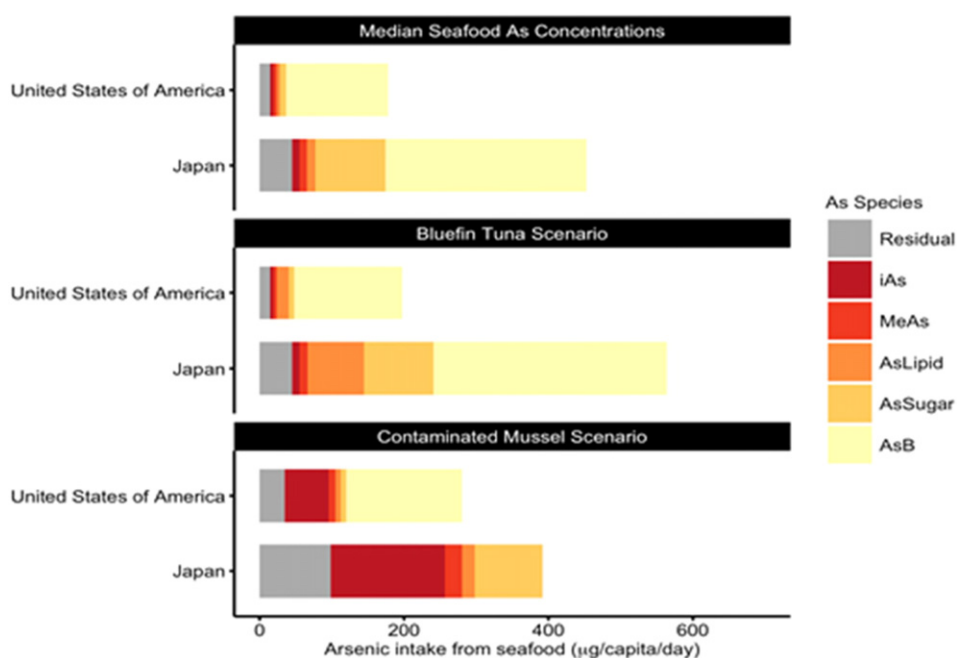


Fig. 3. Hypothetical intake of As species based on seafood consumption data for two populations (Japan and the USA), using estimated distributions of As compounds from median total As concentrations (EFSA, 2009) and distributions from the literature (Fig. 2A), compared with the same diet but where Bluefin tuna having 50% AsLipid and 3.2 mg/kg As wet wt (converted from 5.9 mg/kg As dry weight, present as equal parts fat soluble and water soluble As reported by Taleshi et al., 2010, assuming 80% water content) as the source of pelagic fish, or where mussels with elevated concentrations of iAs (42% of 13.8 mg/kg As wet wt reported by Sloth and Julshamn, 2008) were the source of bivalves.

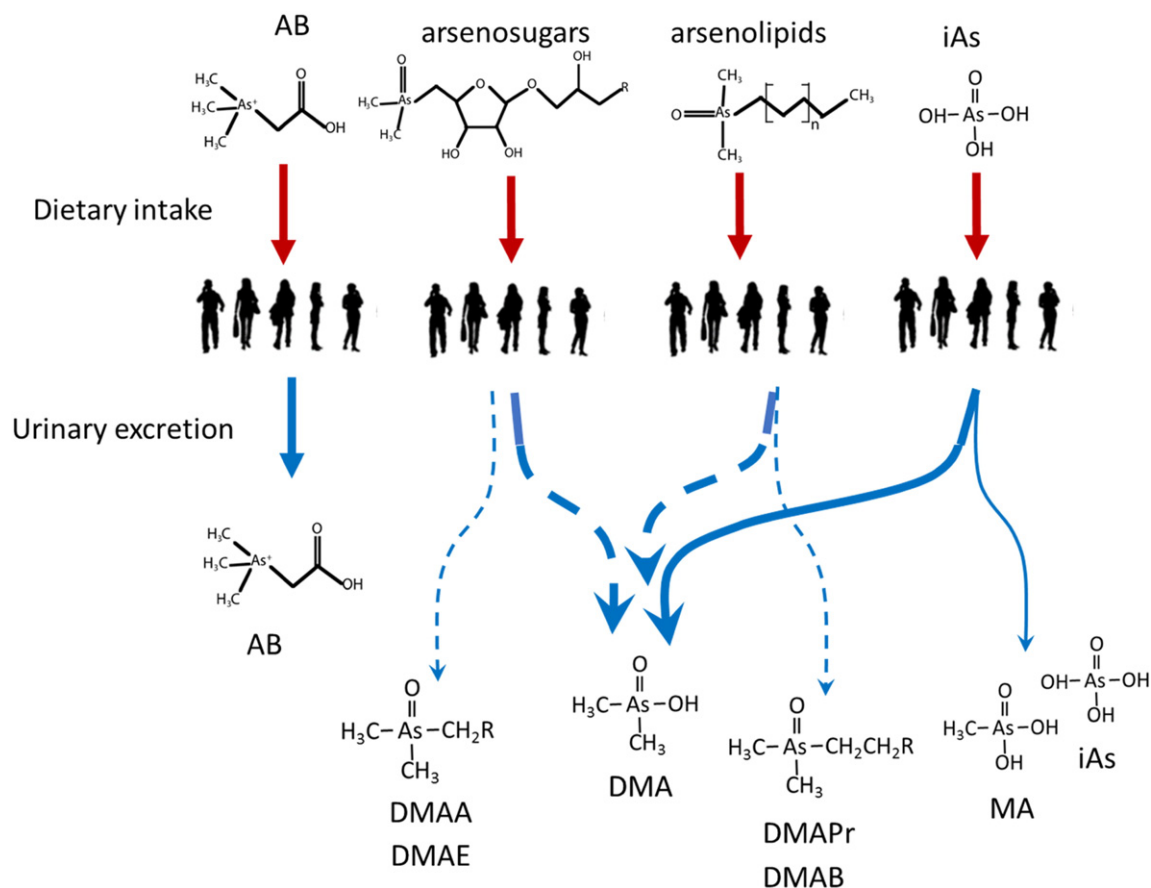


Fig. 4. Major urinary metabolites from ingestion of AB, AsSugars, AsLipids and iAs. Dashed lines represent large variability in excretion between individuals.

(Aylward et al., 2014). While seafood type was not specified in that analysis, tuna, salmon and shrimp are the most highly consumed seafoods in the U.S. (NOAA, 2010). Because AsSugar concentrations are expected to be low in these marine species, AsLipid metabolism may be the source of urinary DMA.

Because DMA is also used as a biomarker for studies of iAs exposure, the presence of urinary DMA from the breakdown of organic As *in vivo* is confounding to these studies (Aylward et al., 2014). Metabolic studies, based on a small number of samples, have suggested the presence of minor As compounds in urine following AsSugar (DMAA, DMAE) (Raml et al., 2009) and AsLipid (DMAPr) (Schmeisser et al., 2006a, 2006b) intake. Neither recent nor habitual seafood consumption, were associated with speciated urinary As or toenail As in a population with a wide range of drinking water As from Nevada (Calderon et al., 2013). Likewise, neither current seafood or seaweed consumption were associated with toenail As in a Japanese population (Tabata et al., 2006). However, studies of recent exposure would not necessarily be expected to find association with a long-term biomarker such as toenails. In an analysis from NHANES data, 24-hour recall of fish and shellfish was associated with urinary As species including DMA (Davis et al., 2014). Additionally, dark fish meat intake based on a food frequency questionnaire was positive related to toenail As in a population-based study from New Hampshire (Cottingham et al., 2013) and a study from Michigan (Slotnick et al., 2008). In a pilot study from Puerto Rico, nail As more so than hair or urine As was related to seafood consumption (Mansilla-Rivera et al., 2014). Elevated total As in urine and blood (Birgisdottir et al., 2013) and maternal blood, infant cord blood and breast milk (Miklavcic et al., 2013) also have been associated with high seafood consumption. Further studies are needed to better characterize biomarker concentrations of arsenic compounds in fish and seafood particularly the AsSugars and AsLipids.

4. Analytical/monitoring considerations

Shortcomings in evaluating exposure to organic As in seafood are largely due to analytical complications in reliably determining the complex distribution of As species in some of these samples. Complete reviews of As speciation techniques have been reported elsewhere (Francesconi and Kuehnelt, 2004, Tyson, 2013, Nearing et al., 2014a, 2014b, Maher et al., 2015); here, we focus on the issues most relevant to determining organic As species in seafood. Speciation analysis for organic As is almost exclusively achieved by liquid chromatography coupled with inductively coupled plasma (ICP) - mass spectrometry (MS) and/or electrospray ionization MS as a detector. This approach consists of four main aspects: extraction, separation, detection and characterization (identification) (See Fig. 5).

4.1. Extraction

Ideally, extraction schemes should quantitatively release As from any sample matrix, preserve each species in an unaltered state, and use an extraction media that is compatible with the intended chromatographic method. In reality, compromise between these goals is necessary, and the extractable concentrations of As species are operationally defined. Due to the broad range of properties of As compounds, using a tailored approach for extracting each As species of interest has been suggested (Francesconi and Kuehnelt, 2004), but where speciation is complex, grouping As species into fractions based on their toxicity (eg. AB, AsSugars, iAs) may be more practical for high throughput monitoring (Feldmann and Krupp, 2011). The inclusion of AsLipids in such an extraction scheme introduces the need for a sequential extraction, and the current lack of AsLipid data in seafood suggests this to be an important addition to current monitoring protocols.

The water soluble As compounds are frequently extracted with MeOH, H₂O, or a mixture of both (Niegel and Matysik, 2010). However, recovery of As compounds by this method can be low for marine algae (Lai et al., 1998, Madsen et al., 2000, Tukai et al., 2002a, 2002b, Kahn et al., 2005) and for oily or fatty fish having high proportions of non-polar arsenicals (Ciardullo et al., 2010, Ruttens et al., 2012). Mildly acidic extractions appear to give higher extraction efficiencies (Foster et al., 2007, Sadee et al., 2016), but this is likely due to acid hydrolysis causing the release of degradation products from As compounds in the lipid and protein fractions. Acidic media can also lead to degradation of different AsSugars to a single riboside species (Gamble et al., 2002, Foster et al., 2007). The same AsSugar degradation has been observed in basic extractions, along with riboside cleavage to form small amounts of DMA (Gamble et al., 2003). High extraction efficiencies therefore, often need to be sacrificed in the interest of preserving species integrity, although reporting of “total AsSugar” may be sufficient for most monitoring needs (Feldmann and Krupp, 2011).

Extraction of AsLipids has been achieved successfully using a number of approaches, often with mixtures of polar and non-polar organic solvents (Amayo et al., 2011, Amayo et al., 2014, Glabonjat et al., 2014, Sele et al., 2015). The AsLipids can be extracted effectively from fish using MeOH/dichloromethane (DCM) (Amayo et al., 2011, Amayo et al., 2014) or MeOH/chloroform (Taleshi et al., 2010) mixtures, which recover the polar AsLipid species. For fish oil, samples have been partitioned by hexane/heptane and MeOH/MeOH:H₂O (Schmeisser et al., 2005, Rimpler et al., 2008, Taleshi et al., 2008, Sele et al., 2013, Sele et al., 2014). Analysis of hexane extracts of fish oil is simplified by silica gel fractionation to remove As-free lipid interferences (Amayo et al., 2013, Amayo et al., 2014). For marine algae, MeOH/DCM extracts also required clean-up using silica gel to improve chromatographic separation (Glabonjat et al., 2014).

Given the diversity of As species in seafood products, a single extraction is unlikely to be optimal over a range of sample matrices, or to extract all As species present with equal recovery (Francesconi and Kuehnelt, 2004). A 2-step procedure could maximize extraction of As

species from most seafood sample types, combining a sequential aqueous extraction to extract polar species and a non-polar solvent to liberate AsLipids. Additional steps which are often applied to improve recoveries include heating, shaking, repeated extraction or sonication. With ICP-MS detection, organic extractants can affect the plasma, and evaporation may be necessary prior to analysis. Further consideration of the extraction scheme is compatibility with the mobile phase used in the chromatographic separation, which may require evaporation/dissolution or pH adjustment of the extract to match the mobile phase. Manipulations may also be applied to convert multiple As compounds to a single species to provide simpler chromatograms and spectra; for example, H₂O₂ is used to convert As(III) to As(V), where it may elute as a single peak, and to oxidize thio-analogs of AsSugars to their oxo-forms (Schmeisser et al., 2004). Addition of H₂S has also been applied to convert AsLipids to their thiol-analogs (Glabonjat et al., 2014), which are more effectively separated by chromatography. Differing extraction efficiencies are often the cause of discrepancies between studies, and as such, known values for As speciation can be defined for a specific extraction method to provide a consistent measure for comparison between laboratories (van Elteren et al., 2007).

4.2. Separation

For seafood samples, a single chromatographic separation, even for the water soluble As species, is often insufficient to characterize all the species present. Separations based on anion exchange, cation exchange and reversed phase columns have all been shown to be useful, depending on the species present. Using a single column, Kohlmeyer et al. reported a HPLC method capable of separating 17 As species in seaweed and oysters (Kohlmeyer et al., 2002). Sloth et al. also reported 23 peaks in cation exchange separations of clam kidney extracts, mostly comprising unidentified species (Sloth et al., 2003).

More realistic is the application of multiple separations. Anion and cation exchange methods can be used complementarily to separate a similar number of species while decreasing the likelihood of co-elution

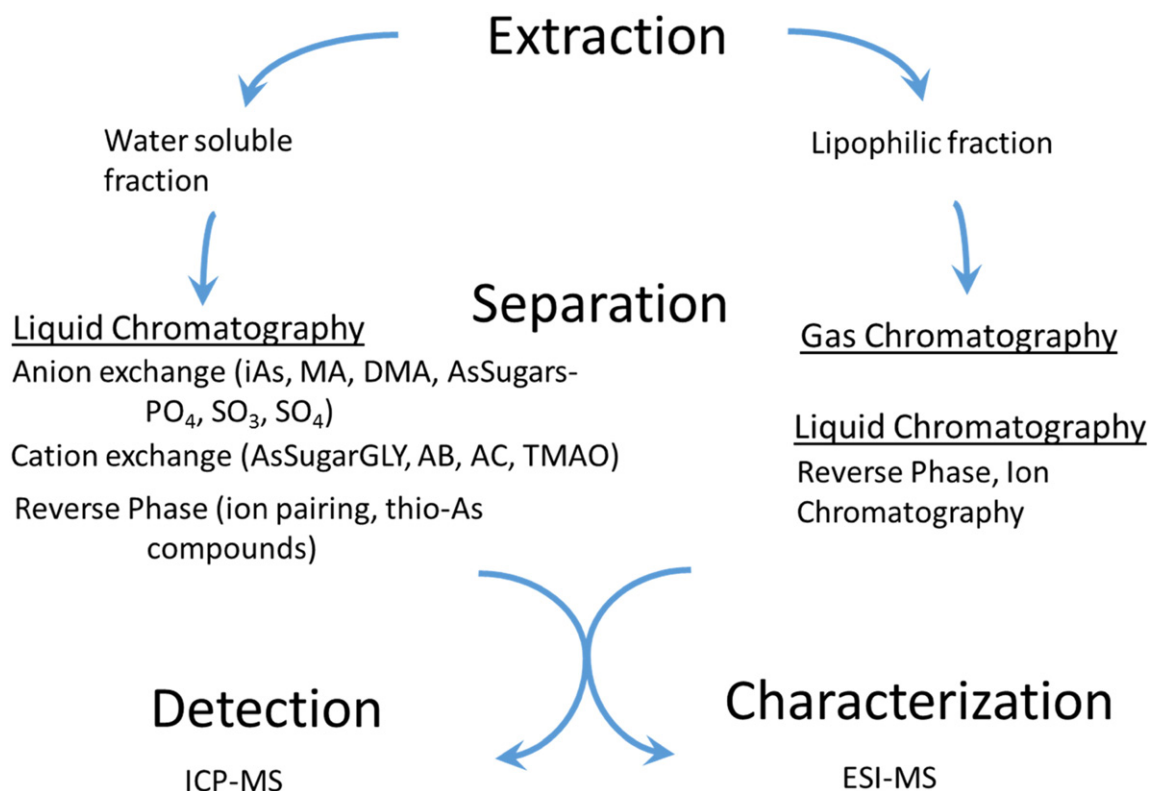


Fig. 5. Broad overview of identification/determination of As compounds in seafood.

(Wuilloud et al., 2006, Ciardullo et al., 2010). DMA, MA, and four commonly occurring AsSugars are all separated using anion exchange columns, while cation exchange chromatography provides effective separations for AB, AC, DMA, TMAO, TETRA and DMAA (Madsen et al., 2000, Sloth et al., 2003). AsLipids require a third separation, usually with a C-8 (Glabonjat et al., 2014) or C-18 column (Amayo et al., 2011, Sele et al., 2014). Gas chromatography has also been applied to AsHC analysis, with both electron impact MS (Raber et al., 2009) and ICP-MS (Sele et al., 2013) detection.

4.3. Detection

Of the four aspects of seafood As speciation discussed here, detection is the most straightforward: ICP-MS is the technique most widely used for detection of organic As species, due to compatibility with HPLC, excellent detection limits and linear range capable of quantitating low ng/L constituents as well as > 1 mg/L-level AB in the same run. Specificity for As-containing compounds is extremely good, especially since the introduction of collision cells that use either He collision or O₂ reaction to reduce interference from ArCl⁺, the most significant interference at *m/z* 75 (As⁺). Separations that involve mobile phases with high organic content (as is the case for AsLipids) can cause problems for the ICP in terms of plasma stability and carbon build-up on the cones, but these issues can be mitigated by adding oxygen to the plasma (Meermann and Kiesshauer, 2011). Changes in sensitivity associated with gradient elutions can also be diminished by addition of organic solvent to the sample flow (Ruiz-Chancho et al., 2012) among other approaches (described in (Sele et al., 2014)).

4.4. Characterization

Electrospray - MS is often used to propose structures for unknown species observed in HPLC-ICP-MS chromatograms. Assigning structure to unknown compounds has been achieved by high resolution MS, an approach that has been applied to AsLipid characterization in multiple matrices ((Taleshi et al., 2014) and refs therein) and AsSugars (Nischwitz et al., 2006). Complete identification of As compounds requires synthesis and further characterization (NMR). This has been achieved for two of the AsSugar compounds (Traar et al., 2009) and the metabolites DMAE (Edmonds et al., 1982) and DMAA (Francesconi et al., 1989), and, recently, nine of the AsLipid compounds (4 AsHC and 5 AsFA) (Taleshi et al., 2014, Arroyo-Abad et al., 2016).

4.5. Standards and reference materials/interlab QA

The two most glaring needs for those performing As speciation analysis of seafood samples are commercially available As species standards and certified reference materials. Commercial standards allow for accurate quantification of species as well as preparation of QC samples such as fortified blanks and fortified analytical portions. Without standards for calibration, determining concentrations of species like AsSugars accurately is challenging. Commercially available organic As species standards (relevant to seafood analysis) are limited to MA, DMA, AB, AC, TMAO and TETRA. One approach for standard-less quantification is to assume that the ICP-MS response for As is independent of the type of As species; one can then use a commercially available As species to prepare the calibration curve to calculate the needed concentration. Although ICP-MS sensitivity has been shown to vary somewhat from species to species (Grotti et al., 2013), this approach easily generates approximate values which are often sufficient-for-purpose. A more labor intensive (also more accurate) approach is to isolate individual species *via* fraction collection and determine the total As content in the fraction using a method that measures As without regard to the molecule it's incorporated into, such as instrumental neutron activation analysis (INAA), although the practicality of this approach is limited (Yu et al., 2015).

Certified reference materials are critically important in method development, ensuring proper method performance and inter-laboratory data quality. There are at least 6 seafood-matrix reference materials currently available that give values for at least one organic As species. Unfortunately, five of the six are only certified for AB (NMIJ CRM 7402-a, NMIJ 7403-a, DORM-4, TORT-3 and NIES-15), and BCR-627 is only certified for AB and DMA. Although not seafood, NIST 2669 and 3669 are urine reference materials certified for MA, DMA, TMAO, AB and AC, and MA, DMA and AB, respectively. New releases are anticipated in the forms of candidate NIST SRM 3232 kelp, which was analyzed for DMA as well as three AsSugars: AsSugGly, AsSugPO₄ and AsSugSO₃ (Yu et al., 2015), which uses a defined extraction procedure approach. NIST is also planning to generate values for As species in mussel SRM 1974 (AB and MA) and SRM 2986 geoduck (DMA and AB) (Yu et al., 2016).

The number of As species identified in marine organisms is well over fifty (Francesconi, 2010), and probably very much higher as a result of the large number of lipophilic As compounds reported in the last few years (Francesconi and Schwerdtle, 2016). Of these compounds, only 6 organic species are currently available commercially, and only 8 are represented in reference materials. Prospects for standards and reference materials for AsLipids are not as bright as for other species. For many As species (including both known and previously unidentified species), the most practical way to procure a supply for use as in-house standard may be to do fraction collection of the peak, concentrate, purify, and characterize by NMR and/or MS/MS.

The analytical difficulties associated with As speciation become apparent in round robin studies, where agreement between laboratories tends to be poor, particularly for seaweed samples where most laboratories overestimated the concentration of iAs in the presence of AsSugars, likely due to peak overlap or misidentification (de la Calle et al., 2012). Considerably better agreement on As speciation in kelp was achieved in an inter-laboratory study where an extraction procedure was prescribed, despite differences in chromatographic methods (Raab et al., 2005).

5. Pre-systemic metabolism and bioavailability

Arsenic is primarily excreted in urine (Aposhian, 1989). The majority of absorption and metabolism data for As in seafood is therefore derived from feeding studies that investigated total As and urinary As species in mammals. These studies have clearly shown that As-containing compounds in seafood are absorbed through the gut and excreted in the urine. The metabolic pathways of organic arsenicals, however, have not yet been defined and specific sites of metabolism are unknown. The role of pre-systemic (*ie.* gut) vs systemic (liver, kidney) metabolism is a key knowledge gap, and further research is required to identify sources of inter-individual differences in As metabolism and excretion. Existing data have identified important differences in the absorption and metabolism of the organic As compounds, which will impact systemic exposure and potential biological effects. We compare bioaccessibility and metabolism of AsSugars, AB, AsLipids and their breakdown products, and highlight knowledge gaps that are critical for assessment of health effects of As exposure from seafood.

5.1. Arsenosugars

Unlike AB, AsSugars are metabolized and broken down to smaller compounds following retention in the body. Absorption and excretion of AsSugars (Le et al., 1994, Francesconi et al., 2002, Raml et al., 2005) is much slower than for AB or AsLipids (Schmeisser et al., 2006a, 2006b), and highly variable between individuals. In studies of a single consumption of seaweed (Le et al., 1994, Ma and Le, 1998, Wei et al., 2003, Van Hulle et al., 2004), or pure AsSugar (Francesconi et al., 2002, Raml et al., 2005, Raml et al., 2009), several volunteers showed no increase or only a slight increase in urinary As concentrations, whereas

others excreted up to 95% of the ingested As (Raml et al., 2009). One study also repeated the consumption experiment with volunteers who had the lowest (4%) and highest (95%) recovery of the ingested As, and results were found to be consistent (Raml et al., 2009). Possible explanations for the long retention times and variability in metabolism of AsSugars may be differences in metabolism by gut microflora, passage across the intestinal barrier, or transformation in the liver.

Insight into AsSugar metabolism has also come from studies of the seaweed-eating sheep from N. Scotland. Unlike the studies of human subjects which are based on a single consumption of seaweed, these sheep are chronically exposed to AsSugars, yet have a similar pattern of As excretion, where urinary concentrations peak about 20 h after ingestion (Hansen et al., 2003). Urinary As concentrations were elevated in all 12 sheep in a seaweed-feeding study (Hansen et al., 2003), providing no evidence of ovine “low and high-excretors”. In the sheep, tissue As concentrations were not elevated to a high degree (Feldmann et al., 2000), and only 4–20% of ingested As was found in feces, suggesting that most As is excreted in urine (Hansen et al., 2003). Although this interpretation is very likely correct, it could not be confirmed owing to the difficulty in obtaining 24 h urine samples from the sheep.

5.1.1. Arsenosugar metabolites detected in humans

Most studies of AsSugar metabolism have observed that DMA(V) is the major metabolite in urine (Le et al., 1994, Van Hulle et al., 2004, Raml et al., 2009), but sites of transformation in the body are unclear. Additional metabolites in urine following AsSugar consumption have been identified as the oxo- and thio-analogs of DMAE and DMAA, and an unknown metabolite (Raml et al., 2005), all of which are thought to be intermediates in the conversion of AsSugars to DMA (Raml et al., 2005). Trace levels of AsSugars in both oxo- and thio-form, TMAO (Francesconi et al., 2002) and its sulfur analog TMAS, as well as thio-DMA have also been observed (Raml et al., 2009). Interestingly, Raml and coauthors found that low-excretors of As primarily excreted intact AsSugars and DMA, whereas high-excretors produced mainly DMA (40–46%), thio-DMAA (15–19%), thio-DMAE (5–9%), and the thio-unknown (3–8%). These data suggest that different metabolic mechanisms occur pre- vs. post-uptake through the gut. Trace levels of AsSugars, as well as the thio-derivatives of DMAA and DMAE were also detected in blood serum of the high excretors, suggesting that these compounds formed in the gut or liver rather than in the kidneys or bladder (Raml et al., 2009).

5.1.2. Bioaccessibility and pre-systemic metabolism of arsenosugars

A handful of studies have employed *in vitro* methods to examine the potential for metabolism of AsSugars in the gut. When exposed to acid and mild heat, replicating gastric conditions, two AsSugar analogs (Gly, SO₃) underwent aglycone cleavage yielding a compound with *m/z* 254, with the As-riboside ring remaining intact (Gamble et al., 2002, Van Hulle et al., 2004). The reaction proceeds slowly (1.4% per hour at 38 °C) suggesting only minor conversion will occur during digestion, and was not observed in seaweed extracts under simulated gastric and intestinal conditions (Almela et al., 2005), likely due to the short incubation times. Incubation of oxo-AsSug-SO₃ extracted from kelp with mouse cecal microflora at body temperature resulted in rapid conversion to the thiol analogs (Conklin et al., 2006). Breakdown of the riboside ring and formation of DMAE has been observed under reducing conditions; it has been proposed to proceed in the gut (Edmonds et al., 1982), but such a process has not yet been observed.

Permeability of a suite of organic As compounds across the intestinal barrier using the Caco-2 cell model are summarized in Fig. 6. *In vitro* studies in the Caco-2 cells found AsSugar-Gly and AsSugar-SO₃ had low intestinal permeability (1.7 and 4.8 %) compared to iAs (62%) (Leffers et al., 2013a, 2013b, 2013c). Permeability of thio-AsSugar-Gly was found to be twice as high as its oxo- analog, but still ~20 times lower than As(III) or AsLipids (Ebert et al., 2016). The AsSugar metabolites, thio-DMA and thio-DMAE, were observed to have much higher

permeabilities, similar to iAs (Leffers et al., 2013a, 2013b, 2013c), whereas oxo-DMAE and both oxo- and thio-DMAA had low transfer rates (Leffers et al., 2013a, 2013b, 2013c). This suggests conversion in the gut plays an important role in AsSugar metabolism, leading to higher uptake of the metabolic intermediates. It is also noted that the Caco-2 model underestimates transfer *via* paracellular (between cell transfer) pathways, and underestimation of the uptake mechanisms of some species relative to intestinal cells *in vivo* cannot be ruled out (Leffers et al., 2013a, 2013b, 2013c).

5.1.3. Systemic metabolism of arsenosugars

While there is evidence of AsSugar transformation to the thiol analog in the gut, this transformation has also been observed in the reducing environment of the liver. Thiol derivatives of AsSugars in kelp extract were produced from incubation with liver cytosol, but with no evidence of breakdown of the As-riboside ring (Hansen et al., 2004a, 2004b). However, conversion of AsSugars to DMA(V) was observed in HepG2 incubations. While only a small proportion (6%) of thio-AsSug-Gly was broken down *in vitro* (Ebert et al., 2016), the functionality of the cell line may be lower than liver cells *in vivo*, explaining the higher transformation seen in humans.

5.2. Arsenobetaine

As the first organic As compound identified in seafood, AB is the best-studied. AB is highly bioavailable; it is absorbed through the gut epithelium following consumption and rapidly excreted unchanged in urine (Cannon et al., 1981, Vahter et al., 1983, Kaise et al., 1985, Francesconi, 2010). *In vitro* incubation of AB with gut microflora for 30 days demonstrated that AB could be broken down to DMA, DMAA and TMAO after 7 days by aerobic gut bacteria (Harrington et al., 2008). This study suggests microbes capable of degrading AB are present in the human gut, but the incubation time is much longer than realistic gut passage time, and this metabolic pathway has not been observed *in vivo*. Because ingested AB is immediately excreted unchanged (Kaise et al., 1985, Edmonds and Francesconi, 1993) and no toxic effects have been associated with AB exposure (Kaise et al., 1985), any metabolism that may occur is expected to be minor.

A handful of studies suggest that AB is formed *in vivo* or accumulated in the body and slowly released. Newcombe et al. found 3 out of 5 volunteers who adhered to a strict rice diet, which excluded seafood and other foods, excreted AB (Newcombe et al., 2010); in a feeding study where 38 individuals consumed a controlled seafood portion (cod, salmon, mussels), AB recovered in urine exceeded the ingested amount (Molin et al., 2012a, 2012b). Urinary AB was also measurable in 25% of individuals who reported no seafood consumption in the past year, based on NHANES data examining seafood consumption in the US population relative to urinary As excretion (Navas-Acien et al., 2011). However, all of these studies rely on diet restriction and self-reporting, which are prone to human error. Radiolabeled AB fed to human volunteers was found to be dispersed among soft tissues without any particular localization, and excretion of 99% of the As was complete after 24h (Brown et al., 1990). A mechanism for AB formation *in vivo* has not been described.

5.3. Arsenolipids

Few studies have investigated bioaccessibility and metabolism of AsLipids. The limited existing data suggest that AsLipids are rapidly absorbed through the gut, but in contrast to AB, are metabolized before excretion. A study conducted with two volunteers who consumed cod liver oil, in which AsLipids accounted for 25–77% of the extracted arsenic, found that As was excreted in urine 6–15 h following consumption, and more than 85% of the consumed arsenic was excreted after two days (Schmeisser et al., 2006a, 2006b). Furthermore, As speciation analysis indicated strong metabolic breakdown; DMA(V) accounted for up

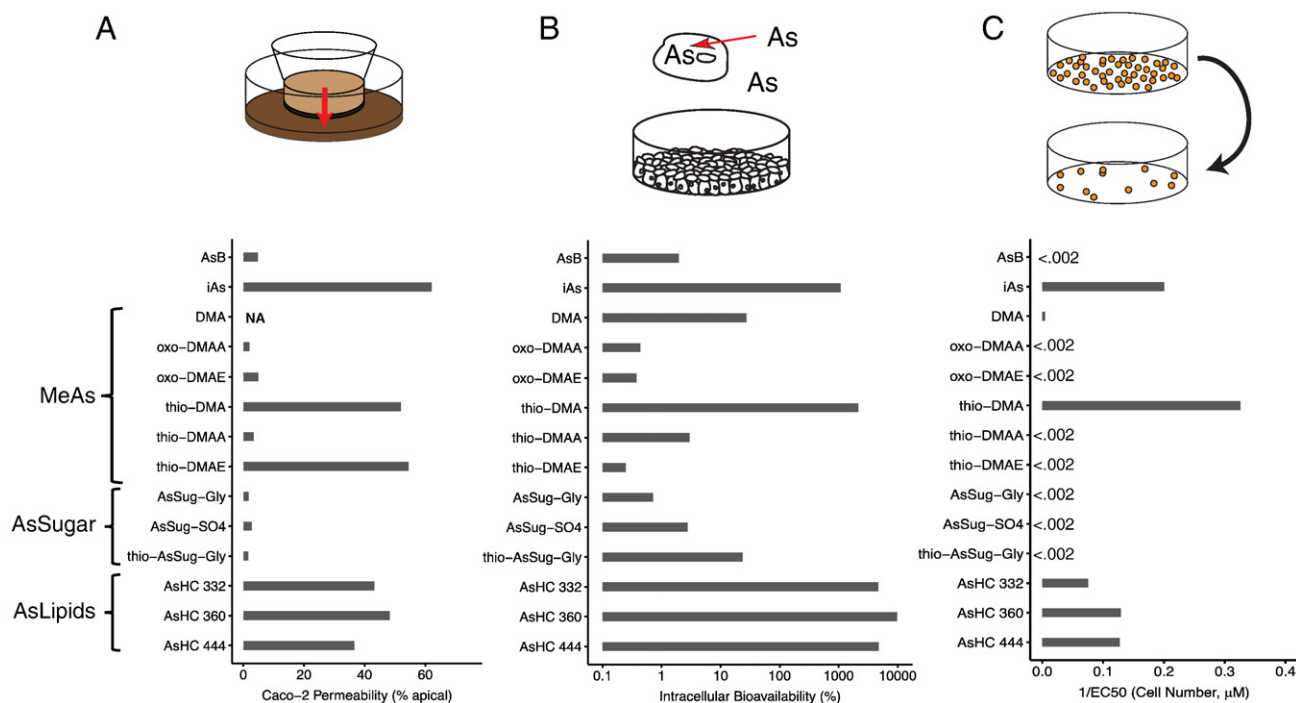


Fig. 6. Comparative permeability, bioaccessibility and effect on cell number for As species are summarized from previously published data (Leffers et al., 2013a, 2013b; Meyer et al., 2014a, 2015b, 2015a, 2014b; Ebert et al., 2016). A) Permeability of As species (apical to basolateral) after 48 h apical incubation with Caco-2 cells. Permeability of As species was determined at the following concentrations: 1 μM iAs; 2.5 μM thio-DMA; 50 μM AsHC332, AsHC 360 and AsHC444; 100 μM thio-AsSug-Gly, and 500 μM AB, AsSug-Gly, AsSug-SO₃, oxo-DMA, thio-DMA, oxo-DMAE and thio-DMAE. Data represent percent applied apical concentration detected on basolateral side. B) Bioavailability of arsenic at 48 h in URO2SA cells. Data are presented as percent of applied concentration detected in cells after 48h incubation. Concentrations of arsenic species applied are as follows: 1 μM AsHC332, AsHC 360, AsHC444, iAs and thio-DMA; 100 μM thio-AsSug-Gly and DMA; and 500 μM AB, AsSug-Gly, AsSug-SO₃, oxo-DMA, thio-DMA, oxo-DMAE and thio-DMAE. C) Effect of As species on URO2SA cell number at 48 h. Data are presented as 1/EC50 values, calculated from cell number data (minimum of 6 concentrations) fit with a Weibull (type 1) 3-parameter curve in R (DRC package).

to 70% of excreted As, and no intact AsLipids were identified in urine. Minor excreted compounds were water-soluble AsFA's with shorter hydrocarbon chains, namely oxo- and thio-derivatives of DMAPr as well as dimethylarsenobutanoic acid (DMAB), with concentrations less than 5%, respectively (Schmeisser et al., 2006a, 2006b).

5.3.1. Bioaccessibility and metabolism of arsenolipids

While the site of AsLipid metabolism *in vivo* has not yet been investigated, AsLipids are readily absorbed by cells in culture. *In vitro* assessment of intestinal bioaccessibility of AsHCs and AsFAs in a Caco-2 intestinal cell model indicated that AsHCs (AsHC 332, AsHC 360, AsHC 444) are intestinally bioavailable (up to 50% permeability) to humans (Meyer et al., 2015a, 2015b). The AsHCs were transported essentially unchanged, with only trace amounts of thiol- and reduced As(III) analogs of these compounds observed on the blood-side of the barrier. Two AsFAs that were studied showed lower but still substantial intestinal bioavailability (up to 22% permeability), and were efficiently biotransformed to polar As metabolites including DMA(V) while passing the *in vitro* intestinal barrier model (Meyer et al., 2015a, 2015b). These As permeabilities were 2–5 times higher than AB or AsSugar permeabilities measured in the same Caco-2 system (summarized in Fig 6A). Unlike water soluble As compounds, for which passage across the intestinal wall is thought to be mediated by aquaglyceroporin transport, AsHC's and AsFA's are thought to permeate by passive diffusion (Meyer et al., 2015a, 2015b).

No studies were identified regarding the toxicokinetics of AsLipids in experimental animals. Nevertheless, two recent papers provide evidence that AsHCs are highly bioavailable to the fruit fly, *Drosophila melanogaster* (Meyer et al., 2014a, 2014b, Niehoff et al., 2016). In all developmental stages of the fruit fly (parental flies, larvae, pupae and flies of the F1 generation), As amounts were vastly higher after incubation with two hydrocarbons, AsHC 332 and AsHC 360, compared to a longer-chained hydrocarbon, AsHC 444 and As(III). These data indicate

that AsHCs, particularly those with shorter hydrocarbon chains and less lipophilicity, are able to accumulate highly in the body of the fruit flies (Meyer et al., 2014a, 2014b).

In a follow-up paper, LA-ICP-MS was applied to assess the accumulation and distribution of arsenic in larvae and adult fruit flies of the F1 generation following administration of AsHC 332 and As(III) in feed (Niehoff et al., 2016). Larvae fed with AsHC 332 showed an inhomogeneous distribution of As, where the high As regions constituted a five-fold enrichment compared to the fed concentration. Larvae fed with As(III) showed a fairly homogeneous distribution of As in the whole organism, which was three-fold less than the concentration consumed. Adult flies fed these compounds exhibited large differences in storage of As(III) and AsHC 332. The most important difference by far was that As was detected in the brain of the fly when they were administered AsHC 332, but not when the administered arsenical was As(III). Moreover, a combination of quantitative elemental imaging of As by LA-ICP-MS and molecular imaging by MALDI-MS revealed that AsHC 332 itself was accumulated in the brain of *Drosophila melanogaster* indicating the transport of the AsHC 332 across the blood-brain barrier.

5.4. Methylated As species and thiolated analogs

While numerous studies report methylated As species distribution in blood and urine as products of iAs metabolism, there is less focus on the absorption and fate of methylated As compounds from food. Methylated As species are a minor component of most seafoods; the most common methylated species, DMA, is largely unchanged in the body (Buchet et al., 1981). Significant methylation of DMA to form TMAO occurs in rodents, but only traces of TMAO (3.5%) were observed in human urine following DMA ingestion, and breakdown of DMA to MMA or iAs has not been observed *in vivo* (Marafante et al., 1987). By the Caco-2 cell model, transport of DMA and MA across the intestinal barrier was low relative to As(V) (3–11% MA(V); 4–6% DMA(V); 3–

25% As(V) after 24 h) (Calatayud et al., 2010). While Caco-2 cells model absorption in the large intestine where physiological pH is neutral to slightly basic, lower pH conditions, which are found in the stomach and small intestine, have been shown to significantly increase (4–8×) absorption of DMA and As(V) (Calatayud et al., 2010), suggesting absorption may occur at other sites. Paracellular transport was also suggested to be significant for both DMA and MMA (Calatayud et al., 2010).

The formation of thioarsenical species from methylated As by gut microflora has been demonstrated *in vitro*, and is an important metabolic step because of the increased bioavailability and cytotoxicity of some of these intermediates. Conversion of oxo- to thio- analogs of DMA (Yoshida et al., 2003, Kubachka et al., 2009) and MA (Rubin et al., 2014) have been observed from gut microbes, as well as during incubation of DMA with liver cells (Meyer et al., 2015a, 2015b). Thio-DMA and TMA were present in urine at dose dependent concentrations in rats that ingested DMA(V), but only in trace concentrations in rats treated with iAs, suggesting the gut is the major site of thiol formation (Adair et al., 2007), because iAs is metabolized in the liver. As discussed previously, thio-DMA and thio-DMAE crossed the intestinal barrier in the Caco-2 cell model much more readily than DMA(V) and oxo-DMAE (Fig. 4A).

AC has a similar structure to AB, but is only present in very small quantities in seafood (Edmonds and Francesconi, 1993). Like AB, AC is efficiently absorbed from the gut in mice and rats, and rapidly excreted in urine; small amounts of consumed AC were retained in tissue in the forms of AB and AsPL (Marafante et al., 1984). *In vitro* incubation of AC in liver cells resulted in biotransformation, with AB as the major product (Christakopoulos et al., 1988). Bioabsorption of the minor species TMAO and TETRA were found to be higher than AB by Caco-2 cell model, with transport of 9–16% after 4 h (Laparra et al., 2007).

6. Toxicity

A summary of the bioaccessibility and cytotoxicity (expressed as effect on cell number) of a suite of organic As species is presented in Fig. 6. Of the four commonly identified AsSugars in seaweed, only two (AsSug-Gly (Sakurai et al., 1997, Andrewes et al., 2004, Leffers et al., 2013a, 2013b, 2013c) and AsSug-SO₃ (Leffers et al., 2013a, 2013b, 2013c)) have been tested and show very low cytotoxicity compared to iAs. A trivalent derivative of AsSug-Gly, (DMA^{III}-AsSug-Gly), showed significant cellular toxicity, (Andrewes et al., 2004) but this As species has never been observed in biological systems. AsSugars also display no genotoxicity (as measured by micronuclei induction), even at cytotoxic levels (Leffers et al., 2013a, 2013b, 2013c). Slight cellular toxicity was observed for the thio-analog of AsSugar-Gly in liver (HepG2) and bladder (UROtsa) cells, expressed as decreased lysosomal integrity (UROtsa) and cell number (HepG2). The higher cytotoxicity of thio-AsSug-Gly compared to its oxo analog is likely due to higher cellular uptake, which is typical of thio vs. oxo pairs. However, cellular concentrations of thio-AsSug-Gly only reached ~10–20% relative to the incubation media concentration (Ebert et al., 2016); by contrast, iAs and AsHC's are pre-concentrated from the media by HepG2 (Meyer et al., 2014a, 2014b). The only *in vivo* study of these compounds in mice administered very high doses of AsSugar-Gly for 40 days, which resulted in disrupted normal neurobehavior, decreased passive avoidance time and motor function, and caused dose-dependent DNA damage and oxidative stress in the blood and brain (Bin Sayeed et al., 2013).

For the AsSugar metabolites, basic cytotoxicity screening has shown that the thio- and oxo- analogs of DMAE and DMAA have relatively low toxicity relative to that of DMA(V), despite high cellular uptake of the thio-derivatives (Raml et al., 2005). Recently, toxicity of the thio analog of DMA(V) has received much attention. Thio-DMA(V) has been observed as a product of both iAs (Raml et al., 2007) and AsSugar metabolism (Raml et al., 2006, Raml et al., 2009), and is suspected to have been misidentified as DMA(III) in urine in the earlier literature (Hansen et al., 2004a, 2004b). Substantial toxicity from this compound

has been observed in skin, bladder, liver and lung cells, which is in part related to its high cellular bioavailability (Naranmandura et al., 2007, Ochi et al., 2008, Naranmandura et al., 2009, Bartel et al., 2011, Naranmandura et al., 2011, Ebert et al., 2014). Thio-DMA(V) has been shown to be a generator of reactive oxygen species in healthy cells (Naranmandura et al., 2007, Naranmandura et al., 2009, Naranmandura et al., 2011), and to disrupt cellular stress response, even at picomolar levels, in cells that are oxidatively stressed (Leffers et al., 2013a, 2013b, 2013c). Thio-DMA(V) showed no genotoxic mode of action in lung cells (Bartel et al., 2011), but DNA damage and changes in gene expression were observed in bladder cells exposed to this compound (Naranmandura et al., 2011); most recently a disturbance of the cellular damage response in DNA-damaged bladder cells was observed at sub-cytotoxic levels (Ebert et al., 2014). Epigenetic effects from longterm exposure to thio-DMA(V) have also been observed at low picomolar levels (Unterberg et al., 2014).

The toxicity of AsLipids in humans and experimental animals has to date not been characterized. In contrast to As(III), AsHCs caused developmental toxicity in the late developmental stages of *Drosophila melanogaster*. The compounds AsHC 332 and AsHC 360 in particular, but also AsHC 444, disturbed the hatching of the flies from pupae (Meyer et al., 2014a, 2014b). Recently the cellular toxicity of AsHCs (Meyer et al. 2014) and AsFAs (Meyer et al., 2015a, 2015b) were examined in human liver (HepG2) and urothelial cells (UROtsa). In addition, the effects of three of the postulated major metabolites (DMA(V), DMAPr, and thio-DMAPr) were investigated in parallel as well as As(III), a toxic reference arsenical. The AsHCs exerted substantial cytotoxicity; effects were observed in the same concentration range as effects induced by As(III). In addition, those arsenicals showed a pronounced cellular bioavailability and data indicate their accumulation especially in the membranes of the cells. This might be attributed to the structural similarities of AsHCs with membrane lipids, especially fatty acids of phospholipids, which also have an amphiphilic character. Unlike As(III), AsHCs had an influence on the cellular energy level, especially by decreasing the cellular energy carrier ATP in liver cells (Meyer et al., 2014a, 2014b). The cytotoxic potential of AsFAs was lower as compared to As(III) and AsHCs. Nevertheless significant cytotoxic effects were observed for AsFAs (Meyer et al., 2015a, 2015b). Since two polar end members are characteristic for AsFAs, the interactions with cell membranes are probably less strong, which is reflected in their lower cellular bioavailability.

The metabolites, DMAPr and thio-DMAPr, showed no cytotoxic or genotoxic effects in the investigated concentration range up to 500 μM. Their cellular bioavailability was low, although as found for other thio-arsenicals, the thio-analogue showed higher bioavailability. So far no genotoxic potential has been identified for AsHCs and AsFAs, or for their metabolites DMAPr and thio-DMAPr. The exhibited cytotoxicity of AsLipids and their metabolites, however, provides impetus to better understand the distribution and breakdown of AsLipids in the body; currently there are no reports of pre-systemic metabolism in the gut, or of levels in the tissues and blood.

Due to a lack of toxicity and chronic exposure data for organic As species in humans or other mammals, health risks from organic As exposure are difficult to assess. Several studies have considered the potential cancer risk from the production of the metabolite DMA(V) (Borak and Hosgood, 2007, Chen et al., 2010, Leffers et al., 2013a, 2013b, 2013c), based on high dose exposure studies in rats to DMA in water (Wei et al., 2002) or diet (Arnold et al., 1999); there is, however, strong evidence that the rat model is not valid for human exposure to DMA, because rats absorb and metabolize As differently, and because these studies cannot evaluate the pathway to DMA (from iAs or organic As compounds) and the effects of intermediates (Cohen et al., 2006). While no conclusions can be drawn on the effects of organic As, evidence of toxicity from AsLipid and organic As metabolites by *in vitro* testing establishes the need for animal and human population studies to evaluate potential health effects of seafood As.

7. Risks and recommendations to assess As exposure from seafood

It has been known for some time that fish are, to those who consume them, sources of a variety of environmental contaminants including mercury and persistent organic pollutants (EFSA, 2015), but fish have been consumed by humans for centuries and are an important source of nutrition as well as part of the cultural traditions of many peoples. Fish are recognized as being an excellent source of protein, essential omega-3 fatty acids, and various vitamins (Domingo, 2016). It has been shown that fish consumption reduces mortality from coronary heart disease and confers neurological benefits on the offspring of women of childbearing age relative to those who do not eat fish (FAO/WHO, 2011). There is a need to balance consumption benefits versus risks but very few of these have included As as a consideration (Sirot et al., 2012), so much more work is required.

Arsenic is among a list of contaminants of emerging concern in seafood, for which there is insufficient data on levels in seafood for risk to be assessed (Vandermeersch et al., 2015). Currently, risk associated with As from seafood can only be assessed based on the iAs component. Some seaweeds, where taxa largely determine As concentration, and some bivalves, where iAs concentration is linked with the location of harvest, have been identified as potential exposure risks for iAs. For organic As, an assessment of exposure risk is currently not feasible – there are simply far too few data on concentrations and distributions of different species in seafood, and there is an almost complete lack of toxicity data and human population studies. The data available, however, demonstrate high concentrations of organic As, present as a wide range of species, in seafood, and indicate metabolism and toxicity of some of these organic As compounds. Collectively, these preliminary results provide a compelling case for further research on these compounds.

This review has identified the following major gaps in our current knowledge and future needs to address the human health risk from organic As exposure:

1. More complete As speciation data is needed across seafood types, particularly for the AsLipids.
2. Standards and reference materials for organic As compounds are crucial for filling the data gap.
3. More advanced toxicity testing is necessary to assess the effects of organic As compounds and their metabolites.
4. Studies on metabolic pathways and individual variability, as well as the distribution of As compounds in the body, are required to examine the toxicokinetics of these compounds.
5. Identification of biomarkers for As in seafood would be helpful both to detect possible confounders in studies of iAs metabolism and to track exposure of organic As.

The assessment of As in food is now being undertaken or being considered by many countries, and in some cases has already led to food regulations. These assessments have rightly focused on iAs because of its known toxicity, relevant data (from drinking water studies) on its epidemiology, and adequate datasets showing its distribution in foods. A full assessment of As in food, however, must consider organic As compounds in seafood, and this area remains a crucial missing link to understanding exposure and informing regulatory practices. We hope that this review combining the distribution of organic As species in seafood with analytical aspects and consumption and toxicity data will provide a valuable information source for researchers and regulators aiming to comprehensively assess human exposure to arsenic in food.

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References

- Adair, B.M., Moore, T., Conklin, S.D., Creed, J.T., Wolf, D.C., Thomas, D.J., 2007. Tissue distribution and urinary excretion of dimethylated arsenic and its metabolites in dimethylarsinic acid- or arsenate-treated rats. *Toxicology and Applied Pharmacology* 222 (2), 235–242.
- Almela, C., Clemente, M.J., Velez, D., Montoro, R., 2006. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food and Chemical Toxicology* 44 (11), 1901–1908.
- Almela, C., Laparra, J.M., Velez, D., Barbera, R., Farre, R., Montoro, R., 2005. Arsenosugars in raw and cooked edible seaweed: Characterization and bioaccessibility. *Journal of Agricultural and Food Chemistry* 53 (18), 7344–7351.
- Amayo, K.O., Petursdottir, A., Newcombe, C., Gunnlaugsdottir, H., Raab, A., Krupp, E.M., Feldmann, J., 2011. Identification and Quantification of Arsenolipids Using Reversed-Phase HPLC Coupled Simultaneously to High-Resolution ICPMS and High-Resolution Electrospray MS without Species-Specific Standards. *Analytical Chemistry* 83 (9), 3589–3595.
- Amayo, K.O., Raab, A., Krupp, E.M., Gunnlaugsdottir, H., Feldmann, J., 2013. Novel identification of arsenolipids using chemical derivatizations in conjunction with RP-HPLC-ICPMS/ESMS. *Anal. Chem.* 85 (19), 9321–9327.
- Amayo, K.O., Raab, A., Krupp, E.M., Marschall, T., Horsfall, M., Feldmann, J., 2014. Arsenolipids show different profiles in muscle tissues of four commercial fish species. *Journal of Trace Elements in Medicine and Biology* 28 (2), 131–137.
- Amlund, H., Berntsen, M.H.G., 2004. Arsenobetaine in Atlantic salmon (*Salmo salar* L.): influence of seawater adaptation. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* 138 (4), 507–514.
- Andrews, P., Demarini, D.M., Funasaka, K., Wallace, K., Lai, V.W.M., Sun, H.S., Cullen, W.R., Kitchin, K.T., 2004. Do arsenosugars pose a risk to human health? The comparative toxicities of a trivalent and pentavalent arsenosugar. *Environ. Sci. Technol.* 38 (15), 4140–4148.
- Aposhian, H., 1989. Biochemical toxicology of arsenic. *Rev Biochem Toxicol* 10, 265–299.
- Aposhian, H.V., Zakharyan, R.A., Avram, M.D., Sampayo-Reyes, A., Wollenberg, M.L., 2004. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxication of the trivalent arsenic species. *Toxicology and Applied Pharmacology* 198, 327–335.
- Arnold, L.L., Cano, M., John, M.S., Eldan, M., van Gemert, M., Cohen, S.M., 1999. Effects of dietary dimethylarsinic acid on the urine and urothelium of rats. *Carcinogenesis* 20 (11), 2171–2179.
- Arroyo-Abad, U., Hu, Z.H., Findeisen, M., Pfeifer, D., Mattusch, J., Reemtsma, T., Piechotta, C., 2016. Synthesis of two new arsenolipids and their identification in fish. *European Journal of Lipid Science and Technology* 118 (3), 445–452.
- Arroyo-Abad, U., Lischka, S., Piechotta, C., Mattusch, J., Reemtsma, T., 2013. Determination and identification of hydrophilic and hydrophobic arsenic species in methanol extract of fresh cod liver by RP-HPLC with simultaneous ICP-MS and ESI-Q-TOF-MS detection. *Food Chemistry* 141 (3), 3093–3102.
- Aylward, L.L., Ramasamy, S., Hays, S.M., Schoeny, R., Kirman, C.R., 2014. Evaluation of urinary speciated arsenic in NHANES: Issues in interpretation in the context of potential inorganic arsenic exposure. *Regulatory Toxicology and Pharmacology* 69 (1), 49–54.
- Baris, D., Waddell, R., Beane Freeman, L.E., Schwenn, M., Colt, J.S., Ayotte, J.D., Ward, M.H., Nuckols, J., Schned, A., Jackson, B., Clerkin, C., Rothman, N., Moore, L.E., Taylor, A., Robinson, G., Hosain, G.M., Armenti, K.R., McCoy, R., Samanic, C., Hoover, R.N., Fraumeni Jr., J.F., Johnson, A., Karagas, M.R., Silverman, D.T., 2016. Elevated Bladder Cancer in Northern New England: The Role of Drinking Water and Arsenic. *J Natl Cancer Inst* 108 (9).
- Bartel, M., Ebert, F., Leffers, L., Karst, U., Schwerdtle, T., 2011. Toxicological Characterization of the Inorganic and Organic Arsenic Metabolite Thio-DMA in Cultured Human Lung Cells. *J. Toxicol.* 2011, 373141.
- Berges-Tiznado, M.E., Paez-Osuna, F., Notti, A., Regoli, F., 2013a. Arsenic and Arsenic Species in Cultured Oyster (*Crassostrea gigas* and *C. corteziensis*) from Coastal Lagoons of the SE Gulf of California, Mexico. *Biological Trace Element Research* 151 (1), 43–49.
- Berges-Tiznado, M.E., Paez-Osuna, F., Notti, A., Regoli, F., 2013b. Biomonitoring of arsenic through mangrove oyster (*Crassostrea corteziensis* Hertlein, 1951) from coastal lagoons (SE Gulf of California): occurrence of arsenobetaine and other arseno-compounds. *Environmental Monitoring and Assessment* 185 (9), 7459–7468.

- Bin Sayeed, M.S., Ratan, M., Hossen, F., Hassan, F., Faisal, M., Kadir, M.F., 2013. Arsenosugar induced blood and brain oxidative stress, DNA damage and neurobehavioral impairments. *Neurochem Res* 38 (2), 405–412.
- Birgisdottir, B.E., Knutsen, H.K., Haugen, M., Gjelstad, I.M., Jenssen, M.T.S., Ellingsen, D.G., Thomassen, Y., Alexander, J., Meltzer, H.M., Brantsaeter, A.L., 2013. Essential and toxic element concentrations in blood and urine and their associations with diet: Results from a Norwegian population study including high-consumers of seafood and game. *Science of the Total Environment* 463, 836–844.
- Borak, J., Hosgood, H.D., 2007. Seafood arsenic: Implications for human risk assessment. *Regulatory Toxicology and Pharmacology* 47 (2), 204–212.
- Brown, R.M., Newton, D., Pickford, C.J., Sherlock, J.C., 1990. Human Metabolism of Arsenobetaine Ingested with Fish. *Hum. Exp. Toxicol.* 9 (1), 41–46.
- Buchet, J.P., Lauwerys, R., Roels, H., 1981. Comparison of the Urinary-Excretion of Arsenic Metabolites after a Single Oral Dose of Sodium Arsenite, Monomethylarsonate, or Dimethylarsinate in Man. *International Archives of Occupational and Environmental Health* 48 (1), 71–79.
- Buchet, J.P., Lison, D., Ruggeri, M., Foa, V., Elia, G., 1996. Assessment of exposure to inorganic arsenic, a human carcinogen, due to the consumption of seafood. *Archives of Toxicology* 70 (11), 773–778.
- Cai, Y., Georgiadis, M., Fourqurean, J.W., 2000. Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy* 55 (9), 1411–1422.
- Calatayud, M., Gimeno, J., Velez, D., Devesa, V., Montoro, R., 2010. Characterization of the Intestinal Absorption of Arsenate, Monomethylarsonic Acid, and Dimethylarsinic Acid Using the Caco-2 Cell Line. *Chemical Research in Toxicology* 23 (3), 547–556.
- Calderon, R.L., Hudgens, E.E., Carty, C., He, B., Le, X.C., Rogers, J., Thomas, D.J., 2013. Biological and behavioral factors modify biomarkers of arsenic exposure in a U.S. population. *Environmental Research* 126, 134–144.
- Caldwell, K.L., Jones, R.L., Verdon, C.P., Jarrett, J.M., Caudill, S.P., Osterloh, J.D., 2009. Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003–2004. *J. Expo. Sci. Environ. Epidemiol.* 19 (1), 59–68.
- Cannon, J.R., Edmonds, J.S., Francesconi, K.A., Raston, C.L., Saunders, J.B., Skelton, B.W., White, A.H., 1981. Isolation, crystal-structure and synthesis of arsenobetaine, a constituent of the western rock lobster, the dusky shark, and some samples of human urine. *Australian Journal of Chemistry* 34 (4), 787–798.
- Carlin, D.J., Naujokas, M.F., Bradham, K.D., Cowden, J., Heacock, M., Henry, H.F., Lee, J.S., Thomas, D.J., Thompson, C., Tokar, E.J., Waalkes, M.P., Birnbaum, L.S., Suk, W.A., 2016. Arsenic and environmental health: state of the science and future research opportunities. *Environ. Health Perspect.* 124 (7), 890–899.
- Caspi, R., Billington, R., Ferrer, L., Foerster, H., Fulcher, C.A., Keseler, I.M., Kothari, A., Krummenacker, M., Latendresse, M., Mueller, L.A., Ong, Q., Paley, S., Subhraveti, P., Weaver, D.S., Karp, P.D., 2014. The Metacyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases nucleic acids research 44(1):D471–80. Retrieved 20/02, 2016. from: <http://www.biocyc.org/META/NEW-IMAGE?type=COMPOUND-IN-PATHWAY&object=CHOLINE>.
- Caumette, G., Koch, I., Reimer, K.J., 2012. Arsenobetaine formation in plankton: a review of studies at the base of the aquatic food chain. *J. Environ. Monit.* 14 (11), 2841.
- Challenger, F., 1945. Biological Methylation. *Chemical Reviews* 36 (3), 315–361.
- Chen, B.C., Chou, W.C., Chen, W.Y., Liao, C.M., 2010. Assessing the cancer risk associated with arsenic-contaminated seafood. *Journal of Hazardous Materials* 181 (1–3), 161–169.
- Choi, B.S., Choi, S.J., Kim, D.W., Huang, M., Kim, N.Y., Park, K.S., Kim, C.Y., Lee, H.M., Yum, Y.N., Han, E.S., Kang, T.S., Yu, I.J., Park, J.D., 2010. Effects of Repeated Seafood Consumption on Urinary Excretion of Arsenic Species by Volunteers. *Archives of Environmental Contamination and Toxicology* 58 (1), 222–229.
- Christakopoulos, A., Norin, H., Sandstrom, M., Thor, H., Moldeus, P., Ryhage, R., 1988. Cellular-metabolism of arsenocholine. *Journal of Applied Toxicology* 8 (2), 119–127.
- Ciardullo, S., Aureli, F., Raggi, A., Cubadda, F., 2010. Arsenic speciation in freshwater fish: Focus on extraction and mass balance. *Talanta* 81 (1–2), 213–221.
- Cleland, B., Tsuchiya, A., Kalman, D.A., Dills, R., Burbacher, T.M., White, J.W., Faustman, E.M., Marien, K., 2009. Arsenic Exposure within the Korean Community (United States) Based on Dietary Behavior and Arsenic Levels in Hair, Urine, Air, and Water. *Environmental Health Perspectives* 117 (4), 632–638.
- Clowes, L.A., Francesconi, K.A., 2004. Uptake and elimination of arsenobetaine by the mussel *Mytilus edulis* is related to salinity. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* 137 (1), 35–42.
- Cohen, S.M., Arnold, L.L., Eldan, M., Lewis, A.S., Beck, B.D., 2006. Methylated arsenicals: The implications of metabolism and carcinogenicity studies in rodents to human risk assessment. *Critical Reviews in Toxicology* 36 (2), 99–133.
- Conklin, S.D., Ackerman, A.H., Fricke, M.W., Creed, P.A., Creed, J.T., Kohan, M.C., Herbin-Davis, K., Thomas, D.J., 2006. In vitro biotransformation of an arsenosugar by mouse anaerobic cecal microflora and cecal tissue as examined using IC-ICP-MS and LC-ESI-MS/MS. *Analyst* 131 (5), 648–655.
- Cottingham, K.L., Karimi, R., Gruber, J.F., Zens, M.S., Sayarath, V., Folt, C.L., Punshon, T., Morris, J.S., Karagas, M.R., 2013. Diet and toenail arsenic concentrations in a New Hampshire population with arsenic-containing water. *Nutr J* 12, 149.
- Cullen, W.R., 2014. Chemical Mechanism of Arsenic biomethylation. *Chemical Research in Toxicology* 27 (4), 457–461.
- Cullen, W.R., Li, H., Pergantis, S.A., Eigendorf, G.K., Harrison, L.G., 1994. The methylation of arsenate by a marine alga *Polyphysa peniculus* in the presence of L-methionine-methyl-d3. *Chemosphere* 28 (5), 1009–1019.
- Dahl, L., Molin, M., Amlund, H., Meltzer, H.M., Julshamn, K., Alexander, J., Sloth, J.J., 2010. Stability of arsenic compounds in seafood samples during processing and storage by freezing. *Food Chemistry* 123 (3), 720–727.
- Davis, M.A., Gilbert-Diamond, D., Karagas, M.R., Li, Z.G., Moore, J.H., Williams, S.M., Frost, H.R., 2014. A dietary-wide association study (DWAS) of environmental metal exposure in US children and adults. *PLoS One* 9 (9).
- Davis, M.A., Higgins, J., Li, Z., Gilbert-Diamond, D., Baker, E.R., Das, A., Karagas, M.R., 2015. Preliminary analysis of in utero low-level arsenic exposure and fetal growth using biometric measurements extracted from fetal ultrasound reports. *Environ. Health* 14, 12.
- Davis, M.A., Slaughter, F., Punshon, T., Ahsan, H., Argos, M., Meharg, A., Gossai, A., Karagas, M.R., in this issue, 2016. Biomarkers of human arsenic exposure through rice. *Sci. Total Environ.*
- de Bettencourt, A.M., Duarte, M.F., Florêncio, M.H., Henriques, F.F., Madeira, P.A., Portela, M.I., Vilas-Boas, L.F., 2011. Possible key intermediates in arsenic biochemistry: synthesis and identification by liquid chromatography electrospray ionization mass spectrometry and high resolution mass spectrometry. *Microchem. J.* 99 (2), 218–234.
- de la Calle, M.B., Baer, I., Robouch, P., Cordeiro, F., Emteborg, H., Baxter, M.J., Brereton, N., Raber, G., Velez, D., Devesa, V., Rubio, R., Llorente-Mirandes, T., Raab, A., Feldmann, J., Sloth, J.J., Rasmussen, R.R., D'Amato, M., Cubadda, F., 2012. Is it possible to agree on a value for inorganic arsenic in food? The outcome of IMEP-112. *Anal. Bioanal. Chem.* 404 (8), 2475–2488.
- de la Calle, M.B., Emteborg, H., Linsinger, T.P.J., Montoro, R., Sloth, J.J., Rubio, R., Baxter, M.J., Feldmann, J., Vermaercke, P., Raber, G., 2011. Does the determination of inorganic arsenic in rice depend on the method? *TrAC Trends Anal. Chem.* 30 (4), 641–651.
- Devesa, V., Martinez, A., Suner, M.A., Velez, D., Almela, C., Montoro, R., 2001. Effect of cooking temperatures on chemical changes in species of organic arsenic in seafood. *J. Agric. Food Chem.* 49 (5), 2272–2276.
- Devesa, V., Suner, M.A., Algora, S., Velez, D., Montoro, R., Jalon, M., Urieta, I., Macho, M.L., 2005. Organoarsenical species contents in cooked seafood. *J. Agric. Food Chem.* 53 (22), 8813–8819.
- Devesa, V., Velez, D., Montoro, R., 2008. Effect of thermal treatments on arsenic species contents in food. *Food Chem. Toxicol.* 46 (1), 1–8.
- Domingo, J.L., 2016. Nutrients and chemical pollutants in fish and shellfish. Balancing health benefits and risks of regular fish consumption. *Crit. Rev. Food Sci. Nutr.* 56 (6), 979–988.
- Duncan, E.G., Maher, W.A., Foster, S.D., Krikowa, F., 2013a. The influence of arsenate and phosphate exposure on arsenic uptake, metabolism and species formation in the marine phytoplankton *Dunaliella tertiolecta*. *Mar. Chem.* 157, 78–85.
- Duncan, E.G., Maher, W.A., Foster, S.D., Krikowa, F., 2013b. Influence of culture regime on arsenic cycling by the marine phytoplankton *Dunaliella tertiolecta* and *Thalassiosira pseudonana*. *Environ. Chem.* 10 (2), 91–101.
- Duncan, E.G., Maher, W.A., Foster, S.D., Mikac, K.M., Krikowa, F., 2014. The influence of bacteria on the arsenic species produced by laboratory cultures of the marine phytoplankton *Dunaliella tertiolecta*. *J. Appl. Phycol.* 26 (5), 2129–2134.
- Ebert, F., Leffers, L., Weber, T., Berndt, S., Mangerich, A., Beneke, S., Burkle, A., Schwerdtle, T., 2014. Toxicological properties of the thiolated inorganic arsenic and arsenosugar metabolite thio-dimethylarsinic acid in human bladder cells. *J. Trace Elem. Med. Biol.* 28 (2), 138–146.
- Ebert, F., Meyer, S., Leffers, L., Raber, G., Francesconi, K.A., Schwerdtle, T., 2016. Toxicological characterisation of a thio-arsenosugar-glycerol in human cells. *J. Trace Elem. Med. Biol.* 38, 150–156.
- Edmonds, J.S., Francesconi, K.A., 1981. Arseno-sugars from brown kelp (*Ecklonia radiata*) as intermediates in cycling of arsenic in a marine ecosystem. *Nature* 289 (5798), 602–604.
- Edmonds, J.S., Francesconi, K.A., 1983. Arsenic-containing ribofuranosides: isolation from brown kelp *Ecklonia radiata* and nuclear magnetic resonance spectra. *J. Chem. Soc. Perkin Trans. 1*, 2375.
- Edmonds, J.S., Francesconi, K.A., 1987. Transformations of arsenic in the marine environment. *Experientia* 43 (5), 553–557.
- Edmonds, J.S., Francesconi, K.A., 1993. Arsenic in seafoods - human health-aspects and regulations. *Mar. Pollut. Bull.* 26 (12), 665–674.
- Edmonds, J.S., Francesconi, K.A., Hansen, J.A., 1982. Dimethyloxarsylethanol from anaerobic decomposition of brown kelp (*Ecklonia radiata*) - a likely precursor of arsenobetaine in marine fauna. *Experientia* 38 (6), 643–644.
- Edmonds, J.S., Shibata, Y., Francesconi, K.A., Rippingale, R.J., Morita, M., 1997. Arsenic transformations in short marine food chains studied by HPLC-ICP MS. *Appl. Organomet. Chem.* 11 (4), 281–287.
- Edmonds, J.S., Shibata, Y., Francesconi, K.A., Yoshinaga, J., Morita, M., 1992. Arsenic lipids in the digestive gland of the western rock lobster *Panulirus cygnus*: an investigation by HPLC ICP-MS. *Sci. Total Environ.* 122 (3), 321–335.
- EFSA, 2009. Scientific opinion on arsenic in food. EFSA panel on contaminants in the food chain. *EFSA J.* 7, 198.
- EFSA, 2014. Dietary exposure to inorganic arsenic in the European population. *EFSA J.* 12, 68.
- EFSA, 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. *EFSA J.* 13, 36.
- FAO/WHO, 2011. Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption. WHO. Geneva, Rome, Food and Agriculture Organization of the United Nations, p. 50.
- FAOSTAT, 2012. GEMS/Food consumption database. from: <http://www.who.int/foodsafety/databases/en/>.
- FDA, 2016. FDA Proposes Limit for Inorganic Arsenic in Infant Rice Cereal. U. S. F. a. D. Administration.
- Feldmann, J., John, K., Pengprecha, P., 2000. Arsenic metabolism in seaweed-eating sheep from Northern Scotland. *Fresenius J. Anal. Chem.* 368 (1), 116–121.
- Feldmann, J., Krupp, E.M., 2011. Critical review or scientific opinion paper: arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? *Anal. Bioanal. Chem.* 399 (5), 1735–1741.
- Fort, M., Grimalt, J.O., Casas, M., Sunyer, J., 2014. Food sources of arsenic in pregnant Mediterranean women with high urine concentrations of this metalloid. *Environ. Sci. Pollut. Res.* 21 (20), 11689–11698.

- Foster, S., Maher, W., Krikowa, F., Apte, S., 2007. A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues. *Talanta* 71 (2), 537–549.
- Foster, S., Thomson, D., Maher, W., 2008. Uptake and metabolism of arsenate by anoxic cultures of the microalgae *Dunaliella tertiolecta* and *Phaeodactylum tricorutum*. *Mar. Chem.* 108 (3–4), 172–183.
- Francesconi, K.A., 2010. Arsenic species in seafood: origin and human health implications. *Pure Appl. Chem.* 82 (2), 373–381.
- Francesconi, K.A., Edmonds, J.S., 1996. Arsenic and marine organisms. *Adv. Inorg. Chem.* 44, 147–189.
- Francesconi, K.A., Edmonds, J.S., 1997. Arsenic and marine organisms. *Adv. Inorg. Chem.* 44 (44), 147–189.
- Francesconi, K.A., Edmonds, J.S., Hatcher, B.G., 1988. Examination of the arsenic constituents of the herbivorous marine gastropod tectus-pyramis - isolation of tetramethylarsonium ion. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.* 90 (2), 313–316.
- Francesconi, K.A., Edmonds, J.S., Stick, R.V., 1989. Accumulation of arsenic in yelloweye mullet (*Aldrichetta forsteri*) following oral administration of organoarsenic compounds and arsenate. *Sci. Total Environ.* 79 (1), 59–67.
- Francesconi, K.A., Gailer, J., Edmonds, J.S., Goessler, W., Irgolic, K.J., 1999. Uptake of arsenic-betaines by the mussel *Mytilus edulis*. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.* 122 (1), 131–137.
- Francesconi, K.A., Kuehnelt, D., 2004. Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* 129 (5), 373–395.
- Francesconi, K.A., Schwerdtle, T., 2016. Fat-soluble arsenic – new lipids with a sting in their tail. *Lipid Technol.* 28 (5–6), 96–98.
- Francesconi, K.A., Tanggaard, R., McKenzie, C.J., Goessler, W., 2002. Arsenic metabolites in human urine after ingestion of an arsenosugar. *Clin. Chem.* 48 (1), 92–101.
- Fricke, M.W., Creed, P.A., Parks, A.N., Shoemaker, J.A., Schwegel, C.A., Creed, J.T., 2004. Extraction and detection of a new arsine sulfide containing arsenosugar in molluscs by IC-ICP-MS and IC-ESI-MS/MS. *J. Anal. At. Spectrom.* 19 (11), 1454–1459.
- Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Parks, A.N., Freeman, D.M., Schwegel, C.A., Creed, J.T., 2003. An investigation of the chemical stability of arsenosugars in basic environments using IC-ICP-MS and IC-ESI-MS/MS. *Analyst* 128 (12), 1458–1461.
- Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Wei, X., Schwegel, C.A., Creed, J.T., 2002. An investigation of the chemical stability of arsenosugars in simulated gastric juice and acidic environments using IC-ICP-MS and IC-ESI-MS/MS. *Analyst* 127 (6), 781–785.
- García-Salgado, S., Quijano, M.A., Bonilla, M.M., 2012a. Arsenic speciation in edible alga samples by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry. *Anal. Chim. Acta* 714, 38–46.
- García-Salgado, S., Raber, G., Raml, R., Magnes, C., Francesconi, K.A., 2012b. Arsenosugar phospholipids and arsenic hydrocarbons in two species of brown macroalgae. *Environ. Chem.* 9 (1), 63–66.
- García-Salgado, S., Raber, G., Raml, R., Magnes, C., Francesconi, K.A., 2012. Arsenosugar phospholipids and arsenic hydrocarbons in two species of brown macroalgae. *Environ. Chem.* 9 (1), 63.
- Geislinger, A., Goessler, W., Pedersen, S.N., Francesconi, K.A., 2001. Arsenic biotransformation by the brown macroalga *Fucus serratus*. *Environ. Toxicol. Chem.* 20 (10), 2255.
- Gilbert-Diamond, D., Emond, J.A., Baker, E.R., Korrick, S.A., Karagas, M.R., 2016. Relation between in utero arsenic exposure and birth outcomes in a cohort of mothers and their newborns from New Hampshire. *Environ. Health Perspect.* 124 (8), 1299–1307.
- Glabonjat, R.A., Raber, G., Jensen, K.B., Ehgartner, J., Francesconi, K.A., 2014. Quantification of arsenolipids in the certified reference material NMJ 7405-a (Hijiki) using HPLC/mass spectrometry after chemical Derivatization. *Anal. Chem.* 86 (20), 10282–10287.
- Granchinho, S.C.R., Franz, C.M., Polishchuk, E., Cullen, W.R., Reimer, K.J., 2002. Transformation of arsenic(V) by the fungus *Fusarium oxysporum melonis* isolated from the alga *Fucus gardneri*. *Appl. Organomet. Chem.* 16 (12), 721–726.
- Grotti, M., Ardini, F., Terol, A., Magi, E., Todoli, J.L., 2013. Influence of chemical species on the determination of arsenic using inductively coupled plasma mass spectrometry at a low liquid flow rate. *J. Anal. At. Spectrom.* 28 (11), 1718–1724.
- Hanaoka, K.I., Goessler, W., Yoshida, K., Fujitaka, Y., Kaise, T., Irgolic, K.J., 1999. Arsenocholine- and dimethylated arsenic-containing lipids in star-spotted shark *Mustelus manazo*. *Appl. Organomet. Chem.* 13 (10), 765–770.
- Hanaoka, K.I., Ohno, H., Wada, N., Ueno, S., Goessler, W., Kuehnelt, D., Schlagenhaufen, C., Kaise, T., Irgolic, K.J., 2001. Occurrence of organo-arsenicals in jellyfishes and their mucus. *Chemosphere* 44 (4), 743–749.
- Hansen, H.R., Jaspars, M., Feldmann, J., 2004a. Arsinothiyl-sugars produced by in vitro incubation of seaweed extract with liver cytosol analysed by HPLC coupled simultaneously to ES-MS and ICP-MS. *Analyst* 129 (11), 1058–1064.
- Hansen, H.R., Raab, A., Francesconi, K.A., Feldmann, J., 2003. Metabolism of arsenic by sheep chronically exposed to arsenosugars as a normal part of their diet. 1. Quantitative intake, uptake, and excretion. *Environ. Sci. Technol.* 37 (5), 845–851.
- Hansen, H.R., Raab, A., Jaspars, M., Milne, B.F., Feldmann, J., 2004b. Sulfur-containing arsenical mistaken for dimethylarsinous acid [DMA(III)] and identified as a natural metabolite in urine: major implications for studies on arsenic metabolism and toxicity. *Chem. Res. Toxicol.* 17 (8), 1086–1091.
- Harrington, C.F., Brima, E.L., Jenkins, R.O., 2008. Biotransformation of arsenobetaine by microorganisms from the human gastrointestinal tract. *Chem. Speciat. Bioavailab.* 20 (3), 173–180.
- Heck, J.E., Andrew, A.S., Onega, T., Rigas, J.R., Jackson, B.P., Karagas, M.R., Duell, E.J., 2009. Lung cancer in a U.S. population with low to moderate arsenic exposure. *Environ. Health Perspect.* 117 (11), 1718–1723.
- Heinrich-Ramm, R., Mindt-Pruffert, S., Szadkowsky, D., 2002. Arsenic species excretion after controlled seafood consumption. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 778 (1–2), 263–273.
- Hirata, S., Toshimitsu, H., 2007. Determination of arsenic species and arsenosugars in marine samples by HPLC-ICP-MS. *Appl. Organomet. Chem.* 21 (6), 447–454.
- Hunter, D.A., Goessler, W., Francesconi, K.A., 1998. Uptake of arsenate, trimethylarsine oxide, and arsenobetaine by the shrimp *Crangon crangon*. *Mar. Biol.* 131 (3), 543–552.
- Julshamm, K., Nilsen, B.M., Frantzen, S., Valdersnes, S., Maage, A., Nedreaas, K., Sloth, J.J., 2012. Total and inorganic arsenic in fish samples from Norwegian waters. *Food Addit. Contam. Part B Surveill.* 5 (4), 229–235.
- Kahn, M., Raml, R., Schmeisser, E., Vallant, B., Francesconi, K.A., Goessler, W., 2005. Two novel thio-arsenosugars in scallops identified with HPLC-ICPMS and HPLC-ESMS. *Environ. Chem.* 2 (3), 171–176.
- Kaise, T., Watanabe, S., Itoh, K., 1985. The acute toxicity of arsenobetaine. *Chemosphere* 14 (9), 1327–1332.
- Khokiattiwong, S., Kornkanitnan, N., Goessler, W., Kokarnig, S., Francesconi, K.A., 2009. Arsenic compounds in tropical marine ecosystems: similarities between mangrove forest and coral reef. *Environ. Chem.* 6 (3), 226–234.
- Kirby, J., Maher, W., 2002. Tissue accumulation and distribution of arsenic compounds in three marine fish species: relationship to trophic position. *Appl. Organomet. Chem.* 16 (2), 108–115.
- Koch, I., Dee, J., House, K., Sui, J., Zhang, J., McKnight-Whitford, A., Reimer, K.J., 2013. Bioaccessibility and speciation of arsenic in country foods from contaminated sites in Canada. *Sci. Total Environ.* 449, 1–8.
- Kohlmeier, U., Kuballa, J., Jantzen, E., 2002. Simultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high-performance liquid chromatography/inductively coupled plasma mass spectrometry. *Rapid Commun. Mass Spectrom.* 16 (10), 965–974.
- Kubachka, K.M., Kohan, M.C., Conklin, S.D., Herbin-Davis, K., Creed, J.T., Thomas, D.J., 2009. In vitro biotransformation of dimethylarsinic acid and trimethylarsine oxide by anaerobic microflora of mouse cecum analyzed by HPLC-ICP-MS and HPLC-ESI-MS. *J. Anal. At. Spectrom.* 24 (8), 1062–1068.
- Lai, V.W.M., Cullen, W.R., Harrington, C.F., Reimer, K.J., 1997. The characterization of arsenosugars in commercially available algal products including a *Nostoc* species of terrestrial origin. *Appl. Organomet. Chem.* 11 (10–11), 797–803.
- Lai, V.W.M., Cullen, W.R., Harrington, C.F., Reimer, K.J., 1998. Seasonal changes in arsenic speciation in *Fucus* species. *Appl. Organomet. Chem.* 12 (4), 243–251.
- Laparra, J.M., Velez, D., Barbera, R., Montoro, R., Farre, R., 2007. Bioaccessibility and transport by Caco-2 cells of organoarsenic species present in seafood. *J. Agric. Food Chem.* 55 (14), 5892–5897.
- Larsen, E.H., Francesconi, K.A., 2003. Arsenic concentrations correlate with salinity for fish taken from the North Sea and Baltic waters. *J. Mar. Biol. Assoc. U. K.* 83 (2), 283–284.
- Larsen, E.H., Quérel, C.R., Munoz, R., Fiala-Medioni, A., Donard, O.F.X., 1997. Arsenic speciation in shrimp and mussel from the mid-Atlantic hydrothermal vents. *Mar. Chem.* 57 (3–4), 341–346.
- Le, X.C., Cullen, W.R., Reimer, K.J., 1994. Human urinary arsenic excretion after one-time ingestion of seaweed, crab, and shrimp. *Clin. Chem.* 40 (4), 617–624.
- Leffers, L., Ebert, F., Taleshi, M.S., Francesconi, K.A., Schwerdtle, T., 2013a. In vitro toxicological characterization of two arsenosugars and their metabolites. *Mol. Nutr. Food Res.* 57 (7), 1270–1282.
- Leffers, L., Unterberg, M., Bartel, M., Hoppe, C., Pieper, I., Stertmann, J., Ebert, F., Humpf, H.U., Schwerdtle, T., 2013b. In vitro toxicological characterisation of the S-containing arsenic metabolites thio-dimethylarsinic acid and dimethylarsinic glutathione. *Toxicology* 305, 109–119.
- Leffers, L., Wehe, C.A., Huewel, S., Bartel, M., Ebert, F., Taleshi, M.S., Galla, H.J., Karst, U., Francesconi, K.A., Schwerdtle, T., 2013c. In vitro intestinal bioavailability of arsenosugar metabolites and presystemic metabolism of thio-dimethylarsinic acid in Caco-2 cells. *Metallomics* 5 (8), 1031–1042.
- Leufroy, A., Noel, L., Beauchemin, D., Guerin, T., 2012. Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. *Anal. Bioanal. Chem.* 402 (9), 2849–2859.
- Li, W.H., Wei, C., Zhang, C., Van Hulle, M., Cornelis, R., Zhang, X.R., 2003. A survey of arsenic species in Chinese seafood. *Food Chem. Toxicol.* 41 (8), 1103–1110.
- Lischka, S., Arroyo-Abad, U., Mattusch, J., Kuhn, A., Piechotta, C., 2013. The high diversity of arsenolipids in herring fillet (*Clupea harengus*). *Talanta* 110, 144–152.
- Lorente-Mirandes, T., Ruiz-Chancho, M.J., Barbero, M., Rubio, R., Lopez-Sanchez, J.F., 2011. Determination of water-soluble arsenic compounds in commercial edible seaweed by LC-ICPMS. *J. Agric. Food Chem.* 59 (24), 12963–12968.
- Lorenzana, R.M., Yeow, A.Y., Colman, J.T., Chappell, L.L., Choudhury, H., 2009. Arsenic in seafood: speciation issues for human health risk assessment. *Hum. Ecol. Risk Assess.* 15 (1), 185–200.
- Lovreglio, P., D'Errico, M.N., Gilberti, M.E., Drago, I., Basso, A., Apostoli, P., Soleo, L., 2012. The influence of diet on intra and inter-individual variability of urinary excretion of arsenic species in Italian healthy individuals. *Chemosphere* 86 (9), 898–905.
- Lunde, G., 1968. Analysis of arsenic in marine oils by neutron activation. Evidence of arseno organic compounds. *J. Am. Oil Chem. Soc.* 45 (5), 331 (–&).
- Lynch, H.N., Greenberg, G.L., Pollock, M.C., Lewis, A.S., 2014. A comprehensive evaluation of inorganic arsenic in food and considerations for dietary intake analyses. *Sci. Total Environ.* 496, 299–313.
- Ma, M.S., Le, X.C., 1998. Effect of arsenosugar ingestion on urinary arsenic speciation. *Clin. Chem.* 44 (3), 539–550.
- Madsen, A.D., Goessler, W., Pedersen, S.N., Francesconi, K.A., 2000. Characterization of an algal extract by HPLC-ICP-MS and LC-electrospray MS for use in arsenosugar speciation studies. *J. Anal. At. Spectrom.* 15 (6), 657–662.
- Mahaffey, K.R., Clickner, R.P., Bodurrow, C.C., 2004. Blood organomercury and dietary mercury intake: national health and nutrition examination survey, 1999 and 2000. *Environ. Health Perspect.* 112 (5), 562–570.

- Maher, W.A., Ellwood, M.J., Krikowa, F., Raber, G., Foster, S., 2015. Measurement of arsenic species in environmental, biological fluids and food samples by HPLC-ICPMS and HPLC-HG-AFS. *J. Anal. At. Spectrom.* 30 (10), 2129–2183.
- Maher, W.A., Foster, S., Krikowa, F., Duncan, E., John, A.S., Hug, K., Moreau, J.W., 2013. Thio arsenic species measurements in marine organisms and geothermal waters. *Microchem. J.* 111, 82–90.
- Mansilla-Rivera, I., Nazario, C.M., Ramirez-Marrero, F.A., Crespo, C.J., Rodriguez-Sierra, C.J., 2014. Assessing arsenic exposure from consumption of seafood from Vieques-Puerto Rico: a pilot biomonitoring study using different biomarkers. *Arch. Environ. Contam. Toxicol.* 66 (2), 162–175.
- Marafante, E., Vahter, M., Dencker, L., 1984. Metabolism of arsenocholine in mice, rats and rabbits. *Sci. Total Environ.* 34 (3), 223–240.
- Marafante, E., Vahter, M., Norin, H., Envall, J., Sandstrom, M., Christakopoulos, A., Ryhage, R., 1987. Biotransformation of dimethylarsinic acid in mouse, hamster and man. *J. Appl. Toxicol.* 7 (2), 111–117.
- Maulvault, A.L., Anacleto, P., Barbosa, V., Sloth, J.J., Rasmussen, R.R., Tediosi, A., Fernandez-Tejedor, M., van den Heuvel, F.H.M., Kotterman, M., Marques, A., 2015. Toxic elements and speciation in seafood samples from different contaminated sites in Europe. *Environ. Res.* 143, 72–81.
- Meermann, B., Kiesshauer, M., 2011. Development of an oxygen-gradient system to overcome plasma instabilities during HPLC/ICP-MS measurements using gradient elution. *J. Anal. At. Spectrom.* 26 (10), 2069–2075.
- Meyer, S., Matissek, M., Muller, S.M., Taleshi, M.S., Ebert, F., Francesconi, K.A., Schwerdtle, T., 2014a. In vitro toxicological characterisation of three arsenic-containing hydrocarbons. *Metallics* 6 (5), 1023–1033.
- Meyer, S., Raber, G., Ebert, F., Leffers, L., Muller, S.M., Taleshi, M.S., Francesconi, K.A., Schwerdtle, T., 2015a. In vitro toxicological characterisation of arsenic-containing fatty acids and three of their metabolites. *Toxicol. Res.* 4 (5), 1289–1296.
- Meyer, S., Raber, G., Ebert, F., Taleshi, M.S., Francesconi, K.A., Schwerdtle, T., 2015b. Arsenic-containing hydrocarbons and arsenic-containing fatty acids: transfer across and pre-systemic metabolism in the Caco-2 intestinal barrier model. *Mol. Nutr. Food Res.* 59 (10), 2044–2056.
- Meyer, S., Schulz, J., Jeibmann, A., Taleshi, M.S., Ebert, F., Francesconi, K.A., Schwerdtle, T., 2014b. Arsenic-containing hydrocarbons are toxic in the *in vivo* model *Drosophila melanogaster*. *Metallics* 6 (11), 2010–2014.
- Micha, R., Khatibzadeh, S., Shi, P.L., Andrews, K.G., Engell, R.E., Mozaffarian, D., Dis, G.B.D.N.C., 2015. Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide. *BMJ Open* 5 (9).
- Miklavcic, A., Casetta, A., Tratnik, J.S., Mazej, D., Kršnik, M., Mariuz, M., Sofianou, K., Spiric, Z., Barbone, F., Horvat, M., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. *Environ. Res.* 120, 7–17.
- Molin, M., Ulven, S.M., Dahl, L., Goessler, W., Fliegel, D., Holck, M., Sloth, J.J., Oshaug, A., Alexander, J., Meltzer, H.M., Ydersbond, T.A., 2014. Urinary excretion of arsenicals following daily intake of various seafoods during a two weeks intervention. *Food Chem. Toxicol.* 66, 76–88.
- Molin, M., Ulven, S.M., Dahl, L., Telle-Hansen, V.H., Holck, M., Skjeggstad, G., Ledsaak, O., Sloth, J.J., Goessler, W., Oshaug, A., Alexander, J., Fliegel, D., Ydersbond, T.A., Meltzer, H.M., 2012a. Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. *Environ. Res.* 112, 28–39.
- Molin, M., Ulven, S.M., Meltzer, H.M., Alexander, J., 2015. Arsenic in the human food chain, biotransformation and toxicology - review focusing on seafood arsenic. *J. Trace Elem. Med. Biol.* 31, 249–259.
- Molin, M., Ydersbond, T.A., Ulven, S.M., Holck, M., Dahl, L., Sloth, J.J., Fliegel, D., Goessler, W., Alexander, J., Meltzer, H.M., 2012b. Major and minor arsenic compounds accounting for the total urinary excretion of arsenic following intake of blue mussels (*Mytilus edulis*): a controlled human study. *Food Chem. Toxicol.* 50 (7), 2462–2472.
- Moreda-Piñeiro, J., Alonso-Rodríguez, E., Moreda-Piñeiro, A., Moscoso-Pérez, C., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D., Bermejo-Barrera, P., 2010. Simultaneous pressurized enzymatic hydrolysis extraction and clean up for arsenic speciation in seafood samples before high performance liquid chromatography-inductively coupled plasma-mass spectrometry determination. *Anal. Chim. Acta* 679 (1–2), 63–73.
- Morita, M., Shibata, Y., 1988. Isolation and identification of arseno-lipid from a brown alga, (wakame). *Chemosphere* 17 (6), 1147–1152.
- Naranmandura, H., Carew, M.W., Xu, S., Lee, J., Leslie, E.M., Weinfeld, M., Le, X.C., 2011. Comparative toxicity of arsenic metabolites in human bladder cancer EJ-1 cells. *Chem. Res. Toxicol.* 24 (9), 1586–1596.
- Naranmandura, H., Ibat, K., Suzuki, K.T., 2007. Toxicity of dimethylmonothioarsinic acid toward human epidermoid carcinoma A431 cells. *Chem. Res. Toxicol.* 20 (8), 1120–1125.
- Naranmandura, H., Ogra, Y., Iwata, K., Lee, J., Suzuki, K.T., Weinfeld, M., Le, X.C., 2009. Evidence for toxicity differences between inorganic arsenite and thioarsenicals in human bladder cancer cells. *Toxicol. Appl. Pharmacol.* 238 (2), 133–140.
- Navas-Acien, A., Francesconi, K.A., Silbergeld, E.K., Guallar, E., 2011. Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. *Environ. Res.* 111 (1), 110–118.
- Nearing, M.M., Koch, I., Reimer, K.J., 2014a. Arsenic speciation in edible mushrooms. *Environ. Sci. Technol.* 48 (24), 14203–14210.
- Nearing, M.M., Koch, I., Reimer, K.J., 2014b. Complementary arsenic speciation methods: a review. *Spectrochim. Acta B At. Spectrosc.* 99, 150–162.
- Newcombe, C., Raab, A., Williams, P.N., Deacon, C., Haris, P.I., Meharg, A.A., Feldmann, J., 2010. Accumulation or production of arsenobetaine in humans? *J. Environ. Monit.* 12 (4), 832–837.
- Niegel, C., Matysik, F.M., 2010. Analytical methods for the determination of arsenosugars—a review of recent trends and developments. *Anal. Chim. Acta* 657 (2), 83–99.
- Niehoff, A.-C., Schulz, J., Soltwisch, J., Meyer, S., Kettling, H., Sperling, M., Jeibmann, A., Dreisewerd, K., Francesconi, K.A., Schwerdtle, T., Karst, U., 2016. Imaging by elemental and molecular mass spectrometry reveals the uptake of an arsenolipid in the brain of *Drosophila melanogaster*. *Anal. Chem.* 88 (10), 5258–5263.
- Ninh, T.D., Nagashima, Y., Shiomi, K., 2007. Water-soluble and lipid-soluble arsenic compounds in Japanese flying squid *Todarodes pacificus*. *J. Agric. Food Chem.* 55 (8), 3196–3202.
- Ninh, T.D., Nagashima, Y., Shiomi, K., 2008. Unusual arsenic speciation in sea anemones. *Chemosphere* 70 (7), 1168–1174.
- Nischwitz, V., Kanaki, K., Pergantis, S.A., 2006. Mass spectrometric identification of novel arsinothioyl-sugars in marine bivalves and algae. *J. Anal. At. Spectrom.* 21 (1), 33–40.
- NOAA, 2010. Fisheries of the United States. N. M. F. S. O. o. S. a. Technology. Silver Spring, Maryland, National Oceanic and Atmospheric Administration.
- NRC, 2014. Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report. N. R. C. o. t. N. Academies, Washington, D.C.
- Nyssola, A., Kerovu, J., Kaukinen, P., von Weymarn, N., Reinikainen, T., 2000. Extreme Halophiles synthesize betaine from glycine by methylation. *J. Biol. Chem.* 275 (29), 22196–22201.
- Ochi, T., Kita, K., Suzuki, T., Rimpler, A., Goessler, W., Francesconi, K.A., 2008. Cytotoxic, genotoxic and cell-cycle disruptive effects of thio-dimethylarsinate in cultured human cells and the role of glutathione. *Toxicol. Appl. Pharmacol.* 228 (1), 59–67.
- OHA, 2015. Technical Report: Soft-shell Clam Advisory for the Oregon Coast. Oregon Health Authority.
- Petursdottir, A.H., Fletcher, K., Gunnlaugsdottir, H., Krupp, E., Kupper, F.C., Feldmann, J., 2016. Environmental effects on arsenosugars and arsenolipids in Ectocarpus (Phaeophyta). *Environ. Chem.* 13 (1), 21–33.
- Petursdottir, A.H., Sloth, J.J., Feldmann, J., 2015. Introduction of arsenic in feed and food with emphasis on inorganic arsenic, and implications for analytical chemistry. *Anal. Bioanal. Chem.* 407 (28), 8385–8396.
- Phillips, D.J.H., Depledge, M.H., 1985. Metabolic pathways involving arsenic in marine organisms: a unifying hypothesis. *Mar. Environ. Res.* 17 (1), 1–12.
- Raab, A., Fecher, P., Feldmann, J., 2005. Determination of arsenic in algae - results of an interlaboratory trial: determination of arsenic species in the water-soluble fraction. *Microchim. Acta* 151 (3–4), 153–166.
- Raab, A., Newcombe, C., Pitton, D., Ebel, R., Feldmann, J., 2013. Comprehensive analysis of lipophilic arsenic species in a Brown alga (*Saccharina latissima*). *Anal. Chem.* 85 (5), 2817–2824.
- Raber, G., Khoomrung, S., Taleshi, M.S., Edmonds, J.S., Francesconi, K.A., 2009. Identification of arsenolipids with GC/MS. *Talanta* 78 (3), 1215–1218.
- Raml, R., Goessler, W., Francesconi, K.A., 2006. Improved chromatographic separation of thio-arsenic compounds by reversed-phase high performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Chromatogr. A* 1128 (1–2), 164–170.
- Raml, R., Goessler, W., Traar, P., Ochi, T., Francesconi, K.A., 2005. Novel thioarsenic metabolites in human urine after ingestion of an arsenosugar, 2',3'-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-ribose. *Chem. Res. Toxicol.* 18 (9), 1444–1450.
- Raml, R., Raber, G., Rimpler, A., Bauernhofer, T., Goessler, W., Francesconi, K.A., 2009. Individual variability in the human metabolism of an arsenic-containing carbohydrate, 2',3'-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-ribose, a naturally occurring arsenical in seafood. *Chem. Res. Toxicol.* 22 (9), 1534–1540.
- Raml, R., Rimpler, A., Goessler, W., Vahter, M., Li, L., Ochi, T., Francesconi, K.A., 2007. Thio-dimethylarsinate is a common metabolite in urine samples from arsenic-exposed women in Bangladesh. *Toxicol. Appl. Pharmacol.* 222 (3), 374–380.
- Reimer, K.J., Koch, I., Cullen, W.R., 2010. Organoarsenicals. Distribution and transformation in the environment. *Met. Ions Life Sci.* 7, 165–229.
- Rose, M., Lewis, J., Langford, N., Baxter, M., Origgi, S., Barber, M., MacBain, H., Thomas, K., 2007. Arsenic in seaweed—forms, concentration and dietary exposure. *Food Chem. Toxicol.* 45 (7), 1263–1267.
- Rubin, S.S.C.D., Alava, P., Zekker, I., Laing, G.D., Van de Wiele, T., 2014. Arsenic thiolation and the role of sulfate-reducing bacteria from the human intestinal tract. *Environ. Health Perspect.* 122 (8), 817–822.
- Ruiz-Chancho, M.J., Taleshi, M.S., Goessler, W., Francesconi, K.A., 2012. A method for screening arsenolipids in fish oils by HPLC-ICPMS. *J. Anal. At. Spectrom.* 27 (3), 501–504.
- Rimpler, A., Edmonds, J.S., Katsu, M., Jensen, K.B., Goessler, W., Raber, G., Gunnlaugsdottir, H., Francesconi, K.A., 2008. Arsenic-containing long-chain fatty acids in cod-liver oil: a result of biosynthetic infidelity? *Angew. Chem. Int. Ed.* 47 (14), 2665–2667.
- Ruttens, A., Blanpain, A.C., De Temmerman, L., Waegeneers, N., 2012. Arsenic speciation in food in Belgium Part 1: Fish, molluscs and crustaceans. *J. Geochem. Explor.* 121, 55–61.
- Ruttens, A., Blanpain, A.C., De Temmerman, L., Waegeneers, N. (2012). Arsenic speciation in food in Belgium Part 1: Fish, molluscs and crustaceans. *J. Geochem. Explor.* 121, 55–61.
- Sadee, B.A., Foulkes, M.E., Hill, S.J., 2016. An evaluation of extraction techniques for arsenic in staple diets (fish and rice) utilising both classical and enzymatic extraction methods. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 33 (3), 433–441.
- Saeki, K., Sakakibara, H., Sakai, H., Kunito, T., Tanabe, S., 2000. Arsenic accumulation in three species of sea turtles. *Biometals* 13, 241–250.
- Sakurai, T., Kaise, T., Ochi, T., Saitoh, T., Matsubara, C., 1997. Study of in vitro cytotoxicity of a water soluble organic arsenic compound, arsenosugar, in seaweed. *Toxicology* 122 (3), 205–212.
- Schmeisser, E., Goessler, W., Francesconi, K.A., 2006a. Human metabolism of arsenolipids present in cod liver. *Anal. Bioanal. Chem.* 385 (2), 367–376.
- Schmeisser, E., Goessler, W., Kienzl, N., Francesconi, K.A., 2005. Direct measurement of lipid-soluble arsenic species in biological samples with HPLC-ICPMS. *Analyst* 130 (6), 948.

- Schmeisser, E., Raml, R., Francesconi, K.A., Kuehnelt, D., Lindberg, A.L., Soros, C., Goessler, W., 2004. Thio arsenosugars identified as natural constituents of mussels by liquid chromatography mass spectrometry. *Chem. Commun.* 16, 1824–1825.
- Schmeisser, E., Rumpfer, A., Kollroser, M., Rechberger, G., Goessler, W., Francesconi, K.A., 2006b. Arsenic fatty acids are human urinary metabolites of arsenolipids present in cod liver. *Angew. Chem. Int. Ed.* 45 (1), 150–154.
- Sele, V., Amlund, H., Berntssen, M.H.G., Berntsen, J.A., Skov, K., Sloth, J.J., 2013. Detection of arsenic-containing hydrocarbons in a range of commercial fish oils by GC-ICPMS analysis. *Anal. Bioanal. Chem.* 405 (15), 5179–5190.
- Sele, V., Sloth, J.J., Julshamn, K., Skov, K., Amlund, H., 2014. Arsenic-containing fatty acids and hydrocarbons in marine oils – determination using reversed-phase HPLC-ICP-MS and HPLC-qTOE-MS. *Talanta* 121, 89–96.
- Sele, V., Sloth, J.J., Julshamn, K., Skov, K., Amlund, H., 2015. A study of lipid- and water-soluble arsenic species in liver of Northeast Arctic cod (*Gadus morhua*) containing high levels of total arsenic. *J. Trace Elem. Med. Biol.* 30, 171–179.
- Sele, V., Sloth, J.J., Lundebye, A.-K., Larsen, E.H., Berntssen, M.H.G., Amlund, H., 2012. Arsenolipids in marine oils and fats: a review of occurrence, chemistry and future research needs. *Food Chem.* 133 (3), 618–630.
- Shibata, Y., Morita, M., 1988. A novel, trimethylated arseno-sugar isolated from the brown alga *Sargassum thunbergii*. *Agric. Biol. Chem.* 52 (4), 1087–1089.
- Shibata, Y., Sekiguchi, M., Otsuki, A., Morita, M., 1996. Arsenic compounds in zoo- and phyto-plankton of marine origin. *Appl. Organomet. Chem.* 10 (9), 713–719.
- Shiomi, K., Kakehashi, Y., Yamanaka, H., Kikuchi, T., 1987. Identification of arsenobetaine and a tetramethylarsonium salt in the clam *Meretrix lusoria*. *Appl. Organomet. Chem.* 1 (2), 177–183.
- Sioen, I., De Henauw, S., Van Camp, J., Volatier, J.L., Leblanc, J.C., 2009. Comparison of the nutritional-toxicological conflict related to seafood consumption in different regions worldwide. *Regul. Toxicol. Pharmacol.* 55 (2), 219–228.
- Siro, V., Leblanc, J.C., Margaritis, I., 2012. A risk-benefit analysis approach to seafood intake to determine optimal consumption. *Br. J. Nutr.* 107 (12), 1812–1822.
- Šlejkovec, Z., Kápolna, E., Ipolyi, I., van Elteren, J.T., 2006. Arsenosugars and other arsenic compounds in littoral zone algae from the Adriatic Sea. *Chemosphere* 63 (7), 1098–1105.
- Šlejkovec, Z., Stajanko, A., Falnoga, I., Lipej, L., Mazej, D., Horvat, M., Faganelli, J., 2014. Bioaccumulation of arsenic species in rays from the northern Adriatic Sea. *Int. J. Mol. Sci.* 15 (12), 22073–22091.
- Sloth, J.J., Julshamn, K., 2008. Survey of total and inorganic arsenic content in blue mussels (*Mytilus edulis* L.) from Norwegian fjords: revelation of unusual high levels of inorganic arsenic. *J. Agric. Food Chem.* 56 (4), 1269–1273.
- Sloth, J.J., Larsen, E.H., Julshamn, K., 2003. Determination of organoarsenic species in marine samples using gradient elution cation exchange HPLC-ICP-MS. *J. Anal. At. Spectrom.* 18 (5), 452–459.
- Sloth, J.J., Larsen, E.H., Julshamn, K.R., 2005. Report on three aliphatic dimethylarsinoyl compounds as common minor constituents in marine samples. An investigation using high-performance liquid chromatography/inductively coupled plasma mass spectrometry and electrospray ionisation tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 19 (2), 227–235.
- Slotnick, M.J., Meliker, J.R., Nriagu, J.O., 2008. Intra-individual variability in toenail arsenic concentrations in a Michigan population, USA. *J. Expo. Sci. Environ. Epidemiol.* 18 (2), 149–157.
- Soleo, L., Lovreglio, P., Iavicoli, S., Antelmi, A., Drago, I., Basso, A., Di Lorenzo, L., Gilberti, M.E., De Palma, G., Apostoli, P., 2008. Significance of urinary arsenic speciation in assessment of seafood ingestion as the main source of organic and inorganic arsenic in a population resident near a coastal area. *Chemosphere* 73 (3), 291–299.
- Suner, M.A., Devesa, V., Clemente, M.J., Velez, D., Montoro, R., Urieta, I., Jalon, M., Macho, M.L., 2002. Organoarsenic species contents in fresh and processed seafood products. *J. Agric. Food Chem.* 50 (4), 924–932.
- Tabata, H., Anwar, M., Horai, S., Ando, T., Nakano, A., Wakamiya, J., Koriyama, C., Nakagawa, M., Yamada, K., Akiba, S., 2006. Toenail arsenic levels among residents in Amami-Oshima Island, Japan. *Environ. Sci.* 13 (3), 149–160.
- Takeuchi, M., Terada, A., Nanba, K., Kanai, Y., Owaki, M., Yoshida, T., Kuroiwa, T., Nirei, H., Komai, T., 2005. Distribution and fate of biologically formed organoarsenicals in coastal marine sediment. *Appl. Organomet. Chem.* 19 (8), 945–951.
- Taleshi, M.S., Edmonds, J.S., Goessler, W., Ruiz-Chancho, M.J., Raber, G., Jensen, K.B., Francesconi, K.A., 2010. Arsenic-containing lipids are natural constituents of sashimi tuna. *Environ. Sci. Technol.* 44 (4), 1478–1483.
- Taleshi, M.S., Jensen, K.B., Raber, G., Edmonds, J.S., Gunnlaugsdottir, H., Francesconi, K.A., 2008. Arsenic-containing hydrocarbons: natural compounds in oil from the fish capelin, *Mallotus villosus*. *Chem. Commun.* 39, 4706.
- Taleshi, M.S., Seidler-Egdal, R.K., Jensen, K.B., Schwerdtle, T., Francesconi, K.A., 2014. Synthesis and characterization of arsenolipids: naturally occurring arsenic compounds in fish and algae. *Organometallics* 33 (6), 1397–1403.
- Taylor, V.F., Jackson, B.P., Siegfried, M.R., Navratilova, J., Francesconi, K.A., Kirshtein, J., Voytek, M., 2012. Arsenic speciation in food chains from mid-Atlantic hydrothermal vents. *Environ. Chem.* 9 (2), 130.
- Terol, A., Ardini, F., Grotti, M., Todoli, J.L., 2012. High temperature liquid chromatography-inductively coupled plasma mass spectrometry for the determination of arsenosugars in biological samples. *J. Chromatogr. A* 1262, 70–76.
- Thomson, D., Maher, W., Foster, S., 2007. Arsenic and selected elements in inter-tidal and estuarine marine algae, south-east coast, NSW, Australia. *Appl. Organomet. Chem.* 21 (6), 396–411.
- Traar, P., Rumpfer, A., Madl, T., Saischek, G., Francesconi, K.A., 2009. Synthesis of naturally occurring arsenic-containing carbohydrates. *Aust. J. Chem.* 62 (6), 538–545.
- Tukai, R., Maher, W.A., McNaught, I.J., Ellwood, M.J., 2002a. Measurement of arsenic species in marine macroalgae by microwave-assisted extraction and high performance liquid chromatography-inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* 457 (2), 173–185.
- Tukai, R., Maher, W.A., McNaught, I.J., Ellwood, M.J., Coleman, M., 2002b. Occurrence and chemical form of arsenic in marine macroalgae from the east coast of Australia. *Mar. Freshw. Res.* 53 (6), 971–980.
- Tyson, J., 2013. The determination of arsenic compounds: a critical review. *ISRN Anal. Chem.* 2013, 835371 (24pp).
- US EPA, 2014. Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003–2010).
- US FDA, 1993. Guidance Document for Arsenic in Shellfish. Food and Drug Administration, Washington, DC.
- Uneyama, C., Toda, M., Yamamoto, M., Morikawa, K., 2007. Arsenic in various foods: cumulative data. *Food Addit. Contam.* 24 (5), 447–534.
- Unterberg, M., Leffers, L., Hubner, F., Humpf, H.U., Lepikhov, K., Walter, J., Ebert, F., Schwerdtle, T., 2014. Toxicity of arsenite and thio-DMA(V) after long-term (21 days) incubation of human urothelial cells: cytotoxicity, genotoxicity and epigenetics. *Toxicol. Res.* 3 (6), 456–464.
- Vahter, M., Marafante, E., Dencker, L., 1983. Metabolism of arsenobetaine in mice, rats and rabbits. *Sci. Total Environ.* 30, 197–211 (SEP).
- van Elteren, J.T., Šlejkovec, Z., Kahn, M., Goessler, W., 2007. A systematic study on the extractability of arsenic species from algal certified reference material IAEA-140/TM (*Fucus* sp., sea plant homogenate) using methanol/water extractant mixtures. *Anal. Chim. Acta* 585 (1), 24–31.
- Van Hulle, M., Zhang, C., Schotte, B., Mees, L., Vanhaecke, F., Vanholder, R., Zhang, X.R., Cornelis, R., 2004. Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed *Laminaria*. *J. Anal. At. Spectrom.* 19 (1), 58–64.
- Van Hulle, M., Zhang, C., Zhang, X., Cornelis, R., 2002. Arsenic speciation in Chinese seaweeds using HPLC-ICP-MS and HPLC-ES-MS. *Analyst* 127 (5), 634–640.
- Vandermeersch, G., Lourenco, H.M., Alvarez-Munoz, D., Cunha, S., Diogene, J., Cano-Sancho, G., Sloth, J.J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J.O., Marques, A., Robbins, J., 2015. Environmental contaminants of emerging concern in seafood—European database on contaminant levels. *Environ. Res.* 143, 29–45 (Pt B).
- Viczek, S.A., Jensen, K.B., Francesconi, K.A., 2016. Arsenic-containing phosphatidylcholines: a new group of arsenolipids discovered in Herring Caviar. *Angew. Chem. Int. Ed.* 55 (17), 5259–5262.
- Wei, C., Li, W.H., Zhang, C., Van Hulle, M., Cornelis, R., Zhang, X.R., 2003. Safety evaluation of organoarsenic species in edible porphyra from the China Sea. *J. Agric. Food Chem.* 51 (17), 5176–5182.
- Wei, M., Wanibuchi, H., Morimura, K., Iwai, S., Yoshida, K., Endo, G., Nakae, D., Fukushima, S., 2002. Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. *Carcinogenesis* 23 (8), 1387–1397.
- Whaley-Martin, K.J., Koch, I., Moriarty, M., Reimer, K.J., 2012. Arsenic speciation in blue mussels (*Mytilus edulis*) along a highly contaminated arsenic gradient. *Environ. Sci. Technol.* 46 (6), 3110–3118.
- Whaley-Martin, K.J., Koch, I., Reimer, K.J., 2013. Determination of arsenic species in edible periwinkles (*Littorina littorea*) by HPLC-ICPMS and XAS along a contamination gradient. *Sci. Total Environ.* 456, 148–153.
- WHO-IARC, 2011. Arsenic in Drinking Water.
- Williams, P.N., Price, A.H., Raab, A., Hossain, S.A., Feldmann, J., Meharg, A.A., 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environ. Sci. Technol.* 39 (15), 5531–5540.
- Wu, X., Gao, M., Wang, L., Luo, Y.J., Bi, R., Li, L.X., Xie, L.T., 2014. The arsenic content in marketed seafood and associated health risks for the residents of Shandong, China. *Ecotoxicol. Environ. Saf.* 102, 168–173.
- Wuilloud, R.G., Altamirano, J.C., Smichowski, P.N., Heitkemper, D.T., 2006. Investigation of arsenic speciation in algae of the Antarctic region by HPLC-ICP-MS and HPLC-ESI-ion trap MS. *J. Anal. At. Spectrom.* 21 (11), 1214–1223.
- Yamauchi, H., Takahashi, K., Mashiko, M., Saitoh, J., Yamamura, Y., 1992. Intake of different chemical-species of dietary arsenic by the Japanese, and their blood and urinary arsenic levels. *Appl. Organomet. Chem.* 6 (4), 383–388.
- Yoshida, K., Kuroda, K., Zhou, X., Inoue, Y., Date, Y., Wanibuchi, H., Fukushima, S., Endo, G., 2003. Urinary sulfur-containing metabolite produced by intestinal bacteria following oral administration of dimethylarsinic acid to rats. *Chem. Res. Toxicol.* 16 (9), 1124–1129.
- Yu, L.L., Davis, C., Ellis, M., Narukawa, T., Paul, R., 2016. Quality assurance standard for assessment of arsenic species in apple juice. Winter Conference on Plasma Spectrochemistry. National Institute of Standards and Technology Material Measurement Laboratory U.S. Department of Commerce.
- Yu, L.L., Wei, C., Zeisler, R., Tong, J.T., Oflaz, R., Bao, H.X., Wang, J., 2015. An approach for identification and determination of arsenic species in the extract of kelp. *Anal. Bioanal. Chem.* 407 (12), 3517–3524.
- Zava, T.T., Zava, D.T., 2011. Assessment of Japanese iodine intake based on seaweed consumption in Japan: a literature-based analysis. *Thyroid Res.* 4, 14.