Nutrition review

Arsenic in the human food chain, biotransformation and toxicology- review focusing on seafood arsenic

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Abstract

Fish and seafood are main contributors of arsenic (As) in the diet. The dominating arsenical is the organoarsenical arsenobetaine (AB), found particularly in finfish. Algae, blue mussels and other filter feeders contain less AB, but more arsenosugars and relatively more inorganic arsenic (iAs), whereas fatty fish contain more arsenolipids. Other compounds present in smaller amounts in seafood include trimethylarsine oxide (TMAO), trimethylarsoniopropionate (TMAP), dimethylarsenate (DMA), methylarsenate (MA) and sulphur-containing arsenicals. The toxic and carcinogenic arsenical iAs is biotransformed in humans and excreted in urine as the carcinogens dimethylarsinate (DMA) and methylarsonate (MA), producing reactive intermediates in the process. Less is known about the biotransformation of organoarsenicals, but new insight indicates that bioconversion of arsenosugars and arsenolipids in seafood results in urinary excretion of DMA, possibly also producing reactive trivalent arsenic intermediates. Recent findings also indicate that the pre-systematic metabolism by colon microbiota play an important role for human metabolism of arsenicals. Processing of seafood may also result in transformation of arsenicals.

Key words: arsenic, seafood, biotransformation, toxicology, human, review.

Introduction

The beneficial effects on health of a diet consisting of a moderate fish and seafood intake are well established [1-3]. On the other hand, fish and seafood may also contain harmful contaminants and other undesirable substances such as mercury and persistent halogenated compounds, which has resulted in a number of risk-benefit assessments of fish and seafood during the last decade [3-8]. However, the role of arsenic (As) has received attention only in few reports [1, 9, 10].

Seafood is the major contributor to As in the diet. Although As in seafood mostly occurs as organic As species, occurrence data on As in seafood are usually reported as total arsenic (tAs). However, due to the differences in toxicity among the different arsenicals, there is a need for speciation data to conduct a risk assessment of As ingested from seafood [11]. Furthermore, there is a need to explore whether the arsenicals present in seafood may be converted during food processing to As species of possible health concern. Of additional importance is to examine whether arsenicals ingested with (sea)food may convert into intermediates or As species of toxicological concern by the intestinal microflora or during metabolism in the body. The aim of the present paper is to review what is currently known about the chemistry, metabolism and toxicity of arsenicals in seafood.

Chemico-biological characteristics of arsenic

Arsenic is a metalloid with the atomic number 33 and belongs to group 15 in the Periodic Table. The German scholar and alchymist Albertus Magnus (1193-1280) is believed to be the first who isolated As in 1250. Arsenic can exist in four oxidation states: -3, 0, +3 and +5, the latter state being the dominant one under oxygenated and normal environmental conditions (Table 1). The majority of As species found in organisms and foods are in the pentavalent oxidation state [11, 12].

The chemistry of As shows similarities with other elements in the same group of the Periodic Table, i.e. nitrogen and phosphorous. For example, arsenate (AsO_4^{3-}), occurring in seawater together with the structurally similar phosphate (PO_4^{3-}), are indistinguishable to marine algae, hence the uptake of arsenate is high [11]. In addition, the As compound arsenobetaine (AB) (Table 1) has structural similarities to glycine betaine [$(CH_3)_3N^+CH_2COO^-$], a nitrogen betaine, which is used as an osmolyte by aquatic organisms to maintain osmotic balance under conditions of changing salinity [13]. These structural similarities might explain the high concentrations of inorganic arsenic (iAs) in marine algae and the higher concentrations of AB in marine organisms compared to freshwater organisms.

Arsenic is not considered to be an essential element for humans. Research on the possible essentiality of As is scarce and mostly conducted in the 1970s and 1980s [14]. However, based on animal studies (chickens, hamsters, goats, miniature pigs and rats) indicating that As deprivation results in depressed growth, abnormal reproduction, growth retardation and fragile red blood cells, a few researchers have hypothesized that As is an essential element for humans at very low dosages [15-17].

Arsenicals in food

Food is the primary contributor for human As intake for those that are not exposed through occupation or drinking water [12, 14, 18]. Already at the beginning of the 20th century reports on high concentrations of As in marine organisms were presented [19]. The As concentrations vary widely depending on the food type, growing conditions (type of soil, water, geochemical activity conditions, use of As pesticides) and processing techniques [12]. Most monitoring data are still reported as total As (tAs), thus not differentiating the various arsenicals, although As in foods occurs as a mixture of inorganic and organic species (organoarsenicals) [11].

When chemically quantified, As (V) and As (III) are usually reported together and referred to as the sum of iAs. Foods of terrestrial origin generally contain concentrations of tAs below 0.02 mg As/kg wet weight (ww) [20] and therefore have low concentrations of iAs. The exceptions are plants that accumulate As by root uptake from the soil (e.g. the rice plant) [21] or by absorption of airborne As deposited on the leaves, e.g. tea [22]. Cereal and cereal products, and in particular rice and rice based products, have the highest iAs concentrations with 0.1-0.4 mg As/kg dry weight (dw) [11, 23, 24]. The proportion of iAs of tAs in rice ranges between 10-93 %, and brown rice seems to have a higher iAs content than white rice, indicating that the As is attached to bran components [25, 26]. The iAs in rice is probably present both as As (III) and As (V) [27, 28]. Rice may also contain minor amounts of organic As such as DMA (V) [28, 29].

Although fish and seafood are the food groups with the highest tAs concentrations, these food groups are generally low in iAs (usually < 0.2 mg As/kg dw) [11, 30]. A few exceptions exist, e.g. some marine algae, with hijiki (*Hizikia fusiforme*) having an extremely high iAs concentration (arsenate > 60 mg/kg) [11]. Bivalves, like blue mussels (*Mytilus edulis*), also have relatively high concentrations of iAs with concentrations ranging between 0.001-4.5 mg As/kg [31].

While terrestrial organisms mainly contain As in the form as iAs, most of the As in marine organisms are organoarsenicals with levels between 1-100 mg As/kg ww [11]. Arsenobetaine (AB), which was first identified in lobster in 1977, is the most prevalent arsenical in marine organisms and particularly in organisms higher in the food web [32]. Fresh-water organisms also contain AB, but at much lower levels (< 0.1 mg As/kg dw) [10]. AB has been detected in some terrestrial foods, like mushrooms [33] and poultry, in the latter possibly originating from seafood in the feed or added as a growth promoter [34, 35].

Arsenosugars are the major arsenicals in marine algae (2-50 mg As/kg dw) and in animals feeding on algae, e.g. mussels that typically contain 0.9-3.4 mg As/kg dw [36]. Arsenosugars are probably a biotransformation product of inorganic arsenate that initially is taken up by the algae from seawater. This represents the most significant bioaccumulation step for As in the environment [11].

Arsenolipids (i.e. arsenic-containing fatty acids, arsenic-containing hydrocarbons, cationic trimethylarsenio fatty acids and arsenosugar-phospholipids; Table 1) have been reported to be present in cod liver oil, capelin and tuna [37-40]. Although many arsenolipids still are unknown, several structures have been elucidated recently [41-43]. Arsenic-containing hydrocarbons seem to dominate in fish species with higher total arsenolipid content (brill and sardine), whereas arsenic-containing fatty acids seems to predominate in fish species with less total arsenolipid content (mackerel and red mullet) [44].

Other organoarsenicals that are present at low concentrations in marine organisms include trimethylarsoniopropionate (TMAP), arsenocholine (AC), methylarsonate (MA), dimethylarsinate (DMA), trimethylarsine oxide (TMAO), and tetramethylarsonium ion (TETRA) (Table 1) [10, 11].

Metabolism of dietary arsenic

The food matrix as well as the presence of other food constituents in the gastrointestinal tract, affects the absorption of arsenicals. In addition, water-soluble arsenicals seem to be more easily absorbed than fat-soluble arsenicals [11]. Studies in rodents indicate rapid and near complete (95 %) absorption of iAs present in drinking water [18], and the absorption rate of iAs from rice was 89 % in a pig model [45].

Early studies on absorption and excretion of As ingested from seafood (i.e. mainly AB), indicated an efficient absorption and that "fish arsenic" was rapidly excreted [19, 46, 47]. Later, one study using isotopically labeled AB showed rapid and almost complete excretion, with less than 1 % of the radioactivity remaining in the body 24 days after ingestion [48]. However, this study presented no accurate quantitative data on AB absorption since the first measurement point was after 24 h when much of the absorbed AB probably already was excreted in urine. An experimental study in human volunteers suggest that both MA and DMA are readily absorbed across the GI-tract with about 75 % excreted in the urine after four days [49]. In another human study, arsenosugars seem to be almost completely absorbed (> 80 %) [50], possibly with considerable inter-individual variation [51]. A metabolic study of arsenolipids in two volunteers showed that arsenolipids were readily absorbed and converted to water-soluble arsenicals; 90 % were excreted in the urine during 66 h [52]. The absorption of tAs, AB and AC has been shown to decrease with increased fat content in seafood, while it seems not to be affected by the protein content [53, 54].

After absorption in the GI-tract, As is taken up in the bloodstream and distributed between the plasma and the erythrocytes where it is bound to hemoglobin and further transported into the cells by the aquaglyceroporins 3, 7 and 9, which are phosphate- and hexose permease transporters [11, 55]. Animal studies have shown that after administration of iAs, it is distributed to all tissues, with high concentrations in the kidney, lung, urinary bladder, skin, blood and liver [56, 57]. Several weeks after administration, As is translocated to hair, nails and skin because of the high affinity of trivalent arsenicals to bind to cystein-components present in the proteins in these tissues [11].

In comparison with iAs, less is known about human metabolism of organoarsenicals. Both arsenite and arsenate are extensively methylated, with MA and DMA being the principle methylated metabolites excreted in urine [12]. Challenger [58] was the first to provide a

chemically plausible scheme for the methylation of iAs. In humans, As (V) enters the cell via the phosphate carrier system, is reduced enzymatically to As (III), and further biotransformed by oxidative methylation in the liver by addition of a methyl group from the methyl donor S-adenosyl methionine (SAM) to form methylarsenate (MA(V)). MA (V) is then enzymatically reduced to methylarsonite (MA (III)), which is added a second methyl group via an oxidative reaction to yield dimethylarsenate (DMA (V)). It is unclear to what extent DMA (V) is further reduced to DMA (III) *in vivo*, since DMA (III) is unstable and difficult to measure [11]. Some studies have reported the presence of trivalent mono- and dimethylated arsenicals in the urine of populations exposed to iAs via drinking water [59-61]. In rodents, DMA (III) is further methylated to trimethylarsine oxide (TMAO), however this arsenical is usually not detected in human urine [62]. The methylation activity occurs in the cytosol of the cell with glutathione (GSH) as an essential co-factor and source of electrons for the reduction [63, 64].

Over the latest decades, extensive work has been carried out to further explore the metabolic pathway of iAs. Hayakawa et al. (2005) proposed another pathway where trivalent methylated As species may be formed prior to their respective mono- and dimethyl pentavalent arsenical. In this scheme, arsenite reacts with glutathione to form arsenic triglutathione, which is methylated to form monomethylarsenic diglutathione that may form methylarsonite (MA (III) or further methylated to dimethylarsenic glutathione may form dimethylarsinite (DMA (III)). The trivalent species may then undergo oxidation to form the pentavalent species methylarsenate and dimethylarsinate [65]. The exact scheme for methylation of iAs has not yet been clearly established.

An arsenic methyl transferase (As3MT) existing in a number of polymorphic forms has been identified [66-68]. This enzyme is crucial as a reducing agent in the methylation of iAs, and thus is at least one of the enzymes involved in this process [66, 69, 70]. There are genetic polymorphisms in the regulation of As3MT, and one of the polymorphism, the genotype

M287T, has been linked to higher percentage of urinary MA and increased risk of cancer and other diseases [71-75]. Large inter-population and inter-individual differences in the methylation pattern in humans have been observed, e.g. the percentage of MA in urine may vary between 1-30 % [11]. These differences could possibly be explained by this enzyme and its polymorphic forms [76]. However the inter-individual differences may also be due to differences in nutritional status of nutrients involved in the one-carbon metabolism, like folate, B-vitamins, zink and selenium [77-80]. Pregnancy has been demonstrated to increase the efficacy of As methylation [81].

Whereas our understanding of the mechanism of methylation has increased by the characterization of arsenic methyl transferases, less is known about the mechanisms underlying the *in vivo* reduction of pentavalent arsenic to trivalent arsenic. Glutathione (GSH) appears to play an essential role. Also some glutathione–S−transferases (GST), i.e. GSTO1 and GSTT1, may take part in the reductive reactions (Fowler et al 2014). Recently, it was shown that glutathione synthase (GS) could promote the reduction of As (V) via arsenolysis of GSH [82]. This reaction requires ADP as an As (V) acceptor forming ADP-arsenate, which upon release from GS As (V) is reduced by GSH to form As (III). The mechanism of DMA (V) reduction to the highly toxic DMA (III) is also unclear, but seems in the rat liver cytosol to require GSH and NADPH in addition to catalysing activity yet not identified, which could be inhibited by thiol reagents [83]. Also in the reduction of DMA (V), GSTs may be involved.

The relative distribution of iAs, MA and DMA in urine in various populations is considered to be fairly constant and in the range of 10-30 %, 10-20 % and 60-80 %, respectively [63, 84]. A high proportion of MA in urine may indicate lower methylation capacity. Age, smoking and gender seem to influence the relative distribution of methylated urinary arsenicals [85-87].

In humans, the organoarsenical AB has been assumed neither to be formed or biotransformed, and thus excreted unchanged in urine [46-48, 88, 89]. In contrast to this assumption, our recent human seafood intervention study indicated endogenous formation of AB, based on a higher estimated total urinary excretion of AB than the amount ingested. Using an ANCOVA model, we estimated that for each µg AB absorbed, about 1.6 and 1.1 µg AB was excreted in those consuming blue mussels and cod, respectively [54]. Another human study also speculated that AB could be produced or accumulated in humans since they found that 3 out of 5 study participants excreted AB despite ingesting an AB-free diet, namely rice [90].

Arsenosugars and arsenolipids have been reported to be metabolised with DMA as the main metabolite, with oxo- and thio-arsenicals as minor urinary metabolites [50, 52, 91, 92]. Consequently, urinary DMA originates not only from the bioconversion of iAs, but also from the metabolism of these organoarsenicals. This is in accordance with recent findings that we observed in volunteers consuming different seafoods [54, 93]. The participants excreted far more DMA than that could have been produced from ingested iAs from the seafood. The enzyme As3MT has also been found to play a major role in the metabolism of organoarsenicals from seafood (oysters) in terms of the urinary DMA excretion [94] following seafood intake, indicating that this enzyme not only takes part in iAs metabolism. It is also possible that the microbiome of the GI-tract can play a role in the (pre-absorptive) metabolism of arsenicals. *In vitro* studies have shown that the microbiome in mouse coecum and human colon microbiota converts As (V) and methylated arsenicals into oxo-arsenicals and thio-arsenicals [95-98]. In our previous study, a multitude of urinary arsenicals excreted after volunteers ingested an identical meal consisting of 150 g blue mussels were identified; totally seventeen different arsenicals were detected [99]. Six of the excreted arsenicals were thio-arsenicals, counting for 10 % of the tAs excreted, and great inter-individual differences in the urinary excretion pattern were found despite an identical diet. A recent study of the human

gut microbiota found that sulfate reducing bacteria were necessary and sufficient for an extensive thiolation of MA (V) to monomethyl monothioarsenate to occur [98]. Here they also found great inter-individual variability in the thiolation capacity between the different human fecal inocula, supporting the role of the colon microbiome in the pre-absorptive As metabolism. One recent *in vitro* study found that dietary composition might have an impact on the presystematic arsenical metabolism; a Westernized diet resulted in higher formation of MA (III) and monomethylmonothio arsonate (MMMTA (V)) than an Asian diet after 48 h incubation of these diets with rice in human colon suspensions [28].

The main excretory route for As in humans is urine, although scarce documentation also indicates that a small amount is excreted via the gallbladder in feces [100]. Glutathione conjungated arsenicals are excreted in rat bile [101-103]. Other excretory routes are generally insignificant, although As to some degree is excreted in sweat as well as by minor excretory routes such as skin, hair and nails [12]. Normal urinary tAs concentrations in non-exposed humans are in the range of 5-50 µg tAs/L, but seafood ingestion may increase the concentration to more than 1 mg tAs/L [76]. The mean tAs plasma concentration in blood has been observed to be < 2 µg/L in populations not exposed through drinking water [54, 104, 105].

AB seems to be rapidly cleared from the blood with similar kinetics and clearance in plasma and erythrocytes. Peak concentration in blood is around 2 h after intake and rapidly decreases thereafter [88, 106]. However, we found that upon daily repeated exposure for two weeks AB accumulated as not all AB was excreted within 24 h [93]. Ingested DMA and MA seem to be excreted mainly in urine in humans, and mostly within one day [49]. Our own and others data suggest that tAs ingested with seafood is eliminated from the body within approximately four days [93, 107]. Upon 15 consecutive days with blue mussel consumption, a remarkably high

level of urinary MA was observed. The urinary MA concentration in this period increased from 0.29 to 24.6 µg As/g creatinine [93].

Mechanisms and toxicity of arsenic compounds occurring in seafood

Despite many years of research, the exact mechanisms of As toxicity are not yet fully understood. The main reason for this is that As undergoes a number of complicated metabolic conversions *in vivo*, and its metabolites and As itself interacts with both intra- and extracellular macromolecules. There is also lack of appropriate animal models since rodents, which usually are the species used in toxicity studies, differ from humans regarding As metabolism [55].

Both iAs as well as MA and DMA have been shown to inhibit mitochondrial respiration, leading to the formation of reactive oxygen species (ROS) which in turn may cause DNA mutations and therefore possibly play a role in cancer development and cell death [76]. Both As (III) and As (V) may bind to thiol groups and membrane lipids, resulting in inhibition of functional groups in enzymes and altered fluidity of membrane lipids, respectively [55]. Some As (V) compounds may substitute structurally similar phosphate groups, thereby affecting enzymes depending on this group for their activity (e.g. interfering in the synthesis of ATP and DNA); thus possibly playing a role in As toxicity [108, 109].

Information concerning the toxicity of As is largely related to the intake of iAs (As (III) and As (V)), which in acute doses can lead to multiple organ failure and death. A chronic, long-term ingestion of iAs with As-contaminated drinking water has been associated with a range of health effects, such as skin lesions, cancer in the lung, bladder, kidney and skin, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism and diabetes [11].

Previously, the methylation of iAs was regarded as a detoxification pathway. However, recent studies have suggested that the main intermediate metabolites MA (III) and DMA (III) are reactive and highly toxic in themselves, resulting in the current view that the metabolic products of iAs metabolism probably have a role in the mechanism of As toxicity [14, 76, 110]. Generally, trivalent arsenicals are more toxic than the pentavalent [14, 111], and MA (III) and DMA (III) are rapidly oxidized to their pentavalent counterparts [112, 113]. These intermediates in the iAs methylation scheme can initiate toxic effects, like DNA-damage [114, 115], or be indirectly genotoxic [110, 116].

A repeated dose of MA has been shown to have effects on the GI-tract, kidney, thyroid and reproductive system [18]. Also, a higher proportion of urinary MA has been associated with higher risks of developing As-related diseases [117-123].

DMA (V) has shown genotoxic effects on cultured mammalian cells [115, 124], and in animal studies been reported to promote carcinogenic effects in the urinary bladder, kidney, liver and the thyroid gland [125, 126]. Both MA (V) and DMA (V) has been classified as "possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) [127].

Although based on scarce documentation [10], the organoarsenical AB is regarded as non-toxic. Due to insufficient information, AB could not be classified as carcinogenic by IARC [127]. In addition, also the compounds TMAO, TETRA, and AC are considered non-toxic; however, the compound TETRA has been found to be more acutely toxic than AB [14, 128].

Although scarce information is available regarding the toxicity of arsenosugars and arsenolipids, concerns have been expressed about their potential toxicity because toxic intermediates may be produced during their biotransformation to DMA [14, 129]. In a recent study on two arsenosugars, DMA (V)-sugar-glycerol and DMA (V)-sugar-sulfate, no

genotoxic or cytotoxic effects were found in cultured human bladder cells [130]. These arsenosugars in the same study were intestinally bioavailable in a Caco-2 model, but it is not known whether the cells had the ability to further metabolise the arsenosugars. For arsenolipids, however, a recent cellular toxicity study of three arsenolipids showed cytotoxicity comparable with that of iAs (V) in human bladder and liver cells [131]. The authors suggest that the high cellular toxicity induced by arsenolipids may be related to their ability to easily pass cell membranes, possibly by passive diffusion. Thio-arsenicals are probably produced by the colon microbiota [95-97], and one recent study found that sulfate-reducing bacteria present in the human gut was necessary for conversion of MA (V) into the thiolated monomethyl monothioarsonate [98]. Thio-arsenicals have been recognized as toxic in studies on human cells [132, 133]. Thio-DMA (V), the sulphur analogue of DMA (V) and a mammalian metabolite after arsenosugar consumption [51, 134-136], has shown strong cytotoxic effects in cultured human lung and bladder cells [130, 137].

Biomarkers of arsenic exposure

Concentrations of tAs or its metabolites in blood, hair, nails and urine are used as biomarkers of As exposure. Blood As is a useful biomarker only in the case of acute As poisoning or in stable, chronic high-level exposure, since As is cleared from blood within a few hours after absorption. Therefore tAs in blood has been considered to reflect only very recent exposure [18]. Since chronic iAs exposure from drinking water has been shown to be correlated with tAs in blood, blood tAs might be a reliable biomarker in such cases [138, 139]. Blood As represents a measure of internal dose and might better reflect actual tissue burdens than urinary As [11]. The concentrations of As in hair and nails can indicate past As exposure, and is therefore considered reliable biomarkers for long-term iAs exposure [11, 140].

Previously, urinary tAs was used as a biomarker of recent iAs exposure [11]. This viewpoint is fading because metabolites from ingested organoarsenicals are excreted in urine. Therefore, a careful diet history must be taken and/or seafood consumption should be terminated at least 2-3 days before urine collection when using urinary tAs as a biomarker for iAs exposure [141, 142]. Also, intake of arsenosugars and/or arsenolipids ingested with seafood results in increased DMA excretion [99, 143-145]. Some studies have used tAs minus AB or the sum DMA+MA+iAs as a biomarker for recent iAs exposure, resulting in misleading results unless a careful dietary history rules out possible organic arsenicals as precursors for urinary DMA. Only when the tAs concentrations are low, can it be assumed that iAs also must be low [146].

Effect of storage and processing on concentration and stability of arsenic compounds

Food preparation may affect the concentrations and stability of As compounds. Cooking and washing foods in iAs-contaminated water have also been shown to increase the iAs concentration in the prepared food [147], especially in rice [25, 148, 149]. However, cooking of rice in water which has not been contaminated with iAs, rinse washing before cooking and cooking with large volume of water seem to decrease the iAs content in the prepared food [147, 150, 151]. Traditional washing and soaking of the Hijiki algae also reduce the iAs content with up to 60 % [152], and likewise, cooking of pasta decreased the iAs content by about 60 % [153]. In our study of seafood, we found that the content of iAs of the blue mussels did not increase following heat processing [154].

Preparation of seafood at high temperatures (above 150 °C), like grilling and roasting, has been shown to decarboxylate (possibly by direct contact to the heat source) AB present in the raw product and produce small amounts of TETRA. [154-157] However, we found that cooking in water (stewing, boiling or steaming) did not decrease the content of AB [154]. One previous study has also indicated that AB can be degraded in an oxidative environment [158].

Storage may also affect the stability of the arsenicals. Seafood products are generally frozen in order to avoid bacterial decomposition after they are caught. During the initial freezing stages, microorganisms may maintain their activity, thus having the potential to transform arsenicals present [152]. Most arsenicals, like As (III), As (V), MA, DMA and AB, seem to be stable in urine at low temperatures (4°C and - 20°C) for up to 2 months, but for longer periods, some arsenicals, like DMA (III), are labile and rapidly oxidizes, even when stored frozen and their rate of conversion depends on the sample matrix [159, 160]. Based on scarce data, it has been suggested that AB in seafood may decompose during the freezing process [161, 162], and limited data suggest that AB decrease whilst DMA increase significantly upon storage by freezing of blue mussels, but not for fish species [154, 163]. Jenkins et al. (2003) found an increase in TMAO after freezing, however in contrast to their findings, Dahl et al. (2010) found a decrease in TMAO after freezing of blue mussels. Dahl et al. (2010) also found increased concentrations of DMA, TMAO and AC in cod after freeze storage for one month [154]. However, the concentrations decreased to approximately the same concentration as the fresh samples after being stored in the freezer for 3 months. In the same study, storage by freezing of salmon for one month resulted in a significant increase in the DMA concentration, whilst storage for three months increased the TETRA concentration for all seafood species studied [154].

Guidelines regarding arsenic intake from food

Some risk assessments have been undertaken over the last 20 years by various agencies and organizations [11, 164]. Most of them were conducted using studies on health effects related to As exposures via drinking water (i.e. iAs) and to exposure to iAs via food [11, 164].

In its hazard characterisation, JECFA modeled data from two recent studies [165, 166] on the same prospective cohort on urinary tract cancer and lung cancer from north-eastern Taiwan

with an average follow-up of 11.5 years. The studies used As in drinking water as the exposure parameter. The lowest benchmark dose lower confidence limit (BMDL) obtained, based on estimated total dietary As exposure, was from the lung cancer study of Chen and co-workers [165]. The modeling gave ranges of BMDL0.5 benchmark dose for 0.5 % increased incidence of cancer over background, of 4.5–7.3 µg/kg body weight per day and ranges of BMDL0.5, 95 % confidence limit for the benchmark dose of 3.0–5.0 µg/kg body weight per day. The lowest BMDL0.5 of 3.0 µg/kg body weight per day was selected as the reference point for risk assessment. A sensitivity analysis showed that this BMDL0.5 could be in the range of 2.0–7.0 µg/kg body weight per day. Based on this assessment, JECFA (2010) noted that the old provisional tolerable weekly intake (PTWI) of 15 µg/kg body weight was in the region of the BMDL0.5 and therefore inappropriate. The old PTWI was therefore withdrawn [164].

Reported mean dietary exposure to iAs in the USA and various European and Asian countries ranged from 0.1 to 3.0 µg/kg body weight per day. Hence, the margin of exposure is small, as it ranges from 1 to 30.

Discussion

Seafood is the main source of As in the diet, and despite limited documentation it is generally accepted that the dominant organoarsenicals in seafood are harmless. Although iAs is regarded as far more toxic than organoarsenicals, biotransformation of organoarsenicals may result in metabolites similar to those occurring in iAs metabolism. Since the toxic action of iAs at least partly seems to be related to formation of toxic trivalent As species, it is important to elucidate whether exposure of organoarsenicals via seafood consumption may pose a risk to humans.

Seafood is generally low in iAs, although seaweed and mussels are important exceptions [10, 30, 31, 54, 99, 167]. From studies where humans have ingested seafood and the urinary excretion of arsenicals have been measured, no indications exist for formation of iAs from ingested organoarsenicals [46, 49, 54, 85, 93, 99, 100, 143, 144]. Scarce data from human seafood studies indicate that a repeated intake of seafood does not result in higher excretion of iAs than that ingested [93, 107]. It is, however, not known whether urinary MA and DMA following seafood ingestion [54, 93] are formed directly or via inorganic As intermediates.

In our recent study following participants through 15 consecutive days, blue mussel consumption increased the urinary MA concentration almost ten times [93], to a concentration even higher than reported in a population exposed via occupation [168]. A higher proportion of urinary MA has been associated with numerous adverse health effects [169]. For instance, increased presence of MA (III) in urine of a Mexican population chronically exposed to iAs in drinking water was associated with a greater risk of As-induced skin lesions [60]. Studies of populations chronically exposed to iAs from drinking water have shown that the relative proportion of urinary MA may increase with increasing dose of iAs exposure [86, 87, 170, 171]. Higher urinary MA associated with increased exposure of iAs in drinking water, has been proposed to be a result of the inhibition of the second methylation step [172]. The finding of increased MA formation following repeated/"chronic" intake of seafood, like shell-fish and seaweed [93] warrants further investigation such as establishment of the mechanism of MA formation from organic arsenicals. Furthermore, it warrants examination of whether reactive and toxic trivalent As intermediates are formed, since this may imply a potential health hazard. One could speculate that repeated ingestion of arsenic from shellfish could cause accumulation of toxic trivalent As species that might inhibit further methylation of MA.

Seafood ingestion seems to be associated with more DMA excreted than ingested. The increased DMA excretion, indicating an endogenous production of DMA, is particularly high

for subjects ingesting seafood high in arsenosugars like bivalves [99, 143, 145, 173, 174], or arsenolipids/arsenosugars [50-52, 136]. Ingestion of seafood containing arsenosugar/arsenolipids results in raised urinary DMA excretion. This shows that DMA may originate from bioconversion of organoarsenicals present in seafood and not only from the methylation process of iAs. Also in this case the mechanism of formation is poorly characterised, and the possibility of intermediate formation of toxic trivalent arsenic species should be further investigated. As a consequence of DMA formation from organoarsenicals in seafood, DMA is not a valid biomarker for iAs exposure unless a careful diet history excluding seafood consumption has been performed. In a recent study arsenolipids, which are major components in fish oil, fish meat and algae, have been shown to be potent cytotoxic agents similar to that of As (III) [131], but more research is needed to establish their possible impact for human health. Both MA and DMA are metabolites of iAs and classified as possibly carcinogenic [175], and since the mechanisms of iAs toxicity are not fully understood, it cannot be ruled out if formation of these arsenicals may indicate a possible health concern associated with seafood intake.

Limited data suggest that AB may be formed endogenously [54, 90]. As the main arsenical present in seafood, it is of great importance for future studies to clarify whether AB can be formed *de novo* as well as identifying its sources in humans.

Generally, the pattern of arsenicals does not seem to be altered much due to processing, however, most seafood processing studies have found an increase in TETRA after heat application [154-157]. Since TETRA is considered to be more toxic than AB, the increase in TETRA in seafood processed at high temperatures should be taken into account when assessing the safety of such products [128].

Conclusion and future perspectives

Seafood arsenicals are mainly organic. Despite limited information on biotransformation and toxicity, they have historically been viewed as harmless. However, recent studies indicate that organoarsenicals undergo biotransformation that might involve formation of trivalent toxic arsenic intermediates. Some of the end products are also more toxic than the parent arsenical. Since the toxicity of arsenicals probably is related to their biotransformation, more knowledge is needed to clarify the pathways by which arsenosugars and/or arsenolipids present in e.g. fatty fish, cod liver oil, shellfish and seaweeds are being biotransformed into DMA, and whether toxic intermediates are formed in the process. Additionally, there is a need for more studies on how repeated doses/"chronic" ingestion of foods with relative high concentration of a variety of organic arsenicals (e.g. shellfish, and seaweed) may impact bioaccumulation and metabolism of these compounds. Since studies, e.g. *in vitro* studies and in mice, emphasize the role of the intestinal microflora in the handling of organoarsenicals, such studies in humans would also be of great importance.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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