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Review

Dietary exposure and neurotoxicity of the environmental free and bound toxin β-*N*-methylamino-L-alanine

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ABSTRACT

The growing evidence supporting a link between exposure to the naturally occurring toxin β-*N*-methylamino-L-alanine (BMAA) and progressive neurodegenerative diseases, has recently arisen the interest of the scientific community. Latest investigations suggest that dietary exposure to this algal toxin may have been largely underestimated. This paper reviews the state of the art regarding BMAA, with special attention paid to its neurotoxicity, its concentration levels in food, and human exposure. As for other environmental toxins, dietary intake is most likely the main route of exposure to BMAA for the general population. However, data concerning BMAA levels in foodstuffs are still scarce. It is concluded that further investigations on dietary intake and potential human health effects are clearly necessary to assess the risks to public health associated with BMAA exposure. Some critical remarks and recommendations on future research in this area are provided, which may help to identify approaches to reduce dietary BMAA exposure.

1. Introduction

Rapid proliferation of microscopic algae in aquatic environments, the so-called harmful algal blooms (HABs), is recognized as a growing problem worldwide. Even when the exact causes of HABs are not yet clear, human impacts combined with climate changes are thought to contribute to the recent increase in the incidence of these episodes (Paerl & Paul, 2012). Although being commonly referred as toxic algae, the microorganisms causing blooms belong to different kingdoms of life: eukaryotic microalgae and prokaryotic cyanobacteria, also known as blue-green algae. Many of the microorganisms responsible for the HABs are known to produce toxins that have a variety of adverse effects, such as skin irritation, diarrhea, hepatotoxicity and neurotoxicity in humans and animals (Dittmann, Fewer, & Neilan, 2013). Consumption of water and food exposed to HABs represents a major route of exposure to these toxins, which may result in serious or even fatal consequences. Particularly, filter-feeders, such as bivalve mollusks may consume HABs organisms and accumulate significant amounts of toxins (Regueiro, Álvarez, Mauriz, & Blanco, 2011; Regueiro, Martín-Morales, Álvarez, & Blanco, 2011).

Among the different classes of HAB toxins identified so far, β-*N*-methylamino-L-alanine (BMAA) has recently triggered an emerging interest because of the increasing evidence that links the exposure to this toxin to progressive neurodegenerative diseases, such as the amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC) (Banack & Cox, 2003; Cox, Davis, Mash, Metcalf, & Banack, 2016; Spencer et al., 1987). This compound belongs to the family of non-proteinogenic amino acids, an extremely diversified group accounting for > 1000 different chemical species (Rodgers, 2014).

It is already known that a number of chemicals may induce or accelerate the development of certain neurodegenerative diseases (Cannon & Greenamyre, 2011). For instance, exposure of humans to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a syndrome that mimics the core neurological symptoms and relatively selective dopaminergic neurodegeneration of Parkinson's disease (PD) (Dauer & Przedborski, 2003). PD has also been linked to exposure to rotenone and paraquat in agricultural workers (Tanner et al., 2011), whereas exposure to lead, mercury, and pesticides have been reported as potential risk factors for ALS (Johnson & Atchison, 2009). However, the role of naturally occurring toxins, such as BMAA, in progressive

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Table 1
Recently reported BMAA-producing organisms.

Species	Strain	Group	Collection place	Concentration (ng/g)	Form	Ref.
Navicula pelliculosa	CCAP 1050/9	Diatom	Massachusetts, USA	–	–	(Jiang, Eriksson, et al., 2014)
Thalassiosira sp.	CCAP 1085/15	Diatom	Loch Linnhe, UK	3.28 ± 2.5 DW	Bound	(Jiang, Eriksson, et al., 2014)
Achnanthes sp.	CCAP 1095/1	Diatom	Millport, Scotland	–	–	(Jiang, Eriksson, et al., 2014)
Proboscia inermis	CCAP 1064/1	Diatom	Brandsfield Strait	–	–	(Jiang, Eriksson, et al., 2014)
Skeletonema marinoi	SAAE08603	Diatom	Gullmarsfjorden, Sweden	1.1 ± 0.4 DW	Bound	(Jiang, Eriksson, et al., 2014)
Skeletonema marinoi	ST28	Diatom	Strömstad, Sweden	1.07 ± 0.8 DW	Bound	(Jiang, Eriksson, et al., 2014)
Phaeodactylum tricornutum	CCAP 1055/1	Diatom	Blackpool, UK	200–1400 DW	Total soluble	(Reveillon et al., 2016a)
Chaetoceros sp.	–	Diatom	Argenton, France	260–1600 DW	Total soluble	(Reveillon et al., 2016a)
Chaetoceros calcitrans	CCMP 1315	Diatom	–	560–1800 DW	Total soluble	(Reveillon et al., 2016a)
Thalassiosira pseudonana	CCMP 1015	Diatom	San Juan Island, WA, USA	170–280 DW	Total soluble	(Reveillon et al., 2016a)
Heterocapsa triquetra	–	Dinoflagellate	Baltic Sea, Sweden	–	–	(Jiang & Ilag, 2014)
Gymnodinium catenatum	IO.13.20	Dinoflagellate	Sesimbra, Portugal	457 ± 186 DW	Bound	(Lage et al., 2014)

DW: dry weight.

neurodegenerative diseases has not been extensively studied.

Here, we present an overview on the recent literature examining the neurotoxicity of BMAA, the main sources of this toxin in nature, as well as the available data regarding its occurrence in food and human exposure through the diet. We will not review analytical methodologies for extraction and detection of BMAA in different sample matrices, as they have been recently summarized by Cohen (2012) and Porojan, Mitrovic, Yeo, and Furey (2016). However, some critical points affecting the accuracy of the reported concentration levels are discussed. Finally, this paper highlights some potential implications of the dietary exposure to BMAA and difficulties faced in its prevention.

2. Sources of BMAA

BMAA was first identified in the seeds of the gymnosperm *Cycas circinalis*, currently known as *Cycas micronesica* Hill, a palm-like tree widespread on the tropical island of Guam in the Western Pacific Ocean (Hill, 1994; Vega & Bell, 1967). In 2003, Cox, Banack, and Murch (2003) found that BMAA was produced by nitrogen-fixing cyanobacteria of the genus *Nostoc* living symbiotically within the coralloid roots of the cycad trees. This finding prompted the search for BMAA in other cyanobacteria, which has resulted so far in the identification of this nonprotein amino acid in numerous groups of cyanobacteria (Cox et al., 2005; Li et al., 2010; Metcalf et al., 2008). For free-living cyanobacteria, Cox et al. (2005) reported the presence of BMAA in all major morphological sections, as well as 95% of the genera and 97% of the strains in a large sample set of cyanobacteria screened. For the symbiotic *Nostoc* strains isolated from relationships with a broad taxonomic diversity of fungi and host plants, they found that BMAA was present in 73% of them. However, several studies were unable to detect BMAA in various cyanobacteria species, including *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena*, among others (Krüger, Mönch, Oppenhäuser, & Luckas, 2010; Kubo, Kato, Hosoya, & Kaya, 2008; Rosén & Hellenäs, 2008).

The ubiquity of BMAA in the cyanobacterial phylum suggests that this amino acid is a fundamental cellular metabolite in cyanobacteria (Berntzon et al., 2013). However, the biosynthetic pathway for BMAA has not yet been elucidated and no genes have been identified so far (Pearson et al., 2016). Some studies indicate that this compound may play a role in nitrogen metabolism in cyanobacteria (Berntzon et al., 2013; Downing, Banack, Metcalf, Cox, & Downing, 2011). Thus, Downing et al. (2011) observed that free BMAA in *Microcystis* PCC7806 increased upon nitrogen deprivation, suggesting that BMAA is produced as a catabolism product to provide nitrogen or that it is synthesized to serve a functional role under starvation conditions.

This neurotoxin has been reported to occur in many different geographical areas including among others, Sweden (Jonasson et al., 2010), UK (Metcalf et al., 2008), the Netherlands (Faassen, Gillissen, Zweers, & Lürling, 2009), USA (Brand, Pablo, Compton, Hammerschlag, & Mash, 2010), South Africa (Esterhuizen & Downing, 2008) and China (Jiao et al., 2014), which highlights the global scale of the problem. Although BMAA-producing cyanobacteria can be found in terrestrial habitats (Cox et al., 2003, 2005), the major exposure pathway to BMAA is thought to be through the aquatic environment during cyanobacterial blooms, as well as through the consumption of aquatic organisms exposed to such blooms (Jonasson et al., 2010; Merel et al., 2013).

Reported BMAA concentrations in cyanobacteria may vary from a few nanogram per gram dry weight (DW) to several thousands of micrograms per gram DW (Cox et al., 2005; Esterhuizen & Downing, 2008; Faassen, 2014; Faassen, Gillissen, & Lürling, 2012; Jonasson et al., 2010). Anyway, controversy surrounds some of these results due to the lack of selectivity of the used analytical methods, such as those based on fluorescence detection (Faassen, 2014; Faassen et al., 2012; Monteiro, Costa, Moreira, Vasconcelos, & Baptista, 2016). This might result in the misidentification of BMAA, which would lead to its overestimation. However, it has been also found that sample matrix, formation of metal ion adducts, and mass spectrometry settings can lead to false negative findings of BMAA, suggesting that mass spectrometry determinations might be underestimated (Glover et al., 2012). Therefore, failure to detect BMAA cannot be always considered proof of absence of the compound. A validated method accepted by the AOAC for determination of BMAA in food stuffs has recently been published (Glover, Baker, Murch, & Brown, 2015).

In addition to cyanobacteria, recent studies have demonstrated the existence of other BMAA-producing organisms in nature, including phytoplankton eukaryotes such as diatoms (Jiang, Eriksson, et al., 2014; Reveillon, Sechet, Hess, & Amzil, 2016a, 2016b; Reveillon et al., 2015) and dinoflagellates (Jiang & Ilag, 2014; Lage et al., 2014). Table 1 summarizes the diatoms and dinoflagellates strains found to produce BMAA.

Jiang, Eriksson, et al. (2014) first reported the presence of BMAA in six axenic cultures of diatoms as well as in diatom containing field samples collected from the Swedish west coast. Reveillon et al. (2016a) also detected BMAA in four non-axenic diatom species. These authors observed the highest BMAA concentrations during the stationary growth phase, for all four species, suggesting BMAA's role as a secondary metabolite. Reported BMAA concentrations in diatoms on a DW basis are lower, in general, than in cyanobacteria, ranging from the nanogram per gram level to the low microgram per gram level (Table 1). However, the comparison on DW is not very appropriate since diatoms contain less protein per milligram than cyanobacteria due to the weight of the diatom frustule (Jiang, Eriksson, et al., 2014).

Diatoms are the most important phytoplankton group in marine environments and generate the vast majority of food that sustains life in the sea (Armbrust, 2009). Therefore, these eukaryotic microorganisms may represent a significant source of BMAA in marine environments. Some diatoms species are already known to produce other toxins such

as the domoic acid, a toxin responsible for the amnesic shellfish poisoning (ASP) (Regueiro, Álvarez, et al., 2011; Tenorio, Uribe, Gil-Kodaka, Blanco, & Álvarez, 2016).

Regarding dinoflagellates, BMAA was detected in a laboratory grown culture of *Gymnodinium catenatum* (Lage et al., 2014), a species known to produce paralytic shellfish poisoning (PSP) toxins in many parts of the world (Hallegraeff, Blackburn, Doblin, & Bolch, 2012). Although lower levels of BMAA (0.457 ± 0.186 μg/g DW) were reported as compared to many cyanobacteria, the global distribution of *G. catenatum* makes it a potentially significant source of BMAA. Recently, an axenic culture of dinoflagellate *Heterocapsa triquetra* has been also shown to produce BMAA (Jiang & Ilag, 2014).

From a general perspective, the emergence of BMAA-producers other than cyanobacteria significantly expands the potential exposure to this neurotoxin in different environments. Thus, while phytoplankton blooms in freshwater environments are dominated by cyanobacteria, dinoflagellates and primarily diatoms are responsible for these phenomena in brackish and marine systems. However, different scenarios involving BMAA-producers are possible in some environments. For example, in the Baltic Sea, cyanobacteria bloom during summer under high nutrient and temperature conditions, whereas diatoms blooms dominate in autumn when temperature decreases, but the nutrient and light are still sufficient. Dinoflagellates become prevalent phytoplankton during spring, coinciding with the decrease in diatoms. With such a situation, the different blooms of cyanobacteria, diatoms and dinoflagellates may make BMAA available in the food web of the ecosystem during the whole year (Jiang, 2015).

In view of the recent findings, further research is definitely necessary to determine other BMAA-producing organisms in nature, which can lead to unexplored pathways for human exposure to this neurotoxin.

3. Chemical identity of BMAA

Structurally, BMAA, also known as L-α-amino-β-methylaminopropionic acid, is an amino acid consisting of a carboxyl group, a primary amine attached to the α-carbon and a methylamine moiety as a part of the side chain (Fig. 1). Therefore, this molecule presents three ionizable groups with estimated pKa values of 1.96, 6.61 and 9.86, respectively, which means that BMAA will be negatively charged in basic media, positively charged in acidic media and as zwitterion around neutral pH.

BMAA is a reactive species that forms excitotoxic carbamates in the presence of bicarbonate (Nunn & O'Brien, 1989), but also react with some aldehydes to form imines (Nunn & Ponnusamy, 2009) and with some transition metal ions yielding stable chelates (Nunn, O'Brien, Pettit, & Pyburn, 1989). Recently, NMR studies of the equilibrium of BMAA with its carbamate adducts have been elucidated under physiological conditions (Zimmerman, Goto, & Krishnan, 2016).

3.1. Free and bound BMAA

In addition to the free, water-soluble molecule, typically referred to as "free BMAA", BMAA is also known to occur as "protein-bound" or "protein-associated", although the exact nature of the binding/association is still unclear and needs further investigation (Faassen et al., 2016; Rosén, Westerberg, Schmiedt, & Hellenäs, 2016).

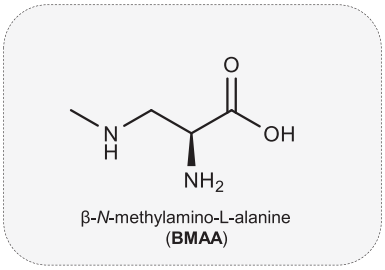

Fig. 1. Structure of BMAA.

Some authors reported that BMAA can be *in vitro* incorporated into the amino acid backbone of human proteins during their synthesis (Dunlop, Cox, Banack, & Rodgers, 2013; Glover, Mash, & Murch, 2014), although the evidence of an actual incorporation is an issue of current debate. Karlsson, Jiang, Andersson, Ilag, and Brittebo (2014) and Karlsson, Jiang, et al. (2015) examined protein association in neonatal rats following subcutaneous injection of BMAA. They found that BMAA passed the neonatal blood-brain barrier and was associated to proteins in the liver and the brain, especially in the hippocampus. The protein-association of BMAA in the hippocampus and other brain areas increased following repeated administration of BMAA whereas the hepatic level of protein-associated BMAA did not increase suggesting that the rate of degradation of BMAA-associated proteins may be different in the neonatal brain and liver.

Other types of association with proteins involving non-covalent bonding have also been reported. Thus, Glover et al. (2014) observed that around 50% of BMAA was released by protein denaturation indicating some non-covalent bonding, whereas a similar amount was only released by hydrolysis after denaturation confirming also the covalent incorporation of this amino acid into the proteins. This protein-associated BMAA may act as an endogenous reservoir that slowly releases BMAA through protein metabolism (Murch, Cox, & Banack, 2004).

Consequently, if a substantial protein-associated pool of BMAA is found, it would be highly relevant to identify which proteins are involved. It is also essential to know which form is detected, whether as a free amino acid or allegedly bound and subsequently freed by different extraction methods, since the release of BMAA could be an artifact of sample preparation (Cohen, 2012).

Even other kind of compounds might be behind the formation of BMAA as reported by Rosén, Westerberg, Schmiedt, et al. (2016). These authors suggested that the main source of BMAA in blue mussels was different from proteins as its concentration increased in some protein-free extracts after storing them at 5 °C. Therefore, the BMAA released was neither free nor protein-bound. Though, the identity of the responsible bound/associated form could not be stablished in this study, it was postulated as a low molecular weight compound (< 3000 Da) where BMAA is not bound through stable peptide bonds.

Taking into account the scarce information regarding the different forms in which BMAA can exist, the terminology proposed by Faassen et al. (2016) seems very appropriate to address the occurrence of BMAA. Thus, "free BMAA" is referred to the fraction obtained simply by extraction with polar solvents, whereas bound forms can either stay in solution as "soluble bound BMAA" or precipitate during extraction as "precipitated bound BMAA". The total BMAA content of a sample is usually obtained by acid hydrolysis of the total sample (Fig. 2). It should be noted that the use of trichloroacetic acid (TCA) to perform protein precipitation may still leave some non-precipitated proteins in

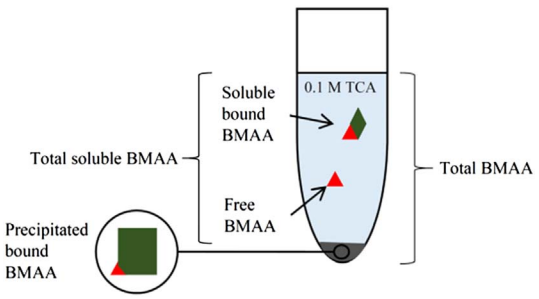

Fig. 2. Terminology proposed by Faassen et al. (2016) for the different BMAA fractions in biota samples.
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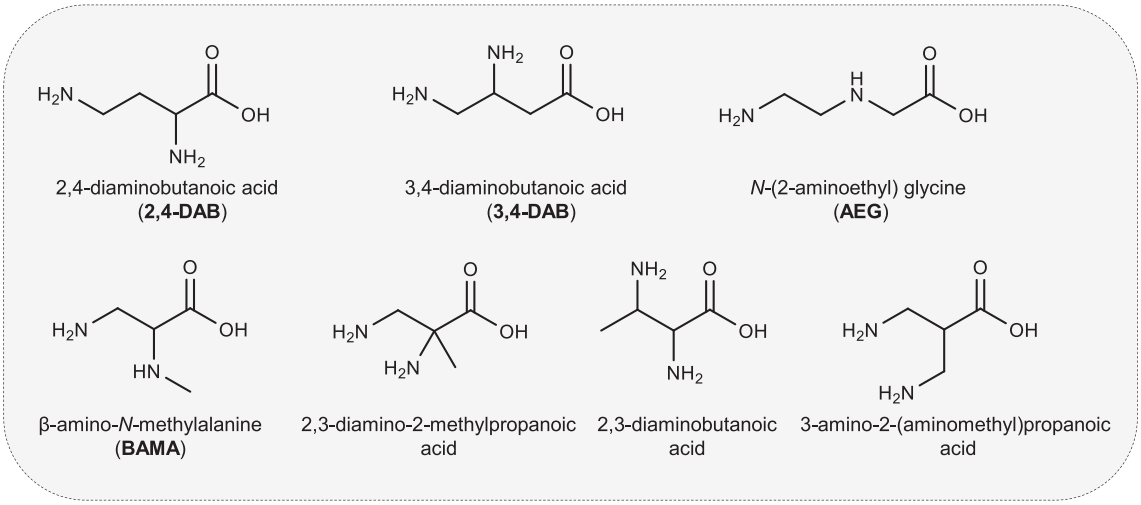

Fig. 3. Structure of some relevant structural isomers of BMAA.

solution. These authors found the soluble bound BMAA to be the major fraction in cycad seeds, seafood and BMAA-exposed *Daphnia magna*, and recommended its inclusion in the total BMAA definition not to underestimate the concentration of this compound. However, they were not able to identify the nature of the BMAA-precursors in the soluble bound fraction (Faassen et al., 2016).

Therefore, further research is still needed to characterize the chemical identities of the BMAA-precursors as wells as to optimize their extraction conditions, which will enable a proper quantification of BMAA.

3.2. BMAA and its isomers

Apart from BMAA, three structural isomers have been so far reported to occur in nature (Fig. 3). These are 2,4-diaminobutyric acid (2,4-DAB), *N*-2(aminoethyl)glycine (AEG) and *β*-amino-*N*-methyl-alanine (BAMA). To date, all of them have been found in different matrices including microalgae and mollusks (S. Banack et al., 2010; Banack et al., 2011; Jiang, Aigret, De Borggraeve, Spacil, & Ilag, 2012; Jiang, Kiselova, Rosén, & Ilag, 2014; Porojan et al., 2016). Among these isomers, only 2,4-DAB, widely present in prokaryotic and eukaryotic organisms, is known to exert neurotoxic effects (Weiss, Christine, & Choi, 1989).

As structural isomers, these compounds present the same monoisotopic mass as BMAA and very similar physicochemical properties, which makes it complicated to distinguish them from BMAA unless methods selective enough are used. Thus, the presence of these or other unknown isomers of BMAA may lead to false positives and/or overestimation. Based on a database search and chemical stability considerations, Jiang et al. (2012) proposed a total of seven structural isomers, including 2,4-DAB, AEG and BAMA, as potential relevant interferents during the analysis of BMAA (Fig. 3). Out of the seven candidates, only 2,4-DAB, AEG and BAMA have been investigated due to of lack of commercial standards for all the others (Beach, Kerrin, & Quilliam, 2015). Mostly, these compounds can be resolved by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), being critical to achieve proper chromatographic separation conditions (Jiang, Johnston, Åberg, Nilsson, & Ilag, 2013; Porojan et al., 2016). The hyphenation of ion mobility spectrometry (IMS) to mass spectrometry allows for an increased selectivity which can contribute to more reliable analytical results. For instance, Beach et al. (2015) used differential mobility spectrometry (DMS) coupled to MS/MS to spectrometrically resolve the co-elution in hydrophilic interaction chromatography (HILIC) of BMAA and BAMA, sharing both compounds the same precursor and product ions (Beach et al., 2015). However, the high resolving power achieved in DMS usually comes at the expense of a lower ion transmission and, consequently, higher limits of detection (LODs) (Regueiro, Giri, & Wenzl, 2016).

In spite of these recent improvements, new analytical developments should address the separation and correct identification of BMAA and its structural isomers, both known and unknown, in order to accurately estimate the exposure to this neurotoxin.

4. Mechanisms of BMAA neurotoxicity

BMAA has been associated with some progressive neurological diseases, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease and Alzheimer's dementia (AD) (S.A. Banack, Caller, & Stommel, 2010; Bradley & Mash, 2009; Pablo et al., 2009). However, several authors highlight the lack of enough scientific evidence to establish a direct link. A special issue on BMAA neurotoxicity is currently being published in Neurotoxicity Research (Cox, Kostrzewa, & Guillemin, 2017).

First animal studies investigating the toxic potential of BMAA *via* oral administration of BMAA led to controversial results. Spencer et al. (1987) after oral administration of BMAA to macaque monkeys (*Macaca fascicularis*) observed that the animals developed corticomoto-neuronal dysfunction, parkinsonian features, and behavioral anomalies, with chromatolytic and degenerative changes of motor neurons in cerebral cortex and spinal cord. On the contrary, experiments in mice did not show behavioral abnormalities (Cruz-Aguado, Winkler, & Shaw, 2006; Perry, Bergeron, Biro, & Hansen, 1989). These contradictory results could be due to the extremely high doses of BMAA administrated by Spencer et al. (1987). However, as discussed in the next section, the fact that BMAA can be highly biomagnified in the food chain before being consumed by humans, suggest that doses of BMAA used by Spencer et al. (1987) in their study might be pretty realistic (Karamyan & Speth, 2008). Also, BMAA might have been highly underestimated as its occurrence in the protein bound fraction is usually much higher than in the free form.

Chang, Chiu, and Kao (1993) found that BMAA at nanomole levels was able to elicit neurotoxic effects on rats and that these toxic effects were mediated by *N*-methyl-D-asparte (NMDA) subtype L-glutamate receptors. These results also showed a decrease in glutamate receptor number due to BMAA treatment, suggesting an involvement of altered glutamate receptor level in the manifestation of BMAA neurotoxicity. Santucci, Zsürger, and Chabry (2009) studied the effects of the BMAA on cell viability by measuring the electrophysiological activity of the mouse retinal neurons by electroretinography recordings. Results evidenced that BMAA induced neuronal cell death *in vivo* supporting a

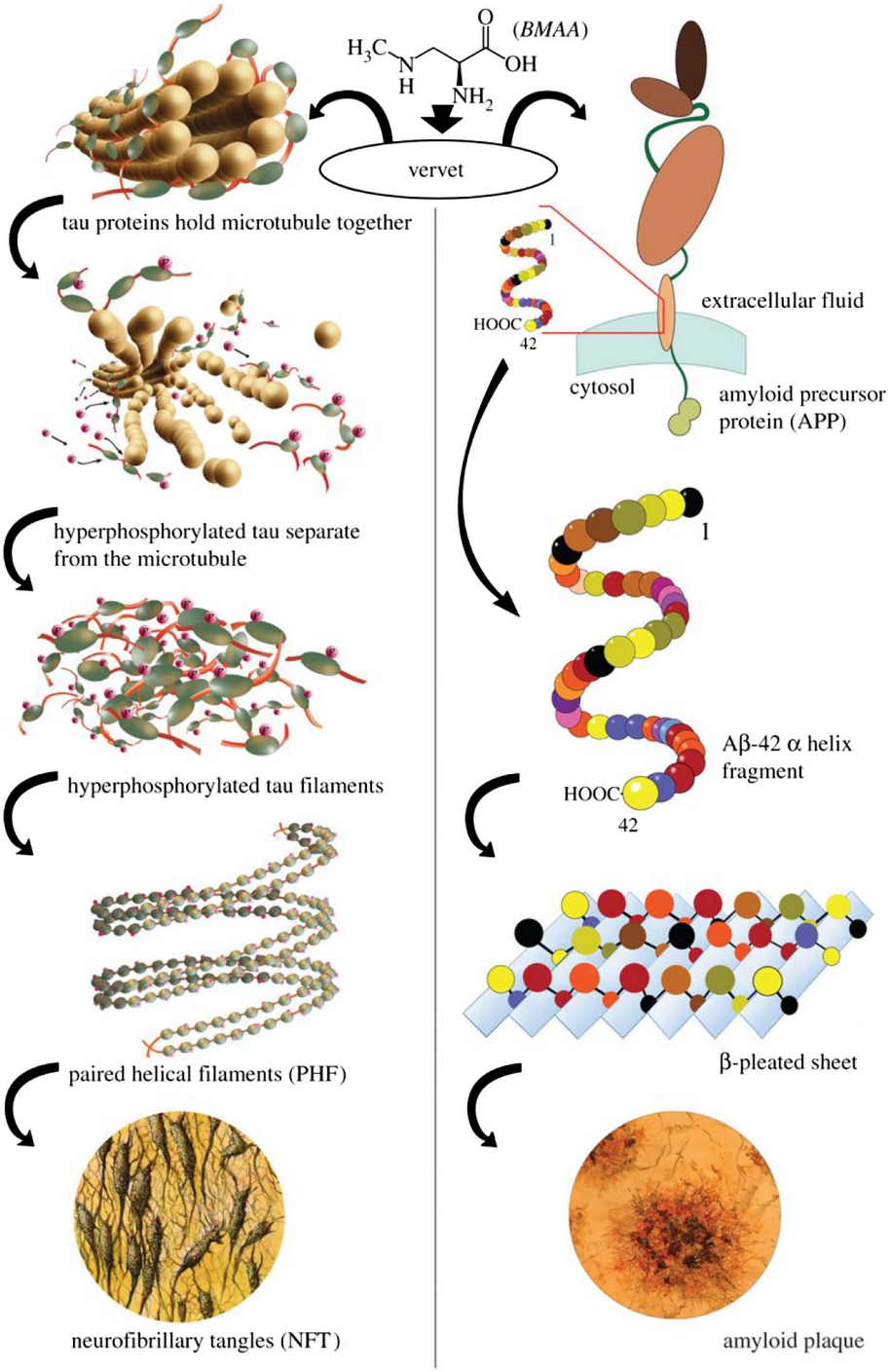

Fig. 4. Theoretical pathways of development of ALS/PDC and AD neuropathology from chronic dietary BMAA exposure. (a) Tau proteins which bind microtubules become hyperphosphorylated, leading to dissociation of hyperphosphorylated tau fragments. These form paired helical filaments, leading to the formation of neurofibrillary tangles. (b) The APP is cleaved, producing β-amyloid (Aβ-42) fragments which are in a α-helix conformation. These change to a β-pleated sheet conformation, oligomerize, forming amyloid plaques.
Reproduced from Cox et al. (2016), licensed under CC BY 4.0.

direct causal link between BMAA and neuronal damages. Karlsson, Roman, Berg, and Brittebo (2011) demonstrated that exposure to BMAA in rats during early life stages may not only induce initial neurotoxicity but also more subtle effects at low doses that manifests as impairments in learning and memory function at adult age. In a follow-up study, the authors (Karlsson, Michno, Ransome, & Hanrieder, 2017) demonstrated the long-term changes in ganglioside expression patterns in the adult hippocampus of animals neonatally treated with BMAA.

Field, Caller, and Stommel (2011) hypothesized that BMAA had a number of toxic effects on motor neurons including direct agonist action on NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, induction of oxidative stress, and depletion of glutathione.

Kisby and Spencer (2011) pointed that BMAA interfered with brain RNA and protein synthesis when the amino acid was incubated with mouse cortical explants or administered intraperitoneally to adult rats (100 mg/kg). While BMAA could act as a glutamate agonist, the amino acid was also taken up by brain tissue, formed a genotoxic metabolite, and regionally interfered with brain RNA and protein synthesis. As has been shown (Lee et al., 2006), low levels of mischarged transfer RNAs (tRNAs) can lead to an intracellular accumulation of misfolded proteins in neurons, which are associated with several pathological conditions including neurodegeneration.

In this regard, Dunlop et al. (2013) demonstrated that BMAA can be mistakenly incorporated in place of L-serine into human proteins. This misincorporation provides a possible mechanism by which BMAA could

initiate misfolding, and the accumulation of aggregate-prone proteins in neurons. Increasing L-serine content *in vitro* has been shown to reduce protein aggregation and apoptosis in human neuronal cell cultures (Dunlop et al., 2013; Main, Dunlop, & Rodgers, 2016). Very recently, Cox and Metcalf (2017) suggested that the high L-serine content of the traditional diet of Ogimi villagers (Japan), rich in seaweeds and tofu, might act as neuroprotective agent contributing to the neurological health indicators of this community.

Tian, Jiang, Wang, Zhang, and Han (2016) stablished a rat model that mimicked most characteristics of ALS/PDC by administering continuous intravenous (i.v.) injections of neurotoxic BMAA. Based on the data obtained, it was demonstrated that continuous i.v. injections of BMAA induced mitochondrial morphology and structural changes, astrogliosis, motor neuronal death, and other relative functional changes, which led to the overexpression of pro-inflammatory cytokines cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α), and resulted in the upregulation of glycogen synthase kinase-3 (GSK3), downregulation of astrocytic glutamate transporter-1 (GLT-1), accumulation of microtubule-associated protein tau and cytosolic aggregates of TAR DNA-binding protein-43 (TDP-43) in degenerating motor neurons. These results suggested that this model could be used as a useful tool for the mechanistic and therapeutic study of ALS/PDC.

Muñoz-Saez et al. (2013) demonstrated that BMAA produced cell death in human neuroblastoma and evoked alterations in GSK3β and TDP-43, two possible biomarkers of neurodegenerative diseases. Okle, Stemmer, Deschl, and Dietrich (2013) suggested an "interaction" of BMAA with intracellular proteins at low non-excitotoxic BMAA concentrations, resulting in dysregulated protein homeostasis and endoplasmic reticulum (ER) stress, and thus most likely in dysfunctional cells. Very recently, Engskog et al. (2017) also observed that BMAA induced alterations in alanine, aspartate and glutamate metabolism, as well as alterations in various neurotransmitters/neuromodulators such as gamma-aminobutyric acid (GABA) and taurine. These results indicated that BMAA could interfere with metabolic pathways involved in neurotransmission in human neuroblastoma cells.

Andersson, Karlsson, Banack, and Brandt (2016) have reported that administration of BMAA to lactating mice and rats results in a mother to off-spring transfer *via* the milk. In another recent study, the authors (Andersson, Ersson, Brandt, & Bergström, 2017) examined the transport of [^{14}C]-labeled L- and D-BMAA in four different human cell lines, namely breast epithelial MCF7 cells, intestinal Caco-2 cells, glioblastoma U343 cells and neuroblastoma SH-SY5Y cells, in order to answer the question whether BMAA could undergo a similar mother-to-infant transport also in humans. Their results suggest that BMAA can be transferred from an exposed mother, *via* the milk, to the brain of the nursed infant.

In a very recent study, Potjewyd, Day, Shangula, Margison, and Povey (2017) reported a previously unexplored mechanism of BMAA toxicity. This mechanism consisted in the *N*-nitrosation of BMAA which resulted in an alkylating agent able to damage DNA *in vitro*, generating single strand breaks and being toxic to human neuroblastoma SH-SY5Y cells under conditions in which BMAA itself was minimally toxic.

Recently, Cox et al. (2016) found that Vervet monkeys subjected to chronic dietary exposure to BMAA (140 days, 210 mg/kg BW per day) developed neurofibrillary tangles and plaques immunopositive for phosphotau AT8 and β-amyloid deposits. These findings indicate that chronic dietary exposure to this toxin can trigger neurodegenerative diseases, particularly Guamanian ALS/PDC (Fig. 4). The authors also found that increasing the amount of L-serine in the diet had a neuroprotective effect, which was in agreement with previous observations *in vitro*. Interestingly, a progressive neurodegeneration with fibrillary inclusions and protein enrichment (including protein previously associated with neurodegeneration) have been also shown in the animals (Karlsson et al., 2012; Karlsson, Berg, et al., 2015).

As shown, nearly all *in vivo* studies on BMAA showed some neurotoxicity of the amino acid with functional disturbances and neurodegenerative changes relevant to motor dysfunction. However, investigations on the presence of BMAA in human brain tissues have yielded contradictory results. While some authors found this toxin in brains of patients who died of neurodegenerative diseases (Cox et al., 2003; Murch, Cox, Banack, Steele, & Sacks, 2004; Pablo et al., 2009), others did not (Montine, Li, Perl, & Galasko, 2005; Snyder et al., 2009). Therefore, the link between BMAA and neurodegenerative diseases is yet to be confirmed and the actual mechanisms whereby BMAA leads to neurotoxicity need to be further elucidated.

5. Dietary exposure to BMAA

Human exposure to BMAA may occur in a variety of ways including consumption of contaminated food and water, recreational water use or even inhalation of contaminated aerosols (Cox et al., 2009; Downing, Contardo-Jara, Pflugmacher, & Downing, 2014; Jiang, Kiselova, et al., 2014). However, dietary intake is recognized as the major exposure pathway to this compound, especially through the consumption of aquatic organisms such as filter-feeding bivalve mollusks (Jiang, Kiselova, et al., 2014; Jonasson et al., 2010; Reveillon et al., 2015). The recent discovery of BMAA-producers among the widespread diatom taxa highly increases the potential exposure to this neurotoxin through the biomagnification in the marine food webs. The oral bioavailability of BMAA has been shown to be almost complete in animal models. Thus, following oral dosing, around 80% of the administered BMAA was rapidly absorbed into the systemic circulation of cynomologus monkeys (Duncan et al., 1992) and rats (Duncan et al., 1991).

A number of investigations have shown the recurrent presence of BMAA in different types of seafood, including bivalve mollusks, crustaceans and fish, all over the world (Tables 2–4).

Brand et al. (2010) determined total BMAA concentrations in seafood collected in South Florida (USA) from water bodies known to have frequent cyanobacterial blooms. A wide range of BMAA concentrations were found, varying from 256 to 293 μg/g wet weight (WW) in mollusks (mussel and oyster), from not detected (ND) to 6976 μg/g WW in crustaceans (crab and shrimp), and from ND to 7351 μg/g WW in different fish species. In Florida Bay, high concentrations of BMAA were measured in pink shrimps (up to 3042 μg/g WW) and much lower in grey snapper (up to 188 μg/g WW). Samples from Biscayne Bay, where the bloom was on the decline, showed some species with no BMAA and others with very high concentrations (up to 6976 and 7351 μg/g WW in blue crab and least puffer, respectively). In Caloosahatchee River, moderate amounts of BMAA were found in mollusks (up to 293 μg/g WW), whereas high concentrations were measured in fish (up to 2559 μg/g WW in bowfin). These levels of BMAA were very high as compared with the concentrations reported by Christensen, Hemscheidt, Trapido-Rosenthal, Laws, and Bidigare, (2012) for oysters and blue crabs (5–47 μg/g DW) collected from Louisiana, Mississippi, and Florida (USA). Even lower were the BMAA concentrations (0.95–13.8 μg/g DW) found by Beach et al. (2015) and by Kerrin, White, and Quilliam, (2017) in mussels and lobsters purchased from local groceries in Canada. Al-Sammak, Hoagland, Cassada, and Snow, (2014) investigated the occurrence of free and bound BMAA in 248 fish samples collected from several Nebraska reservoirs. The highest concentration of free BMAA corresponded to bass (up to 0.416 μg/g DW), whereas the highest level of bound BMAA were found in carp (up to 2.57 μg/g DW).

In Europe, several studies have addressed the occurrence of this neurotoxin in a variety of seafood. Thus, Jonasson et al. (2010) analyzed several fish species collected from the Baltic Sea (Sweden), reporting concentrations in the nanogram per gram level DW. These authors observed that fish species living closer to the bottom of the Baltic Sea (smelt, turbot, fourhorn sculpin, herring and common whitefish) presented, in general, higher levels of BMAA than the pelagic fishes (Atlantic salmon and pikeperch), some of which even did not contain

Table 2
Concentrations of BMAA in mollusks.

Common name	Species	Collection place	Concentration (μg/g)	Form	LOQ (μg/g)	Ref.
Mussel	*Mytilus edulis*	Kattegat Sea, Sweden	0.15–0.2 DW	Total	–	(Jonasson et al., 2010)
Mussel	*Utterbackia imbecillis*	Caloosahatchee River, South Florida, USA	256	Total	0.008	(Brand et al., 2010)
Mussel	*Mytilus galloprovincialis*	Thau lagoon, France	1.8–6 DW	Total	0.013 pmols	(Masseret et al., 2013)
Mussel	–	West coast of Sweden	0.27–1.6 WW	Total	0.15	(Salomonsson, et al., 2013)
Mussel	*Mytilus edulis*	West coast of Sweden	0.08–0.90 WW	Total	< 0.01	(Jiang et al., 2014)
Mussel[b]	*Mytilus edulis*	Scandinavia	ND	Free	0.15	(Salomonsson, et al., 2015)
Mussel[b]	*Mytilus edulis platensis*	South America	ND-0.24 WW	Free	0.15	(Salomonsson et al., 2015)
Mussel	*Mytilus edulis platensis*	South America	ND-0.38 WW	Free	0.15	(Salomonsson et al., 2015)
Mussel	*Perna canaliculus*	Australia	ND-0.15 WW	Free	0.15	(Salomonsson et al., 2015)
Mussel[b]	*Mytilus edulis*	Scandinavia	0.28–0.59 WW	Total	0.15	(Salomonsson et al., 2015)
Mussel[b]	*Mytilus edulis platensis*	South America	4.46–7.08 WW	Total	0.15	(Salomonsson et al., 2015)
Mussel	*Mytilus edulis platensis*	South America	1.69–2.28 WW	Total	0.15	(Salomonsson et al., 2015)
Mussel	*Perna canaliculus*	Australia	0.55–1.14 WW	Total	0.15	(Salomonsson et al., 2015)
Mussel[c]	*Mytilus galloprovincialis*	Thau lagoon, France	1.0–6.6 DW	Total	0.15	(Reveillon et al., 2015)
Mussel[d]	*Mytilus galloprovincialis*	Thau lagoon, France	1.2–9.7 DW	Total	0.15	(Reveillon et al., 2015)
Mussel	*Mytilus edulis* and *galloprovincialis*	Channel, Atlantic and Mediterranean sites, France	0.44–6.7 DW	Total	0.15	(Reveillon et al., 2016b)
Mussel	–	Canada	0.95–1.2 DW	Total	0.066	(Beach et al., 2015)
Mussel	–	Sweden	ND-0.27 WW	Free	0.007	(Rosén, Westerberg, Hellenäs, & Salomonsson, 2016)
CRM-ASP-Mus[e]	*Mytilus edulis*	–	1.2 DW	Total	0.066	(Beach et al., 2015)
CRM-ASP-Mus[e]	*Mytilus edulis*	–	1.7 DW	Total	0.053	(Kerrin, et al., 2017)
Cockle	*Cerastoderma edule*	Ria de Aveiro, Portugal	0.08–0.35 DW	Total	0.00005	(Lage et al., 2014)
Cockle	*Cerastoderma edule*	Ria de Formosa, Portugal	ND-0.43 DW	Total	0.00005	(Lage et al., 2014)
Scallop	*Placopecten magellanicus*	North America	0.18–0.46 WW	Free	0.15	(Salomonsson et al., 2015)
Scallop	*Placopecten magellanicus*	North America	1.12–1.46 WW	Total	0.15	(Salomonsson et al., 2015)
Oyster	*Ostrea edulis*	Kattegat Sea, Sweden	0.006–0.14 DW	Total	–	(Jonasson et al., 2010)
Oyster	*Pinctada margaritifera*	South Florida, USA	275	Total	0.009	(Brand et al., 2010)
Oyster[a]	*Crassostrea virginica*	Caloosahatchee River, South Florida, USA	293	Total	0.009	(Brand et al., 2010)
Oyster	*Crassostrea virginica*	LA, USA	8.6–46.9 DW	Total	1.7	(Christensen, et al., 2012)
Oyster	*Crassostrea virginica*	MS, USA	6.8–9.9 DW	Total	1.7	(Christensen et al., 2012)
Oyster	*Crassostrea gigas*	Thau lagoon, France	0.60–1.6 DW	Total	0.013 pmols	(Masseret et al., 2013)
Oyster	*Ostrea edulis*	West coast of Sweden	0.10–0.28 WW	Total	< 0.01	(Jiang et al., 2014)
Oyster	*Ostrea edulis*	Greece	0.32 WW	Total	< 0.01	(Jiang et al., 2014)
Oyster	*Crassostrea gigas*	France	0.66 WW	Total	< 0.01	(Jiang et al., 2014)
Oyster	*Crassostrea gigas*	Channel, Atlantic and Mediterranean sites, France	0.19–2.4 DW	Total	0.15	(Reveillon et al., 2016b)
Chinese pond mussel	*Anodonta woodiana*	Gonghu Bay of Lake Taihu, China	3.55 DW	Total	–	(Jiao et al., 2014)
Freshwater snail	*Bellamya aeruginosa*	Gonghu Bay of Lake Taihu, China	0.63–3.85 DW	Total	–	(Jiao et al., 2014)
Asiatic clam	*Corbicula fluminea*	Gonghu Bay of Lake Taihu, China	0.80–6.72 DW	Total	–	(Jiao et al., 2014)

DW: dry weight; WW: wet weight; ND: not detected; LOQ: Limit of quantification.
[a] Mantle muscle.
[b] Cooked and canned at room temperature.
[c] Digestive gland.
[d] Remaining flesh.
[e] Certified reference material for domoic acid in mussels, (National Research Council Canada, Halifax, Canada).

Table 3
Concentrations of BMAA in crustaceans.

Common name	Species	Collection place	Concentration (μg/g)	Form	LOQ (μg/g)	Ref.
Blue crab	*Callinectes sapidus*	South Florida, USA	2286	Total	0.407	(Brand et al., 2010)
Blue crab	*Callinectes sapidus*	Biscayne Bay, FL, USA	ND-6976	Total	0.407	(Brand et al., 2010)
Spider crab	*Clupeoides*	Biscayne Bay, FL, USA	ND	Total	0.407	(Brand et al., 2010)
Blue crab	*Callinectes sapidus*	North Florida, USA	4.7–14.1 DW	Total	1.7	(Christensen et al., 2012)
Freshwater crab	*Procambarus clarkii*	Gonghu Bay of Lake Taihu, China	8.76 DW	Total	–	(Jiao et al., 2014)
Crab	*Eriocheir sisensis*	Gonghu Bay of Lake Taihu, China	6.48 DW	Total	–	(Jiao et al., 2014)
Freshwater shrimp	*Macrobrachium nipponense*	Gonghu Bay of Lake Taihu, China	1.04 DW	Total	–	(Jiao et al., 2014)
Siberian Prawn	*Palaemon modestus*	Gonghu Bay of Lake Taihu, China	0.12 DW	Total	–	(Jiao et al., 2014)
Prawn	*Heller*	Gonghu Bay of Lake Taihu, China	5.12 DW	Total	–	(Jiao et al., 2014)
Shrimp	*Panaeus duorarum*	South Florida, USA	1156	Total	–	(Brand et al., 2010)
Pink shrimp	*Panaeus duorarum*	Bay, FL, USA	1247–3042	Total	–	(Brand et al., 2010)
Pink shrimp	*Panaeus duorarum*	South Biscayne Bay, FL, USA	55–942	Total	–	(Brand et al., 2010)
Shrimp	*Caridea*	Sweden	0.13–0.46 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Shrimp	*Caridea*	Northern Atlantic	0.1–0.20 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Lobster tail meat	–	Canada	13.8	Total	0.053	(Kerrin et al., 2017)
Lobster tomalley	–	Canada	3.4	Total	0.053	(Kerrin et al., 2017)

DW: dry weight; WW: wet weight; ND: not detected; LOQ: Limit of quantification.

Table 4
Concentrations of BMAA in fish.

Common name	Species	Collection place	Concentration (μg/g)	Form	LOQ (μg/g)	Ref.
Plaice	*Pleuronectes platessa*	Northeast Atlantic	0.01 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Plaice	*Pleuronectes platessa*	Baltic Sea	0.02 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Baltic Herring	*Clupea harengus*	Baltic Sea	ND-0.02 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Salmon	*Salmo salar*	Norway	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Char	*Salvelinus alpinus*	Sweden	ND-0.01 WW	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Cod	*Gadus morhua*	Norway	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Perch	*Perca fluviatilis*	Northeast Atlantic	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Perch	*Perca fluviatilis*	Sweden	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Crayfish	*Astacus leptodactylus*	Turkey	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Crayfish	*Astacus leptodactylus*	Sweden	ND	Total	< 0.01	(Jiang, Kiselova, et al., 2014)
Puffer fish	*Sphoeroides parvus*	South Florida, USA	1907	Total	0.002	(Brand et al., 2010)
White perch	*Pomoxis annularis*	South Florida, USA	1806	Total	0.002	(Brand et al., 2010)
Grey snapper	*Lutjanus griseus*	Bay, FL, USA	ND-188	Total	–	(Brand et al., 2010)
Smelt	*Osmerus eperlanus*	Baltic Sea	ND-0.24 DW	Total	–	(Jonasson et al., 2010)
Turbot	*Scophthalmus maximus*	Baltic Sea	ND-1.29 DW	Total	–	(Jonasson et al., 2010)
Fourhorn sculpin	*Triglopsis quadricornis*	Baltic Sea	ND-0.124 DW	Total	–	(Jonasson et al., 2010)
Herring	*Clupea harengus*	Baltic Sea	ND-0.010 DW	Total	–	(Jonasson et al., 2010)
Common whitefish	*Coregonus lavaretus*	Baltic Sea	ND-0.059 DW	Total	–	(Jonasson et al., 2010)
Pike-perch	*Sander lucioperca*	Baltic Sea	ND	Total	–	(Jonasson et al., 2010)
Atlantic salmon	*Salmo salar*	Atlantic Ocean	ND	Total	–	(Jonasson et al., 2010)
Anchovy-sardine	*Anchoa* sp.	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Hardhead silverside	*Atherinomorus stipes*	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Pinfish	*Lagodon rhomboides*	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Mojarra	*Gerreidae*	Biscayne Bay, FL, USA	ND-567	Total	–	(Brand et al., 2010)
Grey snapper	*Lutjanus griseus*	Biscayne Bay, FL, USA	ND-188	Total	–	(Brand et al., 2010)
School master snapper	*Lutjanus apodus*	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Band tail puffer	*Sphoeroides spengleri*	Biscayne Bay, FL, USA	ND-455	Total	–	(Brand et al., 2010)
Least puffer	*Sphoeroides parvus*	Biscayne Bay, FL, USA	194–7351	Total	–	(Brand et al., 2010)
Scrawled cowfish	*Acanthostracion quadricornis*	Biscayne Bay, FL, USA	39	Total	–	(Brand et al., 2010)
Sea bream	*Archosargus rhomboidalis*	Biscayne Bay, FL, USA	484–2349	Total	–	(Brand et al., 2010)
Sailors choice grunt	*Haemulon parra*	Biscayne Bay, FL, USA	203–722	Total	–	(Brand et al., 2010)
Bluestriped grunt	*Haemulon sciurus*	Biscayne Bay, FL, USA	ND-3776	Total	–	(Brand et al., 2010)
Redfin needlefish	*Strongylura notata*	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Great barracuda	*Sphyraena barracuda*	Biscayne Bay, FL, USA	ND	Total	–	(Brand et al., 2010)
Bowfin	*Amia calva*	Caloosahatchee River, South Florida, USA	554–2559	Total	–	(Brand et al., 2010)
Alligator gar	*Atractosteus spatula*	Caloosahatchee River, South Florida, USA	857–2140	Total	–	(Brand et al., 2010)
Largemouth bass	*Micropterus salmoides*	Caloosahatchee River, South Florida, USA	1130–2388	Total	–	(Brand et al., 2010)
Fish	*Aristichthys nobilis*	Gonghu Bay of Lake Taihu, China	0.12–0.38 DW	Total	–	(Jiao et al., 2014)
Fish	*Carassius auratus*	Gonghu Bay of Lake Taihu, China	0.25–7.55 DW	Total	–	(Jiao et al., 2014)
Fish	*Coilia ectenes taihuensis*	Gonghu Bay of Lake Taihu, China	2.76–10.60 DW	Total	–	(Jiao et al., 2014)
Fish	*Cyprinus carpia*	Gonghu Bay of Lake Taihu, China	0.47–15.74 DW	Total	–	(Jiao et al., 2014)
Fish	*Erythroculter ilishaeformis*	Gonghu Bay of Lake Taihu, China	1.11–35.91 DW	Total	–	(Jiao et al., 2014)
Fish	*Hemiramphus kurumeus*	Gonghu Bay of Lake Taihu, China	23.41 DW	Total	–	(Jiao et al., 2014)
Fish	*Hypophthalmichthys molitrix*	Gonghu Bay of Lake Taihu, China	1.05–12.93 DW	Total	–	(Jiao et al., 2014)
Fish	*Neosalanx taihuensis*	Gonghu Bay of Lake Taihu, China	1.87–4.54 DW	Total	–	(Jiao et al., 2014)
Fish	*Parabramis pekinensis*	Gonghu Bay of Lake Taihu, China	3.77 DW	Total	–	(Jiao et al., 2014)
Fish	*Parasilurus asotus*	Gonghu Bay of Lake Taihu, China	2.71 DW	Total	–	(Jiao et al., 2014)
Fish	*Pelteobagrus fulvidraco*	Gonghu Bay of Lake Taihu, China	2.13–8.32 DW	Total	–	(Jiao et al., 2014)
Fish	*Protosalanx hyalocranius*	Gonghu Bay of Lake Taihu, China	1.25 DW	Total	–	(Jiao et al., 2014)
Fish	*Pseudorasbora parva*	Gonghu Bay of Lake Taihu, China	0.07–9.61 DW	Total	–	(Jiao et al., 2014)
Fish	*Rhodeus sinensis*	Gonghu Bay of Lake Taihu, China	0.44 DW	Total	–	(Jiao et al., 2014)
Fish	*Günther*	Gonghu Bay of Lake Taihu, China	0.07–4.79 DW	Total	–	(Jiao et al., 2014)
Carp	–	NE, USA	0.476–2.57 DW ND-0.103 DW	Bound Free	< 0.0032	(Al-Sammak et al., 2014)
White crappie	–	NE, USA	ND-0.629 DW ND-0.332 DW	Bound Free	< 0.0032	(Al-Sammak et al., 2014)
Bass	–	NE, USA	0.3–0.72 DW 0.117–0.416 DW	Bound Free	< 0.0032	(Al-Sammak et al., 2014)
Shad	–	NE, USA	0.099 DW 0.196 DW	Bound Free	< 0.0032	(Al-Sammak et al., 2014)
Walleye	–	NE, USA	0.604–0.67 DW	Bound	< 0.0032	(Al-Sammak et al., 2014)

(continued on next page)

Table 4 *(continued)*

Common name	Species	Collection place	Concentration (μg/g)	Form	LOQ (μg/g)	Ref.
			0.095–0.25 DW	Free		
Catfish	–	NE, USA	0.32 DW	Bound	< 0.0032	(Al-Sammak et al., 2014)
			0.254 DW	Free		
Wiper	–	NE, USA	ND	Bound	< 0.0032	(Al-Sammak et al., 2014)
			0.227 DW	Free		
Bluegill	–	NE, USA	0.056 DW	Bound	< 0.0032	(Al-Sammak et al., 2014)
			0.36 DW	Free		
Shark cartilage supplements	–	–	ND-264.9 DW	Total	0.01	(Mondo et al., 2014)

DW: dry weight; WW: wet weight; ND: not detected; LOQ: Limit of quantification.

any BMAA. Moreover, they observed that BMAA concentrations in brain tissue of smelt, turbot and fourhorn sculpin were up to 82-fold higher than the corresponding muscle tissue. Farmed mussels and oysters from the western coast of Sweden were also included in the same study. The levels of BMAA in these mollusks were significantly higher than in fish, ranging from 0.006 to 0.2 μg/g DW. In 2013, Salomonsson, Hansson, and Bondesson, (2013) reported higher concentrations (0.27–1.6 μg/g WW) of BMAA in mussel samples collected from the same region after applying a DW/WW factor of 0.20. In a following study, Salomonsson, Fredriksson, Alfjorden, Hedeland, and Bondesson, (2015) quantified free and total BMAA in mussels and scallops purchased from different grocery stores in Uppsala (Sweden). The levels of free BMAA in mussels were up to 0.46 μg/g WW, whereas the levels of total BMAA varied between 0.29 and 7.08 μg/g WW. The highest level of free BMAA corresponded to an imported cooked mussels sample from South America. The authors quantified other types of seafood, such as crab, crayfish, shrimps, Atlantic salmon, sea bass, sea bream, whitefish, pikeperch and sea trout, but no BMAA was detected. Jiang, Kiselova, et al. (2014) determined total BMAA concentrations in seafood purchased in several supermarkets in Stockholm. Mussels were originally from the western coast of Sweden, whereas the rest of seafood came from the Baltic Sea, Norway, France, Greece, Northern Atlantic, Northeast Atlantic and Turkey. The concentration levels quantified in mollusks ranged from 0.08 to 0.90 μg/g WW in mussels and from 0.10 to 0.66 μg/g WW in oysters, with no significant differences according to the origin. The authors also found BMAA in cooked shrimps from Sweden (0.13–0.46 μg/g WW) and from Northern Altantic (0.11–0.20 μg/g WW). Levels up to 0.02 μg/g WW were detected in plaice, herring and char, whereas BMAA was undetectable (< 0.01 μg/g) in other samples (Atlantic salmon, cod, perch and crayfish).

In France, BMAA was detected in shellfish for the first time in Thau Lagoon (Southern France) by Masseret et al. (2013), who reported levels of total BMAA ranging from 0.27 to 6 μg/g DW in mussels and from 0.60 to 1.6 μg/g DW in oysters. The authors selected this region, a bivalve farming area with narrow connections to the Mediterranean Sea, due to its possible association with one significant amyotrophic lateral sclerosis cluster. In a later study, Reveillon et al. (2015) reported concentrations of total BMAA varying from 1 to 9.7 μg/g DW in mussels collected in the same area. To assess whether BMAA was present in the three French coasts (Channel, Atlantic and Mediterranean sites), Reveillon et al. (2016b) screened 97 bivalve mollusks samples (mussels and oysters) and systematically found BMAA at concentration levels up to 6.7 μg/g DW.

Recently, Lage et al. (2014) reported for the first time the presence of BMAA in Portuguese seafood. The total BMAA concentration in cockles from the rias of Aveiro and Formosa fluctuated from 0.079 to 0.35 μg/g DW and from ND to 0.43 μg/g DW, respectively.

In China, Jiao et al. (2014) monitored total BMAA concentrations in mollusks, crustaceans and various fish species at different trophic levels of ecosystems in Gonghu Bay in Lake Taihu, some of which were widely consumed by humans. Over the entire sampling period, BMAA levels up to 6.72, 8.76 and 35.91 μg/g DW were quantified for mollusks, crustaceans and fish, respectively. The highest level in fish corresponded to a specie (*Erythroculter ilishaeformis*) that feed on other fish species. The authors found higher BMAA contents in the higher trophic levels of the aquatic ecosystem, demonstrating once more the biomagnification of BMAA. Since shark belongs to a trophic level at the top of the food chain, the biomagnification of BMAA is even more evident as observed by Mondo et al. (2014), who analyzed 16 commercial shark cartilage dietary supplements, detecting this toxin in 15 of them at concentrations ranging from 86 to 265 μg/g DW.

As can be observed in Tables 2–4, reported BMAA concentrations in seafood vary from below the LODs to approximately 7000 μg/g (Brand et al., 2010). The huge variation in these data might be due to different factors, such as environmental conditions (especially the existence of recent algal blooms), genetic conditions and ages of the organisms, tissues selected for analysis and, mainly, biomagnification processes in the different trophic levels. However, it might be also due to insufficient analytical performance, which would have led to overestimation and/or misidentification of BMAA in some studies. For instance, it can be noted that in all cases for all the different seafood categories considered, the highest concentration levels were reported by Brand et al. (2010), who found concentration values approximately two to three orders of magnitude higher than any other study. These authors used a method based on the derivatization of BMAA with 6-aminoquinolyl-*N*-hydrosuccinimidyl carbamate followed by liquid chromatography separation and fluorescence detection. Although they claimed to confirm the presence of BMAA by LC-MS/MS, the MS/MS fragmentation of the corresponding BMAA fluorescent derivative produces only reagent-specific fragment ions, which are common to all the derivatives of interferents with the same nominal mass as BMAA. Therefore, it cannot be confirmed the accuracy of the exceptionally high concentration values reported by Brand et al. (2010).

Currently, there is no official Tolerable Daily Intake (TDI) established by the European Food Safety Authority (EFSA) or other international body due to the lack of suitable data on the chronic effects of BMAA in animals or humans. In view of the acute toxicity of this toxin, is was decided to estimate a tentative acute reference dose (ARfD) for risk assesment purposes. Altough intraperitoneal injection is usually considered not relevant for dietary risk assesment, the only point of departure (POD) available in the literature was a lowest-observed-adverse-effect-level (LOAEL) obtained from a single dose intraperitoneal exposure test on mice (Al-Sammak, Rogers, & Hoagland, 2015). Based on this LOAEL (2 g/kg BW) and using a uncertainty factor of 10,000 (10 for interspecies variation, 10 for intraspecies variation, 10 for the use of a LOAEL instead of a NOAEL, and 10 for the use of a non-oral exposure route), a ARfD of 200 μg/kg BW can be derived.

Among the different seafood types screened for BMAA (Tables 2–4), mussels were by far the predominant product so they were considered for dietary exposure estimations in the present work. As BMAA exerts acute toxic effects, it is important to identify a high portion size rather than a long term average consumption in order to effectively protect the health of the consumers. Based on the EFSA scientific opinions on marine biotoxins in shellfish (EFSA, 2008, 2009), a 400 g of shellfish meat was selected as the high portion size to be used in the acute risk assessment of BMAA. Using this portion size and considering only the

highest BMAA concentrations reported in each study, the intake would range from 60 to 3880 μg BMAA (equivalent to 1 and 65 μg/kg BW in an adult person of 60 kg, respectively). The concentration value reported by Brand et al. (2010) was not included in this estimation due to the reasons mentioned above. As can be observed, these intakes are between 200- and 3-fold lower than the estimated ARfD, so accounting just for the acute toxic effects, BMAA intake does not represent a critical concern for the health of mussel consumers.

Nevertheless, considering the potential long-term effects of BMAA, more toxilogical and occurrence data are needed to properly assess the risk related to the dietary intake of this compound and to establish a TDI. This risk might be particularly high for those communities that traditionally consume higher than average amounts of fish and shellfish.

Some recent studies have tried to establish correlations between dietary exposure to BMAA and the high incidence of ALS clusters (Banack, Metcalf, Bradley, & Cox, 2014; Field et al., 2013; Masseret et al., 2013). For instance, Field et al. (2013) investigated three ALS patients who developed the disease within a relatively short time and within close proximity to each other (Annapolis, Maryland, USA). One common factor was the frequent consumption of blue crab in which BMAA was identified. Therefore, the authors concluded that the presence of BMAA in the Chesapeake Bay food web and the lifetime consumption of blue crab contaminated with BMAA may be a common risk factor for sporadic ALS in all three patients.

Masseret et al. (2013) found a significant ALS cluster, surrounding the Thau lagoon, the most important area of shellfish production and consumption along the French Mediterranean coast. BMAA was identified in mussels (1.8 μg/g to 6.0 μg/g) and oysters (0.6 μg/g to 1.6 μg/g) in the region, suggesting its involvement in the development or progression of the disease.

While it is not yet possible to ascertain a direct link between shellfish consumption and the existence of these ALS clusters, these results add new data to the investigation of potential association of consumption of BMAA-contaminated seafood with health risks.

6. Challenges faced in BMAA research

The findings of this review indicate that even though significant research effort has been devoted in recent times to this topic, further research is still required to address several important gaps in the actual state of knowledge.

Thus, the metabolic fate and biotransformation products of BMAA in different organisms remain largely unknown. Downing, Esterhuizen-Londt, and Grant Downing (2015) studied the metabolism of BMAA in the aquatic plant *Ceratophyllum demersum* using stable isotopically labeled BMAA, *via* the analysis of label distribution patterns. They observed that during depuration, cellular BMAA concentrations decreased considerably, without excretion of the toxin back into the environment and without catabolism of BMAA. These results suggested that BMAA was metabolized *via* covalent modification and sequestered inside the plant as an unknown BMAA derivative. This may have an influence on the assessment of BMAA bioaccumulation, since the existence on unknown derivatives may lead to its underestimation. Nunn and Ponnusamy (2009) reported that rat liver and kidney homogenates, but not brain homogenates, formed methylamine and 2,3-diaminopropanoic acid when incubated with BMAA. In contrast, BMAA was suggested to be metabolized in rodent brain tissue by the cytochrome P450 (CYP) to formaldehyde (Kisby & Spencer, 2011). Engskog et al. (2017) observed that BMAA interferes with several metabolic pathways in human neuroblastoma SH-SY5Y, but they were not able to detect any BMAA metabolite.

Given the ubiquity of BMAA-producers, *i.e.*, cyanobacteria, diatoms and dinoflagellates, in the environment, proper monitoring programs should be developed and implemented in order to evaluate the real exposure to this neurotoxin. So far, only a few studies have analyzed BMAA in seafood, suggesting than dietary exposure might be higher than previously thought.

The climate change seems to play also an important role in the increasingly common phytoplankton blooms observed worldwide during the last decades, which can highly contribute to the widespread of BMAA (Holtcamp, 2012).Therefore, future research should also address the impact of climate change on bloom occurrence. Increased runoff and altered hydrologic regimes may increase nutrient concentration in surface waters, favoring the occurrence of cyanobacterial blooms and overall eutrophication (Merel et al., 2013). Climate change may also help spreading some BMAA-producers to latitudes outside of their usual range. Generalized and predictive models that address the overall spread or re-distribution of BMAA-producers due to climate change are needed.

It is also important to identify other potential routes for exposure to BMAA in order not to underestimate its total intake. For example, some agricultural lands are irrigated from water bodies covered in cyanobacterial blooms, so there is a chance that this toxin can finally get into vegetables, milk or meat. Thus, studies assessing the occurrence of BMAA in food other than seafood are still lacking and might be relevant for the total dietary exposure.

Considering the challenge towards the analysis of BMAA, there is still a need for robust and reliable analytical methods. As we have previsouly discussed, the existence of numerous isobaric and even isomeric interferences can highly complicate its identification and quantification, leading to overestimation. Therefore, effective sample clean-up procedures and selective detection techniques are crucial for succeeding in this purpose. The uncertainty related to the occurrence of unknown bound forms of BMAA should be also addressed in order to assess the relevance of these species for the dietary intake of BMAA. In this regard, the use of more comprehensive identification techniques, such as IMS coupled to high-resolution mass spectrometry (LC-HRMS), might be highly valuable.

To properly address the BMAA problem, an interdisciplinary approach including toxicology, microbiology, ecology and analytical chemistry, among others, would be fundamental. In addition, efficient management measures should include epidemiological studies in collaboration with public health agencies.

7. Conclusions

The present work provides a detailed overview of the principal sources for human exposure to BMAA and their contribution to the overall exposure to this neurotoxin. Although other routes are possible, dietary intake of BMAA-contaminated food has been identified as the major contributor. To date, most of available data refer to contaminated fish and shelfish, particurlay fillter-feeding bivalve molusks. Therefore, more investigations are necessary to assess the ocurrence of BMAA in other relevant foodstuffs. The recent discovery of BMAA-producing organisms in the diatom group, highly increases the potential exposure to this neurotoxin in marine and brackish environments, so proper monitoring programs should be implemented. From the analytical point of view, robust and selective methods should be developed in order to estimate accurately the intake of BMAA through the diet. The identifcation of unknown BMAA bound forms is of paramount importance to disclose their bioavailability and toxicity. Although recent investigations have shed some light on the connection between BMAA and neurodegenerative diseaseases, it should be highlighted that more toxicological data are still neccesary to establish a TDI for this toxin.

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