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A critical review of the postulated role of the non-essential amino acid, β-*N*-methylamino-L-alanine, in neurodegenerative disease in humans

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Abstract

The compound BMAA (β-*N*-methylamino-L-alanine) has been postulated to play a significant role in four serious neurological human diseases: Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC) found on Guam, and ALS, Parkinsonism, and dementia that occur globally. ALS/PDC with symptoms of all three diseases first came to the attention of the scientific community during and after World War II. It was initially associated with cycad flour used for food because BMAA is a product of symbiotic cycad root-dwelling cyanobacteria. Human consumption of flying foxes that fed on cycad seeds was later suggested as a source of BMAA on Guam and a cause of ALS/PDC. Subsequently, the hypothesis was expanded to include a causative role for BMAA in other neurodegenerative diseases including Alzheimer's disease (AD) through exposures attributed to proximity to freshwaters and/or consumption of seafood due to its purported production by most species of cyanobacteria. The hypothesis that BMAA is the critical factor in the genesis of these neurodegenerative diseases received considerable attention in the medical, scientific, and public arenas. This review examines the history of ALS/PDC and the BMAA-human disease hypotheses; similarities and differences between ALS/PDC and the other diseases with similar symptomologies; the relationship of ALS/PDC to other similar diseases, studies of BMAA-mediated effects in lab animals, inconsistencies and data gaps in the hypothesis; and other compounds and agents that were suggested as the cause of ALS/PDC on Guam. The review concludes that the hypothesis of a causal BMAA neurodegenerative disease relationship is not supported by existing data.

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Introduction

The non-essential amino acid, β-methylamino-L-alanine (BMAA) (Figure 1A), has been postulated as a causal factor for degenerative neurological diseases that affect large numbers of individuals (Bradley and Mash 2009; Papapetropoulos 2007). As such, BMAA has been the subject of considerable research for over four decades following the discovery and description of an unusually high incidence of neurological disease in Guam (Arnold, Edgren, and Palladino 1953; Kurland 1961), neighboring islands and a few other scattered localities (Garruto and Yase 1986). The neurological disease is known as Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/ PDC). The "BMAA hypothesis" links exposure to this environmental amino acid to Amyotrophic Lateral Sclerosis (ALS), Parkinsonism, and Alzheimer's disease (AD) globally. A search of the Internet revealed a large number of non-peer-reviewed sites concerned with these diseases that discuss BMAA as a possible causal factor in their development. There are, however, numerous questions regarding the assumptions regarding this hypothesis, as well as several competing hypotheses, that need to be evaluated if a valid assessment of BMAA's role in disease is to be verified. The review presented here is an attempt to critically examine the BMAA hypothesis, as well as present an overview of other hypotheses dealing with possible environmental causes of neurodegenerative disease in Guam. The review provides (1) a history of observations concerning ALS/PDC in Guam; (2) development of the BMAA hypothesis; (3) similarities and differences of ALS/PDC in Guam to other neurodegenerative diseases; (4) issues concerning the different analytical approaches utilized to identify BMAA in the environment and tissues; (5) an evaluation of the effects of BMAA in laboratory animals; (6) inconsistencies and questions regarding the BMAA hypothesis; and (7) a brief review of current alternative hypotheses of causal factors related to ALS/PDC.

Neurological disease in Guam

Guam is the southernmost and largest island (209 sq mi and about 30 mi long) in the Marianas Island chain in the western Pacific Ocean. Indigenous population, the Chamorros, utilize rice, corn (introduced by the Spanish), breadfruit, yams, taro, and cycads as their primary plantderived foods. The consumption of wild animals was generally reserved for ceremonies and medicinal uses. Guam was a US possession during the years before World War II. Japan successfully invaded Guam in 1941 and the occupation lasted 31 months. The island's Chamorro population stood at 22,300 in 1940 and experienced over 1,000 deaths during the Japanese occupation. During this period, the Chamorros did not have access to their normal food (especially rice and corn) and turned to cycad tree roots as a source of flour for tortillas and other staples. The population was concentrated in Umatec, at the southern tip of the island, where groundwater was scarce. The US recaptured Guam in 1944 and the Island is currently a US Territory (Rogers 1995).

A neurodegenerative disease with a spectrum of symptoms that affected Chamorros in Guam, Rota, and other Mariana Islands was initially described in a 1936 case history of a patient exhibiting symptoms of both Parkinsonism and dementia. In more recent times, Chamorros referred to the disease as Lytico-Bodig, a name derived from the Spanish

"paralytico" or paralysis, and "bodega" a shop where one of the first patients worked prior to World War I (Yase 1978). The neurological dysfunctions resemble one or more of three major diseases: ALS, Parkinson's disease, and a dementia that is often referred to as AD in the more-recent ALS/PDC literature. It should be noted that Parkinsonism refers to neurological symptoms involving movement that are similar to those seen in Parkinson's disease. The disease rendered patients incapable of normal movement, produced cognitive deficits, and almost always led to premature death. Initial investigations in Guam centered on clinical aspects of approximately 90 patients, and it was postulated that classic (sporadic) ALS was being observed (Arnold, Edgren, and Palladino 1953; Koerner 1952). Koerner (1952) did note "a spasm or rigidity occurred in seemingly more cases than have been described," a 20% incidence of "psychosis," and an unusually high familial incidence of the disease. Kurland and Mulder (1954a, 1954b) studied the epidemiology and symptoms of the disease, and the incidence of ALS in the Chamorros was found to be significantly higher at that time (150/100,000) than in any Caucasian population (1–2/100,000). Difficulty in articulating words (dysarthria) was noted in many patients and two ALS patients in Guam also displayed symptoms of Parkinsonism. In addition, other members of families with cases of ALS exhibited signs of Parkinsonism. The familial nature of the disease was also noted, and it was estimated that one-third of the families examined had affected members. Based on their investigations of ALS in the Marianas, (Kurland and Mulder 1954a,1954b) concluded that the disease incidence was also elevated on Rota, but markedly less frequent on other Mariana Islands. These studies were followed by investigations into the Parkinsonism-Dementia Complex (PDC) (Hirano, Malamud, and Kurland 1961a, 1961b). A series of 47 patients exhibiting significant mental deterioration was studied, and Parkinsonism was present in 85% of these subjects. The average age of the onset of PDC was later than ALS (50–60 as compared to 40–50 years old). It was estimated that the syndrome was responsible for approximately 7% of Chamorro deaths. Hirano, Malamud, and Kurland (1961a, 1961b) also noted that almost all patients exhibited hyperreflexia, over 30% of PDC patients displayed evidence of a type of motor neuron disease (MND) affecting cells that control voluntary muscle movement, and 9 of the 47 individuals examined were also diagnosed as exhibiting ALS, a common type of MND. The brains of 22 patients who died with ALS were examined by Malamud, Hirano, and Kurland (1961) and the findings were consistent including granulovacuolar degeneration in Ammon's horn, loss of neurons in the hippocampus, loss of pigmentation in the substantia nigra, demyelination of the pyramidal tracts, and the absence of both inter-cellular senile plaques typical of Alzheimer's disease and Lewy bodies that are protein tangles found in Parkinson's disease. Evidence of neurofibrillary changes was seen in spinal cords including those with symptoms of ALS, a type of pathology not previously reported with sporadic ALS. The distribution of the disease in Guam during 1953 was correlated with location where prevalence in the village of Umetac was 670/100,000 compared to the nearby town of Merizo where it was 100/100,000 (Kurland and Mulder 1954a). The neurological and pathological features of ALS/PDC were assessed and summarized by Elizan et al. (1966) and Hirano et al. (1966) based upon data gathered from 1957 to 1964. Among their conclusions, findings indicated that the clinical aspects of Guam ALS did not differ markedly from sporadic ALS with the exception of the following: There was an inverse relationship between age at onset of ALS and longevity as compared to lack of such a relationship reported with sporadic ALS. The earlier onset of

ALS in Guam was approximately 45 years of age compared with a range of 52–59 years elsewhere. Further, the unusual incidence of spasticity (pyramidal tract dysfunction) was the initial sign of ALS. Elizan et al. (1966) and Hirano et al. (1966) reported that PDC was invariably fatal and marked by progressive mental deterioration, slowness of movement (bradykinesia), rigidity and/or lack of facial movements and expression, and tremors. These signs of the illness were present in multiple combinations in many patients. In approximately 1/3 of the cases, dementia alone was the only sign of the disease for varying periods of time. Stanhope, Brody, and Morris (1972) investigated the epidemiology of ALS and PDC during 1950–1969 in Guam and found that the disease was associated with 30% of all adult mortality in men and 11% in women during that period. The PDC appeared later in life than ALS and was considered as being unique to Guam with the rates being 115/100,000 in males and 32/100,000 in females. Deaths attributable to PDC were estimated to be 15% for males and 8% for females. Although there was a strong familial factor in the occurrence of these diseases, Stanhope, Brody, and Morris (1972) did not observe any Mendelian pattern of inheritance. Garruto, Yanagihara, and Gajdusek (1985b) noted that onset of ALS/PDC in individuals occurred later between 1960 and 1980. In contrast to Reed and Brody (1975) who found a preponderance of male patients compared to females during 1945–1972, Garruto, Yanagihara, and Gajdusek (1985b) noted that this gender-based incidence of the disease had largely disappeared during this time period. McGeer et al. (1997) examined the mortality rates for ALS/PDC during the period 1991–1995 and observed that the ALS/PDC was still present in Guam although the incidence appeared to be declining. McGeer et al. (1997) hypothesized that the disease is strongly linked to genetic-based susceptibility with unknown modifying factors. In summary, ALS/PDC was historically present in Guam as seen in isolated case histories but no apparent epidemiological studies were conducted pre-WWII. Data obtained after this point indicate a high incidence of ALS/PDC that peaked during the 1960’s. Since that time, the incidence of both diseases has been significantly lower, to a greater extent for ALS than PDC.

ALS/PDC in New Guinea and Japan—In addition to Guam, populations with an incidence of ALS/PDC were detected in both Irian Jaya (western New Guinea) and Kii Peninsula of Japan. Gajdusek (1963) noted MND in a number of cases of bulbar or pseudobulbar palsy in Irian Jaya in the Kepi region in the southern coastal plain. These patients exhibited many symptoms associated with ALS but some cases with Parkinsonism and dementia symptoms were also found. The disease appeared to be limited to only the Auyu and Jaquai tribes, while other tribal groups in close geographical proximity did not apparently suffer from any of these MND symptoms. Gajdusek and Salazar (1982) identified a high incidence of ALS/PDC (approximately 150/ 100,000) in these groups and suggested that low levels of dietary calcium (Ca) and magnesium (Mg) might play a role in the etiology of the disease which is discussed in the “Environmental Trace Element Levels” Section below. A connection between exposures to cycads and occurrence of a high incidence of ALS/PDC in the Auyu people was suggested by Spencer, Palmer, and Ludolph (2005). Exposure was thought to be through the use of cycad-derived poultices used to treat skin wounds (Spencer et al. 1987c). Spencer, Palmer, and Ludolph (2005) reported a significant decline in the incidence in 7 villages along the Ia River during 1987–1990 compared to the 1973 survey of Gajdusek and Salazar (1982). Data suggested that this is due

to increased westernization and associated decrease in the use of traditional healers who used cycad seeds as poultices. These findings, however, were not supported by observations made during 2001–2007 and summarized by Griapon and Togodly (2008), Hirata (2008), and Okumiya et al. (2008). These investigators did not attempt to evaluate the incidence of ALS/PDC, but identified a notable number of cases involving different levels of paralysis among Auyu and Jaquai populations, many with the disease onset at 2000 or later.

Elevated levels of ALS on the Kii Peninsula of Japan have been suspected since the 1920's (Araki, Iwahashi, and Kuroiwa 1967). Kimura et al. (1963) collected data from one village, Mitogawa, and found an ALS incidence of 330/100,000 as well as neurological signs indicative of muscular atrophy in 11% of the population. Araki, Iwahashi, and Kuroiwa (1967) surveyed the incidence of ALS in other population centers on the Kii Peninsula. In the assessment of 21 communities, Araki, Iwahashi, and Kuroiwa (1967) did not find elevated incidences of ALS, but did note increased ALS rates in the towns of Miyama and Nanto that are northeast of Mitogawa. The city of Owase that lies between them did not show an elevated ALS rate. Shiraki (1969) reported on the histopathology of four ALS cases from the Kii area and an equal number from Tokyo. The similarity of the "ALS" from Kii to the ALS/PDC in Guam was confirmed by histological changes, indicating that multiple parts of the brain were simultaneously affected, including the pyramidal tracts of the spinal cord, substantia nigra, and cerebrum. Varying degrees of neurofibrillary degeneration, similar to that seen in AD, was also detected in all of the Kii cases. Evidence indicated that these cases resembled the pathology seen in Guam ALS/PDC. Kuzuhara et al. (2001) provided clinical reports and neuropathological features in a family from Hohara that suffered from Parkinsonism, AD, and ALS. The neuropathology in this family was similar despite differences in gross symptomology and neurofibrillary tangles, confirming the ALS/PDC connection. Spencer et al. (1987b) demonstrated that seeds from the cycad, *Cycas revoluta,* were used medicinally in the Kii Peninsula and suggested that chemicals in cycads might serve as a common factor in Guam and Kii MND.

An additional symptom of ALS/PDC is the occurrence of a retinopathy that has only been found in Guam and the Kii Peninsula. This takes the form of an retinal epitheliopathy (RPE) involving linear tracks of retinal depigmentation with intermittent pigment clumping (Steele et al. 2015). This will be discussed in the "Inconsistencies and questions regarding the BMAA hypothesis" Section below.

Epidemiological patterns of the different symptomologies of ALS/PDC—Plato et al. (2002, 2003) examined the incidence of ALS and PDC in Guam through 1999 and found that after a peak rate had been reached during the 1950s, the incidence of ALS declined, reaching 4–5/ 100,000 in the 1980–1984 period and falling below 3/100,000 since that point and maintained at this level through 1999. The situation with PDC is slightly different as the decline began in the 1960s (males) and 1970s (females) then decreased through the 1980s at a reduced rate as compared to ALS. Galasko et al. (2002) studied the incidence of ALS, Parkinsonism, PDC and dementia in Guam during 1997–2000 with emphasis on cases of PDC and late-life dementia. Data demonstrated that many of these patients exhibited no overt signs of Parkinsonism, and it was postulated that their disease might be attributed to AD rather than PDC, indicating a possible change in identity of the

disease over time. McGeer et al. (1997) found senile plaques present in the brains of 3/19 Chamorros (2/12 with PDC; 0/3 with ALS and ¼ who were asymptomatic of any neurodegenerative disease). Miklossy et al. (2008) noted the presence of senile plaques in 4/13 PDC cases in subjects who died prior to 1987 in contrast with 17/22 cases where death occurred between 1987 and 2006. The Guam population being examined more recently is significantly older, and the factor of increased age needs to be considered when characterizing neurodegenerative disease in Guam. The average age at death in the 1946–90 population was 51.5 ± 2.9 years vs. 70.8 ± 1.8 in the population that died post1990.

The incidence and characteristics of neurodegenerative diseases of individuals born in Guam who had migrated to California have been reported in several studies. Torres, Iriarte, and Kurlund (1957), Eldridge et al. (1969), and Garruto, Gajdusek, and Chen (1980) demonstrated a similar incidence of ALS in Guam emigrants who were living in California as found in Guam itself. Garruto, Gajdusek, and Chen (1980) noted that the latency period for the disease might be as long as three decades based on a maximum time between emigration and onset of the disease, and that a minimum residence in Guam before emigration was 18 years. Brody, Hirano, and Scott (1971) examined the pathology of ALS in Americans who had resided in Guam during WWII. Examination of the brains of three individuals who had died of this disease indicated typical cellular changes associated with sporadic ALS, not the wide distribution of neurofibrillary tangles (NFTs) found in Guam ALS. A survey of the death certificates of 96,000 construction workers who had lived in Guam between 1945 and 1955 did not indicate any alteration in ALS incidence compared to the United States rate (Brody, Edgar, and Gillespie 1978).

The BMAA hypothesis—Its genesis and subsequent history

Use of dietary cycads, discovery of BMAA, and initial animal toxicity studies

Cycads (Family Cycadacae) are primitive dioecious plants and found worldwide in tropical and subtropical regions. Cycads are used as a food staple and for medicinal purposes although their toxicity was well known (Whiting 1963). All populations that utilize the root, stem, or fruit to obtain an edible starch developed methods for significantly reducing toxicity and these techniques all involve sequential washing and grinding. These procedures are well documented (Carr and Carr 1981) and were employed in the Mariana Islands, Indochina, India, various areas in Africa, South America (Colombia and the Amazon region), Central America, Australia, the Ryukyu Islands near Japan (where it was exported to Japan as food), and the United States (produced in Florida and commercially as food prior to 1929). The medicinal uses of cycads are varied and include wound healing, pain relief, curing of ulcers, and as a general “restorative.” The treatments may involve ingestion and/or topical application. Toxicity associated with ingestion of raw or improperly prepared cycads has been described in humans, various livestock species including both mammals and birds, and pets. Due to the common occurrence and toxicity of cycad species in the genus *Zamia,* the paralysis has been termed the “Zamia staggers” (Whiting 1963).

Flour derived from Cycad roots has historically been a major food staple for the Chamorros. Whiting (1964) noted that cycads were ingested in all three areas in which ALS/PDC was reported, but did not reach any conclusions since it was no longer consumed in Japan, and

detailed information on cycad use in the areas affected in Irian Jaya was lacking. In Guam, the toxicity of cycads to both animals and humans has been common knowledge and the preparation of flour from the cycad species eaten in Guam *(Cycas circinalis* same as *C. micronesica)* involved repeated washings over a period of at least 3 weeks to ensure that it was safe for consumption (Duncan et al. 1990; Whiting 1963). Several toxins have now been implicated in cycad-induced neurotoxicity including cycasin and its metabolite, methylazoxymethanol (MAM) that produce paralysis in cattle (Whiting 1963) and mice (Hirono and Shibuya 1967), and central nervous system (CNS) developmental defects in rodents (Laqueur and Spatz 1968). Rao, Adiga, and Sarma (1964) isolated a neurotoxic compound in several species of plants (chicklings or grass peas) in the genus *Lathyrus* that were known to induce irreversible paralysis in humans, and identified it as a non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminoproprionic acid (BOAA). During an investigation of compounds in *C. circinalis* seeds, Vega and Bell (1967) isolated a novel type of amino acid, β-methylamino-L-alanine (BMAA). Vega and Bell (1967) characterized cycad BMAA as the L-isomer and tested a small number of chicks and rats after intraperitoneal (i.p.) injection. Signs of neurotoxicity in both species, leading to convulsions, weakness and a dragging gait in the rats were detected following administration (Bell, Vega, and Nunn 1967; Vega, Bell, and Nunn 1968). Both BMAA and BOAA are similar in structure to glutamic acid that, in addition to being a component of proteins, is a vital neurotransmitter involved in cognitive functions. Excessive neuronal release and impaired uptake of glutamate induce excitotoxicity and are associated with ALS and AD (Hynd, Scott, and Dodd 2004).

The discovery of BMAA provided additional support to the initial suggestions of a causal relationship between cycad use and neurodegenerative diseases as noted by Whiting (1963) and Kurland (1972). Reed et al. (1987) postulated a connection between food and ALS/PDC but other factors such as trace elements and genetic factors were not considered, and specific food types were not delineated. The cycad hypothesis was also extended to other areas outside the Marianas where ALS/PDC was found. Although foods derived from cycads were not commonly consumed in Japan, investigators reported that cycad seeds *(Cycas revoluta)* were used for medicinal purposes in the Kii Peninsula where ALS/PDC occurred (Spencer et al. 1987b). Obendorf and Spencer (2000) examined the occurrence of cycad use for medicinal purposes on Irian Jaya and reported widespread utilization of crushed cycad as a poultice for open sores or abrasions. This use was found in all areas investigated and included the type of cycads in the region where Auyu and Jakai subjects suffered from ALS/PDC.

Duncan et al. (1988, 1990) analyzed levels of BMAA in 30 samples of processed cycad flour purchased in Guam and Rota and found ranges of 146 μg/g to <4 μg/g. A single 24-hr wash of unprocessed flour containing 1140 μg/g BMAA resulted in a residual of 126 μg/g, which is the equivalent of having removed 90% of BMAA such that BMAA in processed flour was only 0.005% by weight. Based on these findings, Duncan et al. (1988, 1990) calculated that the amounts of flour that would have to be consumed to achieve the doses administered to primates to produce features resembling Parkinsonism were unrealistic based upon data from Spencer, Ohta, and Palmer (1987a), who reported features resembling Parkinsonism after BMAA doses of 200–350 mg/kg orally for multiple weeks. Duncan et al. (1988, 1990) also found no marked differences in BMAA levels in flour processed in villages with and

without ALS/PDC. These findings cast significant doubt on the relevance of the BMAA hypothesis. Spencer et al. (1990) subsequently referred to the doses used in their 1987 studies in the macaque monkeys as “huge subconvulsant doses” and suggested that the changes observed “fall short of a model of human disease,” and that attention needs to be directed at the possible presence of other “slow (neuro) toxins” in cycad flour.

Biomagnification

Cox and Sacks (2002) hypothesized that BMAA was biomagnified in the environment leading to levels in flying foxes (large herbivorous bats) *(Pteropus mariannus)* that were sufficient to produce high exposures in humans consuming these animals. Supporting evidence demonstrated that BMAA levels in different parts of the cycad increased from roots (2–37 μg/g) that were the source of the flour, to an average of 1161 μg/g in the edible seed covering (sarcosta) ingested by bats (Banack and Cox 2003a). Flying foxes bats were, in turn, eaten by Chamorros during various ceremonies and celebrations. The BMAA in flying foxes underwent an additional bioaccumulation, and average levels of 2660 ± 1908 μg/g (SEM) were detected in dried skin samples from three preserved museum specimens (Banack and Cox 2003b). The presence of high levels of BMAA in flying foxes led Monson, Banack, and Cox (2003) and Cox, Banack, and Murch (2003) to indicate that biomagnification of BMAA from cycad roots to flying foxes and subsequent consumption of the animals may have resulted in sufficient BMAA exposure to account for the ALS/PDC in Guam. Both studies present a graph showing a parallel decrease in ALS/PDC incidence and flying fox populations. Murch, Cox, and Banack (2004a) found that BMAA in tissues existed in two forms —free and protein-bound, suggesting that a slow release of inactive bound BMAA as a toxic free molecule might explain the long latency of ALS/ PDC in Guam. Banack, Murch, and Cox (2006) reported on flying fox tissues that had been either frozen or preserved in formalin and stored in alcohol. The flying foxes examined comprised a series from Guam and demonstrated significant inter-individual variation in muscle, liver, kidneys, skin and hair. The levels in three samples of dried skin for Guam flying fox specimens were shown to be 479 ± 689 μg/g that are considerably lower than levels noted earlier. Banack, Murch, and Cox (2006) also found 3085 μg BMAA in 250 ml of flying fox stew broth and estimated that a human ingesting 1 L would receive 12.4 mg BMAA (0.18 mg/kg in a 68 kg individual).

Deposition in animals

The possibility that BMAA was responsible for neurodegenerative dysfunction led to studies of its potential to cross the blood-brain barrier (BBB). Three studies were designed to address this question. The transport mechanism and pharmacokinetics of BMAA were investigated in rats by Duncan et al. (1991) who reported that after the intravenous (iv) injection of 100 mg/kg BMAA•HCl, plasma levels fell to 5% of the initial levels within 30 min and calculated that <0.08% of total dose was present two hrs after injection. After oral dosing of similar amounts of BMAA, plasma levels rose to approximately 80% of iv levels indicating rapid transport across intestinal walls. The clearance rates were similar to that of iv administration. BMAA did cross the BBB to a limited extent, and average levels of 4–10 μg/g were seen in brain tissues. This represents an uptake approximately 0.08% of

administered dose. BMAA was cleared from the brain with a $t_{1/2}$ of approximately 0.7 days, not indicating selective brain deposition or retention. A similar pattern of bioavailability after oral administration of 1–100 mg/kg was seen in individual cynomologus monkeys *(Macaca fascicularis)* (Duncan et al. 1992). Smith et al. (1992), using in situ perfusion, demonstrated that BMAA was transported across the BBB of rats by a large neutral amino acid carrier. Results indicated that this type of amino acid carrier generally functions as an amino acid exchanger rather than to facilitate accumulation, consistent with measured brain clearance. The neutral amino acid uptake mechanism is enhanced in infants (Banos, Daniel, and Pratt 1978) and may be related to the findings by Karlsson et al. (2009a) who administered radiolabeled BMAA iv to pregnant mice on gestation day 14 and to postnatal day 10 animals by the subcutaneous (sc) route. Karlsson et al. (2009a) showed that there was little deposition of BMAA in the maternal brain in contrast to greater concentration in liver. This situation was reversed in the 14-day fetus where there was significant concentration detected in the CNS and little in the liver. In 10-day-old pups, high levels of BMAA were seen in the hippocampus, cerebellum, brain stem, and spinal cord.

Occurrence in human brains

Levels of BMAA in postmortem brain samples (superior frontal gyrus) of 8 Chamorros who suffered from ALS/PDC and 15 Canadians who died from a variety of diseases were determined by Murch et al. (2004b) who observed a relationship between neurodegenerative disease and presence of either free or protein-bound BMAA. BMAA was present in one ALS and five PDC brain samples of affected Chamorro people and values ranged from 149 to 1190 μg/g of protein-associated BMAA. One of the two unaffected people displayed a lower, but detectable level of protein-associated BMAA (82 μg/g). Analysis of the tissue samples from Canadians included two obtained from individuals who exhibited AD and both contained free and bound BMAA, contrasting with a lack of BMAA in brains of 13 people who were not diagnosed with AD. A subsequent paper by Pablo et al. (2009) examining archived brain tissues of subjects who died in Miami, Florida: 13 with ALS, 12 with AD, 8 with Huntington's disease, and 12 from other causes. All of the 25 ALS and AD brains showed detectable BMAA in either the frontal cortex or temporal cortex with levels averaging 141 ± 14 μg/g. Of the 20 individuals with either Huntington's Disease or an absence of any other neurological disease, only three displayed detectable levels of BMAA, single samples in the frontal cortex, temporal cortex or caudate. Evidence indicated the potential effects of BMAA to include causation of ALS. These results reveal an interesting pattern with detectable levels of BMAA found in all patients with ALS/PDC, ALS, or AD (32/32) and only detected in 4/34 people without one or more of these diseases at the time of death.

Global distribution

BMAA presence in brains of Canadians and people living in the United States raised the question of possible source(s) of this compound in populations not using cycads or flying foxes for food. Lindblad (1990) demonstrated that symbiotic nitrogen-fixing cyanobacteria (genus *Nostoc)* resided in the coral-loid roots of a cycad species, *Zamia skinneri,* and Cox et al. (2003) reported that BMAA was produced by these cyanobacteria. The association of

BMAA and cyanobacteria was tested by Cox et al. (2005) who analyzed 30 samples of different cyano-bacterial species/strains representing 21 genera and at least 23 species. These cyanobacteria were found in a wide variety of habitats including fresh, brackish, and marine waters, soil, hot springs, and limestone caves. Unlike other studied cyanobacterial toxins that are generally limited to a small number of species, BMAA was detected in all but one sample. BMAA-producing cyanobacteria include *Prochlorococcus marinus,* a picoplanktonic marine species that constitutes a significant part of the biomass in temperate oceans (Partensky et al. 1999), and both *Cylindrospermopsis raciborskii and Microcystis sp.,* two of the most common cyanobacteria in freshwaters (Briand et al. 2004; Falconer and Humpage 2005; Zurawell et al. 2005). Subsequently, several studies were conducted that provided additional evidence for the widespread association of BMAA with diverse species of cyanobacteria. Twenty-seven different isolates of 12 cyanobacterial species found in a variety of freshwaters in South Africa were analyzed for the presence of BMAA by Esterhuizen and Downing (2008), and BMAA at levels ranging from 0.1 to 2757 μg/g was detected in all but one, apparently confirming the ubiquitous nature of this compound. Metcalf et al. (2008) identified BMAA in 12 freshwater cyanobacterial samples from different locations within the United Kingdom and reported levels ranging from 8 to 287 μg/g dry weight in algal blooms and aggregations (scums and mats) and also identified either free or protein-associated BMAA in all samples. A wide range of known cyanobacterial toxins (including the neurotoxins, saxitoxin, and anatoxin-a) in 11/12 of these test samples was observed. Cervantes Cianca et al. (2012) analyzed 18 cyanobacterial strains representing 9 genera by using three different extraction methods and noted BMAA ranging from <10 to >30 μg/g. In addition to cyanobacteria, there are two groups of eukaryotes that have also been found to contain BMAA. BMAA at pg and ng/g levels was reported from United States and European waters (Jiang et al. 2014b; Reveillon et al. 2015, 2016). Lage et al. (2014) isolated BMAA and its isomers, 2,4-diamino butyric acid (DAB) (Figure 1B) and N-(2-aminoethyl)glycine (AEG), from a culture of the dinoflagellate *Gymnodinium catenatum* derived from Portuguese marine waters and protein-associated BMAA was identified at levels of 0.5 μg/g dry weight. It should be noted that Esterhuizen-Londt and Downing (2011) did not find any free BMAA in raw waters that had blooms with high levels of BMAA and suggested that the lack of BMAA might either be due to continual re-uptake by other organisms, or adsorption onto various particulates.

Possible trophic transfer of BMAA in the Baltic Sea and Florida Bay has been demonstrated. Jonasson et al. (2010) found levels of BMAA in cyanobacteria and zooplankton at different sites in the Baltic Sea. Detectable levels (<1 μg/g) were present in muscle tissue of 4 of 7 species of fish. Further, levels of BMAA (0.2–2 μg/g) were detected in mussels and oysters collected off the western coast of Sweden. Baptista et al. (2015) exposed mussels to BMAA-containing cyanobacteria, and detectable levels of the compound were found in whole animals. Brand et al. (2010) investigated the distribution of BMAA in freshwater and saltwater systems in Florida (southeastern United States). Various vertebrate and invertebrate samples were tested from two salt water areas with histories of cyanobacterial blooms, and in a river system where cyanobacterial blooms are common. High levels of BMAA were present in marine invertebrates and species of fish. BMAA ≥6900 μg/g was found in blue crabs; ≥3000 μg/g shrimp, and ≥3000 μg/g grunts (an edible species of fish). Freshwater

largemouth bass contained levels ≥2300 μg/g. These concentrations were similar in magnitude to those recorded in flying foxes by Cox et al. (2006). Al-Sammak et al. (2014) noted detectable levels of BMAA or DAB in 33 of 248 fish tissue samples from a variety of species in the central United States. The highest concentrations of bound BMAA reported were 2.57 μg/g in carp, 0.72 μg/g in bass, and 6.7 μg/g in aquatic plants. A study of BMAA distribution in Lake Taihu, China that has extensive populations of *Microcystis spp.* indicated the presence of BMAA in cyanobacteria at levels of 7.14 μg/g during blooms and an apparent biomagnification to levels of 12.92 μg/g in planktivorous fish, and 35.91 μg/g in a piscivorous fish (Jiao et al. 2014). Metcalf et al. (2013) performed an analysis of the feathers of the Lesser Flamingo *(Phoeniconaias minor)* in East Africa because benthic cyanobacterial mats form a significant part of their food, and noted BMAA and its isomer DAB, at levels ≥8.5 μg/g. Four freshwater gastropod species were exposed to 100 ug/L concentrations of BMAA under lab conditions (Downing et al. 2014). Both uptake over a 24-hr period and depuration was measured demonstrating that uptake from water occurred in all species. This phenomenon was not related to the disappearance of BMAA in cultures but due to an increase in gastropod levels.

Human exposure

The global distribution of BMAA coupled with its ability to bioconcentrate led to studies investigating its presence in foods and dietary supplements. Jiang et al. (2014a) demonstrated detectable levels of BMAA in some species of fish, crustaceans, and gastropods sold as food in Swedish supermarkets. Mondo et al. (2014) examined levels of BMAA in dietary supplements containing shark cartilage and reported the presence of both BMAA and mercury. BMAA was found at levels of approximately 150 μg/ g in general agreement with earlier studies on shark fins. Roney et al. (2009) noted BMAA in cyanobacterial species *Nostoc flagelliforme* that is used to make a soup used for celebrations. It is difficult to draw firm conclusions from the data presented because of the 18 samples listed, BMAA was detected and measured in 4 (0.027-.659 μg/g), not quantified in one, not detected in 6, and not tested in 7. A study in wheat *(Triticum aestivum)* demonstrated that seedlings absorb BMAA from irrigation waters (Contardo-Jara, Schwanemann, and Pflugmacher 2014). McGorum et al. (2015) analyzed levels of BMAA in horses exposed to terrestrial cyanobacteria (primarily *Phormidium).* The presence of BMAA, DAB, and AEG was tested in neural tissues of 6 animals suffering from equine grass disease, 2 with equine MND and 7 controls. None of the toxins were found in any tissues tested. Glover, Cohen, and Murch (2015) utilized LC-MS/MS and isotopically labeled D3,15N2-BMAA to quantify BMAA, AEG, and DAB content in bulk natural health products containing Spirulina or other cyanobacteria species. Several products were determined to contain BMAA, AEG, and DAB at levels ranging from less than 1 to more than 100 μg/ g. However, two previous studies found no detectable levels of BMAA in cyanobacteria-based *(Spirulina* sp.) dietary supplements (McCarron et al. 2014; Scott et al. 2009).

Caller et al. (2009) noted the existence of a northeast US cluster of 9 ALS cases in a town that was close to a lake that contained both *Microcystis* and *Anabaena sp.*, two genera known to produce BMAA, and postulated a possible causal link between BMAA and ALS.

However, it was not possible to identify BMAA in the lake but suggested that this may have been due to methodological issues. Similar clusters had been identified in the areas of lakes with algal blooms, but no specific localities were mentioned (Caller et al. 2009). Banack et al. (2015) revisited the same lake and found both microcystins and BMAA in fish brain (0.043 μg/g) and liver and muscle (1.28 μg/g). Torbick et al. (2014) conducted a spatial analysis of water quality using Landsat data on chlorophyll-a, Sechi depth, and total nitrogen, and related these indicators of water quality to ALS "hot spots." Data indicated an association of poorer water quality with ALS incidence.

Cox et al. (2009) examined mats of cyanobateria in the deserts of Qatar and found BMAA present, although no quantitative data were given. This finding led to speculation of a possible link between inhaled BMAA and a reported increase in ALS incidence in soldiers who were stationed in Qatar (Beard and Kamel 2015). Links between BMAA exposure and ALS due to consumption of blue crabs (Field et al. 2013) and exposure to aerosolized cyanobacteria from cooling towers (Stommel, Field, and Caller 2013) also have been suggested.

Neurotoxicity of BMAA in biological test systems

Numerous investigators used *in vitro* approaches to assess the potential actions of BMAA on mammalian central nervous system tissues (CNS). Such studies are valuable in determining possible mechanisms of toxicity at the cellular level, but less so in relating effects to environmental exposures in whole animals. In nearly all of these investigations, high BMAA concentrations (≥100 μM) were required to produce cellular damage and toxicity. These concentrations are not physiologically relevant and are therefore extraordinarily difficult to interpret in terms of expected *in vivo* responses. It should be noted that the toxicity of glutamate and BMAA was compared in *vitro,* and data demonstrated that glutamate was approximately 10-fold more toxic than equimolar concentrations of BMAA (Chiu et al. 2012; Staton and Bristow 1997). Given these inherent problems, this review will be limited to *in vivo* investigations with the exception of studies that are central to the hypothesis of BMAA-induced protein misfolding as a primary event associated with neurodegeneration, and the possible role of serine (Figure 1C) in protein misincorporation.

Protein misincorporation

Protein misfolding is generally thought to be a critical factor in the formation of aberrant proteins in the CNS in many diseases including AD, Parkinson's disease, Huntington's, ALS, Progressive Supranuclear Palsy (PSP), Kuru, and Creutzfelt-Jacob (Abdel-Salam 2014; Ashraf et al. 2014; Dobson 2001; Ellisdon and Bottomley 2004; Soto 2003). Types of misfolded proteins include tau proteins that generate NFTs in the form of hyperphosphorylated tau protein helical filaments in AD, ubiquinated protein inclusions found in ALS, and alpha-synuclein inclusions (Lewy bodies) in the substantia nigra in Parkinsonism. One of the mechanisms advanced as responsible for misfolded proteins is incorporation of essential amino acids in the wrong positions, or misincorporation of non-essential amino acids such as BMAA into proteins. Several *in vivo* and *in vitro* studies suggested BMAA incorporation into proteins. Xie, Basile, and Mash (2013) used BMAA

distribution and tissue kinetics after iv injection of a dose of 3.8 mg/kg radiolabeled compound. These investigators interpreted the long residence time of the acid-insoluble "protein-bound form" in brain tissue as indicative of protein incorporation. Dunlop et al. (2013) investigated the potential of BMAA to be misincorporated into proteins using human lung fibroblast line (MRC-5), a human neuroblastoma cell line (SH-SY5Y), and human umbilical vein endothelial cell (HUVEC) cultures. The studies used cell cultures incubated with 31nM ^{3}H-BMAA and 200–1000 μM BMAA before 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and liquid chromatography mass spectrophotometry/mass spectrophotometry (LC-MS/MS) analysis. Protein incorporation was determined by lysing cells, isolating the protein, and quantifying the BMAA associated with the protein fractions. The association of BMAA with proteins was positively related to protein synthesis and reduced in the presence of cycloheximide (a protein synthesis inhibitor). Dunlop et al. (2013) concluded that BMAA was misincorporated into proteins as a substitute for serine, but evidence for actual misincorporation rather than a strong chemical association was not provided. The study also showed a correlation between BMAA in culture medium (200–1000 μM) versus BMAA associated with proteins (nmoles). However, data were not reported in the units of nmoles of BMAA per μg protein in the absolute quantitation format that would enable a more accurate comparison between the radiolabel data (low nM) and the LC-MS/MS data (mediumhigh μM). Glover, Mash, and Murch (2014) expanded the findings of Dunlop et al. (2013) by providing data on protein synthesis after incubation of BMAA in the PURExpress *in vitro* protein synthesis construct where essential amino acids were omitted. The level of protein synthesis with all additional amino acids placed into the PURExpress construct was considered baseline positive. Omitting all amino acids (negative control) resulted in approximately 80% of that value apparently corresponding to the presence of all functional enzyme proteins in the PURExpress system itself. Of the nine single amino acid omissions tested in the presence of BMAA, small-to-no differences from the negative control value were found when isoleucine, cysteine, or glutamine were missing from the culture. The remaining six amino acids may be divided into two groups. No reduction in protein synthesis occurred in the absence of alanine (Figure 1D). The absence of phenylalanine, proline, glutamate, threonine or serine resulted in synthesis of approximately 90% of the total protein produced in the positive control. A second analysis calculates the relative incorporation of BMAA into proteins based on the LC-MS/MS measurement of the free BMAA in denatured samples and complete protein hydrolysates. These data appear to show approximately 24% incorporation of the available BMAA into the *E. coli* proteins in experiments that included all amino acids, and somewhat less with the other 6 amino acids mentioned above. Surprisingly, the experiments involving either all amino acids including BMAA, or BMAA replacing serine or alanine, yielded almost the same amount of BMAA incorporation (approximately 20%), apparently indicating that BMAA was utilized by the PURExpress system as an essential amino acid. This inherent low discrimination by the PURExpress enzymes between essential and artificial amino acids is confirmed by the 7 and 3% yield of incorporation of the BMAA isomers AEG and DAB, respectively, into the *E. coli* proteins. Human DNA templates were shown to be more error-prone than standard *E. coli* sequence provided by the PURExpress kit. Further discussion of this bioassay is found in the "Inconsistencies and questions concerning the BMAA hypothesis" below.

In vivo studies

In vivo studies are further divided into sections based upon species group (primates and rodents) and age of the experimental populations (adult or neonatal) since these are fundamental factors in the extrapolation of toxicity data to human populations.

Primates

There have been three primate studies of differing relevance to the BMAA hypothesis. Dastur (1964) exposed three rhesus monkeys *(Macaca mulatta)* to cycad flour made from *Cycas circinalis* for up to 10 months. The response of the animals varied from no effects in a 9-year-old animal to muscle weakness and wasting in the youngest (6 months old). Spencer et al. (1987c) examined fifteen macaques *(Macaca fascicularis*) that were exposed to 100–350 mg/kg/day BMAA•HCl (neutralized with sodium bicarbonate) orally for 2.5–12 weeks. At doses ≥200 mg/kg, motor neuron dysfunction in the forelimbs was followed by muscle weaknesses and loss of muscle mass. Six animals exhibited unilateral or bilateral extensor hindlimb posturing. After a month of BMAA administration, all animals displayed stooped posture, tremors, and weakness in extremities. Long-term treatment resulted in periods of immobility with a blank stare and crouched posture. The overt symptoms did not progress after exposure ended (Spencer, Kisby, and Ludolph 1991). Two of the animals responded to oral anti-Parkinsonian drugs within 30 min. At the cellular level, there was disruption in the motor cortex as well as displacement of Nissl bodies, chromatolysis of giant Betz cells, and similar changes in the large anterior horn cells of the spinal cord. Interestingly, the substantia nigra and hippocampus, two of the most affected areas in ALS/PDC, were similar to controls. Cox et al. (2016) utilized 32 adult vervet monkeys *(Chlorocebus sabaeus)* that were exposed to BMAA for 140 days. Four dose groups of eight animals received 21 mg/kg or 210 mg/kg BMAA; 210 mg/kg of BMAA + 210 mg/kg of serine; or fruit alone. Serine was used to test for a potential protective effect. Animals were on a low-protein diet supplemented with fruit that was used as a vehicle for the BMAA dosing. At necropsy, brain homogenates were analyzed for the presence of BMAA, and 14 regions of the brain were analyzed for the presence of NFTs and β-amyloid deposits. NFTs were found to a greater extent in the high-dose BMAA groups than in the low dose or control animals. β-amyloid deposits were also found in one to three animals in each of the treated groups.

Adult rodents

Perry et al. (1989) examined the impact of BMAA exposure in female CD-1 mice at 2 months of age. Animals were administered 500 mg/kg by gavage for 18 days, followed by 500 mg/kg on alternate days for 4 weeks, and 1000 mg/kg on alternate days for 30 days. The final accumulated dose was 31 g/kg, 15 g being the L-isomer. There were no marked differences in weight, behavior, critical neurochemical level alterations (dopamine, noradrenaline or serotonin), and no apparent evidence of pathological changes. Cruz-Aguado, Winkler, and Shaw (2006) provided 1 mg/day BMAA in the diet of 6-month-old male CD-1 mice for 1 month. The calculated concentration that animals received over the course of the exposure was a total of approximately 28 mg/ kg. No adverse effects were observed in tests of motor function or behavior.

There have been a number of studies using direct intracerebroventricular, intracisternal, or intrastriatal injections of BMAA. These studies bypass the BBB and deliver high doses of BMAA directly to brain tissue. Such studies are extremely difficult to relate to environmental exposures, and although significant adverse effects were noted, it is not possible to assess their biological relevance.

Immature animals

Seawright et al. (1990) administered doses of racemic BMAA (500–4000 mg/kg) by the intraperitoneal (i. p.) route to young Wistar rats weighing approximately 85 g and found dose-related changes in motor behavior (slight to severe ataxia) at doses ≥1000 mg/kg. De Munck et al. (2013a,2015) used the i.p. route to expose weanling (approximately postnatal day (PND) 21) rats to BMAA at doses of 100–350 mg/kg for 5 consecutive days. Testing during the course of 100 days indicated changes in ambulation, tail lift, and strength tests at all dose levels with 100 mg/kg inducing significantly reduced effects compared to higher dose levels of 200–350 mg/kg that induced similar extent of effects. At a dose level of 300 mg/kg, De Munck et al. (2013a, 2015) observed disorganization of the endoplasmic reticulum, increased concentrations of glycogen synthase kinase-3β in lumbar spinal neurons, and DNA-binding protein 43 in the motor cortex. Dose-related elevation in glutamate and decreased levels of GABA were also noted. De Munck et al. (2013b) also reported decreased antioxidant capacity and increased glutathione (GSH) levels and catalase activity in livers indicating the presence of oxidative stress in animals dosed with 300 mg/kg BMAA.

Dawson et al. (1998) administered sc injections of 100 or 500 mg/kg on PND 2 and PND 5, or 500 mg/kg on PND 5 to rats. Animals exposed to 500 mg/kg administered sc exhibited hyperactivity in the open field test. Karlsson et al. (2009a) detected BMAA uptake in the brain of late term fetal mice following a dose of 7.3ug/kg ^{3}H-BMAA administered iv to the pregnant dam on gestation day 14 or administered sc to PND 10 pups for two consecutive days. To test BMAA-induced behavioral changes, PND 9–10 animals were administered two consecutive doses of BMAA•HCl sc at doses of 200 or 600 mg/kg BMAA•HCl (150 and 460 mg/kg BMAA, respectively) (Karlsson, Roman, and Brittebo 2009b). Exposed and control rats were tested at 10 weeks in the multivariate concentric square field test, an environment where a large number of behavioral tests can be run. There were some significant differences observed in a test for cognitive or memory function in which animals were exposed to a puff of air, and at 2 weeks postexposure, BMAA animals disregarded the stimulus to a greater extent than the controls. At 20 weeks after exposure, radial arm tests showed longer acquisition times in the 600 mg/kg BMAA•HCl group during the first trial but no differences in retention 2 weeks after acquisition. Karlsson et al. (2011) looked at effects of lower doses (50 and 200 mg/kg) using the same protocols as their Karlsson, Roman, and Brittebo (2009b). There were few dose-related effects seen, the exception being the radial arm maze test in which treated animals failed to collect as many pellets as controls. At the 460 mg/kg BMAA dose level, neurodegeneration in the hippocampus (Karlsson et al. 2012), changes in neuropeptides and proteins in the striatum (2014a, 2014b; Karlsson et al. 2013), metabolite changes (Hanrieder et al. 2014), and intra-cellular fibril formation in the hippocampus (Karlsson et al. 2015) were noted.

Inconsistencies and questions regarding the BMAA hypothesis

It is difficult to exaggerate the potential importance of the hypothesis that BMAA is a causal factor in the incidence of three devastating neurodegenerative diseases that affect millions of people worldwide. It has been stated that BMAA is produced by most species of cyanobacteria (Brand 2009; Cox et al. 2005) and is therefore a global toxin, bioaccumulates and produces human disease through both dietary and environmental exposures (Banack and Murch 2009; Bradley et al. 2013; Cox et al. 2016). As the hypothesis begins to enter the public domain through popular science-oriented magazines (Discover Magazine (McAuliffe 2011), The Asian Scientist (Lim 2012), and the Scientific American (Eplett 2015), the potential dangers associated with eating foods or living in environments that may contain high levels of BMAA are increasingly noted. Before public health actions are taken based on the hypothesis, it is imperative that fundamental issues and inconsistencies concerning the central assumptions are discussed and resolved:

- Issues with the measurement and quantification of BMAA.
- Extent of the evidence for considering ALS/ PDC as a single neurodegenerative disease with symptoms of three different neurodegenerative diseases occurring globally.
- Significance of the retinopathy discussed previously that was seen in ALS/PDC patients in Guam.
- Basis of the flying fox hypothesis of high exposures to BMAA in Guam being a decisive factor in the cause of ALS/PDC.
- Dosing parameters and exposure levels.
- Strength of the evidence for a relationship of neurodegenerative disease and the presence of BMAA in brains.
- Evidence for BMAA formation by most species of cyanobacteria.
- Evidence for BMAA misincorporation into proteins.

BMAA measurement and methods standardization

There are numerous sample preparation and analytical detection methods for BMAA; however, there has not been a standardization of these methods (Faassen et al. 2016) which may have contributed to the BMAA controversy. Early methods used less specific techniques such as liquid chromatography (LC) hyphenated with either ultraviolet-visible (UV-Vis) or fluorescence spectroscopy for detection (LC-FD) (Montine et al. 2005; Murch, Cox, and Banack 2004a, 2004b; Pablo et al. 2009) for quantification of BMAA. These methods are not selective and should not be used unless the concentrations of positive samples are verified with a method such as LC-MS (Faassen 2014). Later methods largely focused on the use of mass spectrometry (LC/MS or LC/MS/MS) with better agreement regarding BMAA detection and to some extent quantitation. BMAA is not well retained on reverse-phased analytical columns, and therefore, typically derivatized or separations are conducted on hydrophilic interaction chromatography columns (HILIC). Baseline separation of BMAA from its isomers is necessary to prevent misidentification, and it is good practice

to separate analytes from early eluting, polar background materials such as salts and other lower molecular weight organic compounds associated with environmental and tissue compounds. Recent investigations found inconsistency regarding which forms of BMAA were extracted and analyzed between studies (e.g., free, soluble bound, precipitated bound, and total forms) (Faassen et al. 2016; Rosen et al. 2016) and may explain some of the discrepancy noted in the literature regarding BMAA's occurrence in water, algae, and tissues. Most methods are also likely subject to some degree of matrix interference, but few used standard addition or isotope dilution to help compensate for these issues.

ALS/PDC in Guam: Relationship(S) to ALS, Parkinsonism and AD

During the history of scientific investigations into the degenerative neuronal disease(s) in Guam, the characterization of the disease has changed. At first, it was considered two distinct diseases, ALS, and Parkinsonism-Dementia (PD); then one disease, ALS/PDC, specific to Guam and two other areas; and finally as a combination of three distinct diseases, ALS, Parkinsonism, and AD. The identity of the ALS/PDC found in Guam is a fundamental issue because if it is a separate disease, the role of BMAA in Guam and the Kii Peninsula, even if shown to be a causal factor in ALS/PDC, may be of limited concern worldwide.

Koerner (1952) considered the ALS cases examined in Guam as being typical sporadic ALS, although the existence of a familial incidence of the disease was noted. Kurland and Mulder (1954a, 1954b) studied the familial nature of the ALS and found that although most of the 46 patients observed had a typical form of ALS, some showed symptoms of Parkinsonism. Elizan et al. (1966) evaluated the neuropathology in 176 patients in Guam with ALS or PDC and noted that the average age at onset of ALS (45 years old) was earlier than PDC (54 years old). The clinical features of the Guam "Parkinsonism" differed from the disease seen elsewhere in a variety of parameters: Initial bradykinesia was not associated with rigidity at the onset; rigidity was relatively minor; there was minimal tremor; patients had a high incidence of cognitive dysfunction; and the disease rapidly progressed. Six brains of a subpopulation of individuals diagnosed with Parkinsonism were examined, and the characteristic ubiquinated Lewy Bodies were not reported, the central nervous system lesions being indistinguishable from those seen in PDC. In a group of 32 cases of ALS, 5% of cases developed PDC, whereas in a group of 72 PDC cases, 38% developed ALS an average of 1.7 years after onset of PDC. Over time, the transition of a PDC symptomology to that of ALS occurred in all patients. Evidence indicated that the symptoms of neurological disease in Guam formed a "continuum." A total of 153 ALS or PDC cases were studied by Hirano, Malamud, and Kurland (1961a, 1961b) and Malamud, Hirano, and Kurland (1961). Pathological analyses of the brain were conducted on subsets of 64 who exhibited progressive cognitive dysfunction and identified as primarily PDC; and 57 considered to be ALS cases with significant loss of motor function. The common features noted in the PDC brains included atrophy and loss of neurons in cerebrum and globus pallidus, and loss of pigmentation and neurons in substantia nigra. The brains of subjects in Guam diagnosed with ALS exhibited degeneration of the anterior horn cells in the spinal cord, demyelination of anterior spinal roots and pyramidal tracts, and atrophy of the affected muscles in cases exhibiting upper neuron dysfunction. NFTs were present in cerebral tissue of both ALS and PDC groups, but to a greater extent in PDC brains. These findings were confirmed in studies

by Hirano and Zimmerman (1962), Anderson et al. (1979), Shankar et al. (1989) and Matsumoto, Hirano, and Goto (1990). Anderson et al. (1979) showed the frequency and distribution of NFTs in Chamorros who did not exhibit clinical signs of neurodegenerative disease and found that it was common and widespread, occurring to a significant extent regardless of familial history of ALS/ PDC. NFTs also occurred in younger individuals than in populations in Japan and England.

NFTs are considered the key histological marker in ALS/PDC and are composed of tau proteins that function to stabilize microtubules in axons, microtubules in turn constituting a critical component of cellular structure. In ALS/PDC, the normally single-stranded tau proteins become hyperphosphorylated; assume paired, helical structures; lose contact with the microtubules; and form NFTs. The presence of the NFTs disrupts the function of the microtubules and dysfunction and/or cell death results. Ubiquinated TAR DNA-binding protein 43 (TDP43) may also play a role in ALS/PDC NFT formation as found in brains of individuals diagnosed with the disease (Geser et al. 2008). Hirano et al. (1966) investigated the neuropathology of ALS and PDC in 35 ALS, 47 PD, and 29 subjects who did not display symptomology associated with neurodegenerative diseases. Both PD and ALS cases demonstrated histopathological changes in substantia nigra and cerebral cortex. PD patients contained more NFTs in cerebral cortex and subcortical nuclei than ALS cases where there was a greater incidence of NFTs in the brain stem. There was evidence of NFTs in the spinal cord in more than half the PD cases although no apparent evidence of cord involvement was noted during life. ALS patients seldom developed full dementia, but NFTs were detected in the subcortical region in all brains examined. The distribution of NFTs, therefore, appeared to be related to the symptomology exhibited by individuals. The brains of 5/29 people considered to be controls exhibited evidence of pathologic changes associated with PDC, and NFTs were observed in various areas of the brain including the hippocampus, cerebral cortex, thalamus, and substantia nigra. Differences between ALS/PDC and ALS, Parkinsonism, and AD include the familial occurrence in Guam (Koerner 1952; Kurland and Mulder 1954a, 1954b; Hirano et al. 1966; Morris et al. 2001; Zhang et al. 1996) and the common mixed disease syndrome in Guam (Malamud, Hirano, and Kurland 1961; Murakami 1999), both being extremely rare elsewhere. Additional factors, indicating that ALS/PDC is distinct from sporadic ALS, Parkinsonism, and AD, include the occurrence of cerebral NFTs in younger population in Guam than elsewhere, the absence of betaamyloid plaques that are characteristic of AD; the absence of ubiquinated Lewy bodies characteristic of Parkinsonism (Hirano et al. (1961b); and the observation that the typical “pill rolling” movements in Parkinsonism were rare in ALS/PDC (Brody et al. 1971). Although similar brain areas are affected in AD, Parkinsonism, ALS, and ALS/ PDC, the fundamental differences in the pattern of occurrence, age at onset of the diseases, and absence of key cellular characteristics of AD and Parkinsonism indicate that ALS/PDC is a separate disease entity.

Additional studies addressed ALS/PDC as a separate disease. Hof et al. (1991) compared the distribution of NFTs in cerebral tissues of five Guam PDC and nine AD cases. In both PDC and AD cases, NFTs were present in the neocortex but the distribution of the protein damage was significantly different. In ALS/PDC, NFTs were more numerous in the supragranular layers II-III than the deeper infragranular layers V-VI, whereas the opposite was noted in

non-Guam AD cases. Hof et al. (1994) and Umahara et al. (1994) examined NFT distribution in brains of ALS/PDC patients and reported that regardless of the primary symptoms, there were moderate to high numbers of NFTs in the hippocampus. A major difference between the ALS and PDC cases was higher NFT numbers in the cortex of subjects primarily exhibiting PDC symptoms as compared to those showing ALS where the NTF density was higher in the spinal cord. Hof et al. (1994) suggested that these differences might reflect the extent of damage in different neural circuits affected in patients with PDC compared to those exhibiting symptoms of ALS. A similar type of CNS distribution of tau protein was found in cases of ALS/PDC on the Kii Peninsula providing additional evidence that ALS/PDC occurs in both areas (Kuzuhara et al. 2001; Mimuro, Kokubo, and Kuzuhara 2007). Studies that examined the relationship of NFT density and degree of dementia demonstrated a positive relationship to neuronal dysfunction (Brion 1998). During the course of an investigation on the ultrastructural appearance of NFTs in ALS/PDC patients, Kato et al. (1992) found that NFTs in the spinal cord reacted with antibodies for tau protein, ubiquitin, and paired helical fragments. Buee-Scherrer et al. (1995) characterized the type of tau protein in both AD and PDC cases in Guam and showed that hyperphosphorylated tau protein triplets were similar. Ikemoto, Hirano, and Akiguchi (2000) reviewed cases of ALS that also exhibited cognitive dysfunction and/or dementia and noted similar to typical ALS, there was the presence of ubiquitin, a protein that forms intra-cytoplasmic inclusions mediating cellular dysfunction and/or death. In contrast, CNS damage in ALS/PDC was associated with the hyperphosphorylated tau NFTs. This tauopathy is a hallmark of ALS/PDC and is absent in sporadic ALS. Ikemoto, Hirano, and Akiguchi (2000) concluded that the ALS/PDC is a tau protein-related disease, whereas the sporadic ALS with or without associated dementia is associated with ubiquinated neuronal inclusions indicating that these conditions represent two distinct diseases with similar areas of CNS cellular damage and site-associated clinical symptoms. Kokubo and Kuzuhara (2003) compared sporadic ALS patients with Kii Peninsula ALS/PDC patients and reported significant differences between ALS and ALS/PDC including the presence of increased brain atrophy and both dementia and Parkinsonism in ALS/PDC patients as compared to ALS alone. The clinical signs of ALS and PDC were similar as was the significantly reduced cerebral blood flow. Evidence indicated that ALS/PDC is a “single tauopathy of frontotemporal degeneration” (Table 1).

The characteristics of neurodegenerative disease in Guam appear to be changing over time. Oyanagi et al. (1994) analyzed the brains of 27 asymptomatic and 43 cases of Guam ALS, ALS/PDC, or PDC who died in 1979–1982. The numbers of NFTs in the CNS differed significantly in the varying disease states, high in the PDC and ALS/PDC and low or not present in both ALS cases and tissues from the asymptomatic individuals. Ubiquinated inclusion bodies in the anterior horn cells were detected in Guam ALS and ALS/PDC. Oyanagi et al. (1994) concluded that sporadic ALS occurred in Guam and that NFTs were a background feature in the Guam population. Morris et al. (2001) examined brain tissues of 16 ALS cases diagnosed after 1950. Only tau-positive, ubiquitin-negative, spinal cords were identified in 1/10 cases and two cases with severe hippocampal neurofibrillary tangles also displayed senile plaques, indicating sporadic ALS histopathology rather than a tau-related anterior horn disease. Both the mean onset and survival times were comparable to sporadic ALS. Morris et al. (2001) noted a strong familial relationship for ALS similar to that

reported by McGeer et al. (1997) for PDC cases. In contrast to differences reported in the studies discussed immediately above, Rodgers-Johnson et al. (1986) examined a series of 279 Guam patients with ALS and 293 with PDC who exhibited the onset of symptoms occurring between 1950 and 1979. During 1970–1979, there was an increase in the age where the diseases were diagnosed, but no marked changes in clinical or histological features during the entire period of the study. Data of Morris et al. (2001) and Rodgers-Johnson et al. (1986) were obtained from more recently deceased populations compared to those reported in previous investigations. The presence of sporadic ALS cases in Guam may reflect both diseases that are exhibiting patterns increasingly similar to those found elsewhere and the reasonable possibility that some cases of ALS/PDC may have always been a combination of that disease and sporadic ALS.

BMAA-associated retinopathy and abnormal eye movements

Several studies described a retinal pigment epitheliopathy (RPE) that appears to be associated with ALS/PDC both in Guam and on the Kii Peninsula. The condition manifests itself as linear tracks of retinal depigmentation with intermittent pigment clumping and is similar to ophthalmomyiasis interna posterior, a condition produced by larva of parasitic flies in the Family *Oestridae.* No traces of any parasite were found in a patient with RPE in Guam (Steahly and Peterson 1982). Cox et al. (1989) noted that the incidence in clinically normal Guamanians was 6/37 (16%) compared to an incidence of 26/49 (53%) in patients with ALS/ PDC. Hanlon and Steele (1993) screened 741 individuals during 1990–1992. This study population included a number of ethnic groups with the Chamorros as the majority. Retinopathy was identified in 85 of 531 Chamorros without neurodegenerative symptoms (16%) compared to 38 of 72 (53%) who were diagnosed with some type of ALS/ PDC. Linear tracts of the retina exhibited reduced pigmentation, but no evidence of parasites was detected. Campbell et al. (1993) undertook a histopathological examination of eyes from 7 of the 26 neurologic patients with RPE reported in the Cox et al. (1989) study. The pathology consistently showed tracks with hypo-pigmentation, lack of inflammation, and no signs of insect larvae. The etiology of these tracks remains unknown at this time, but the onset of the retinopathy appears to have ended after WWII. Additional indication of an ALS/PDC relationship to the retinopathy is provided by Kokubo, Ito, and Kuzuhara (2003, 2006) that found RPE in 5/17 ALS/PDC patients on the Kii Peninsula compared to 1/115 neurologically normal people. As is the case with Guam ALS/PDC, no apparent evidence of parasite infection was observed in any of the patients exhibiting the retinopathy. Karlsson et al. (2008) noted the affinity of BMAA to melanin and suggested that this reaction might be a link between the compound and retinopathy. The affinity for ocular melanin binding of diverse compounds is well known (Leblanc et al. 1998) and resulted in the use of hair in forensic studies (Appenzeller and Tsatsakis 2012; Nakao et al. 2002; Pragst and Balikova 2006). The significant correlation of retinopathy with ALS/PDC seen in both Guam and the Kii Peninsula is indications that it may be a component of the ALS/PDC disease, providing another finding of ALS/PDC being a separate disease.

Another ocular effect associated with ALS/PDC is a disturbance of supranuclear ocular and eyelid motility resembling supranuclear palsy (Steele, Richardson, and Olszewski 1964). These were noted in Guam patients with ALS/PDC and include abnormal saccades and

vestibular-ocular reflex, and alterations in eyelid movement and reflexes (Lepore et al. 1988; Steele et al. 2015; Troost and Daroff 1977). One or more of these effects were seen in all of the patients examined. Lepore et al. (1988) considered the eye and eyelid dysfunction as evidence of ALS/PDC being a disease with widespread degeneration in the CNS and symptomology reflecting different cell populations affected.

Flying fox hypothesis

Central to the BMAA hypothesis is the possibility of sufficient human exposure to BMAA to induce neurodegenerative disease. Cox and Sacks (2002) and Monson, Banack, and Cox (2003) have indicated that a primary source of BMAA exposure in Guam was consumption of flying foxes *(Pteropus mariannus).* This is primarily based on two factors: bioaccumulation of BMAA resulting in high levels in these animals (Banack and Cox 2003b) and a postulated biologically significant association of decreased flying fox populations with the observed fall in ALS/PDC incidence (Cox and Sacks 2002).

Data on BMAA bioaccumulation in flying foxes are based on three skin samples obtained from dried museum specimens collected approximately 50 years prior to the analyses (Banack and Cox 2003b). The concentration of BMAA in skin samples ranged from 7502 to 1287 μg/g (mean 3556 ± 3430 μg/g). The method used to quantify the BMAA was HPLC-FD, an analytical strategy that is prone to overestimation of BMAA levels (Faassen, Gillissen, and Lurling 2012). Banack and Cox (2003b) use the data from the specimen with the highest BMAA concentration to calculate possible levels in intact fresh weight specimens weighing 500 g. No further studies on flying fox tissues were reported at the time, and BMAA bioaccumulation in this species was based on these limited data from dehydrated specimens. The weight of dried tissues cannot, however, be utilized for extrapolations to fresh animals since the samples of dried skin employed weighed significantly less than fresh weight of equal areas of normal skin containing fluids. BMAA levels in these preserved skin samples were not correlated to levels of BMAA in hydrated skin in living animals. Banack, Murch, and Cox (2006) reported on a similar number of dried skin samples from an unspecified source using HPLC-FD for quantification, and BMAA levels were considerably lower than those reported in 2003 (479 μg/g vs 3556 μg/g). This difference necessitates additional investigation since the magnitude of flying fox BMAA levels played a major role in the contention that consumption of these animals was a critical factor in its role as a cause of ALS/PDC. Data on the influence of cooking process on levels of BMAA are difficult to draw conclusions from, since they are based on a single example, which is reflected in some of the tissue data before and after cooking.

Throughout their ranges, flying foxes are herbivores subsisting on a varied diet of fruits, leaves, and plant flowers. Wiles (1987a) referred to 30 species of plants that compose the diet of flying foxes in Guam. Banack (1998) noted that the diet of related species on Samoa included 69 plant species. This raises the critical question of dietary importance of cycad seeds as opposed to flowering trees and other forms of vegetation. If the flying fox-BMAA connection is based on cycad fruit in their diets, the levels of consumption by *P. mariannus* need to be considered.

Reduced flying fox populations have been linked to the observed decrease in the occurrence of ALS/PDC (Cox and Sacks 2002). These presumed population numbers are derived by using estimated forest cover as a proxy for population density. This extrapolation assumes that the historical distribution of cycads and other vegetation remained constant from 1900, and there does not appear to be data supporting this contention. Wiles (1987b) found that flying fox populations were not common in 1931 due to the introduction of firearms, and this would imply that the species was already reduced at the time of WWII. Further, evidence for the assumption that cycads are evenly distributed in Guam is lacking. Stone (1970) noted that cycads are primarily found on limestone soil in forests dominated by *Artocarpus mariannus* and *Ficus prolixa* in the northern part of Guam. Wiles (1987b) reported that *C. circinalis* were present in southern Guam ravine forest habitat.

Emphasis has been placed on human consumption as the key factor in the decreased numbers of flying foxes in Guam and over-hunting was certainly a major factor during the first half of the twentieth Century. However, the extirpation of *P. mariannus* has been due, to a significant extent, to the accidental introduction of the brown tree snake *(Boiga irregularis)* at some point during the 1950's. This species of snake initiated the extinctions of many other forms of wildlife in Guam including at least 12 species of birds and 3–5 species of reptiles (Fritts and Rodda 1998). It is an arboreal predator known to prey on young bats left in trees, while their mothers are foraging. Observations of bat colonies indicate that juvenile animals were unable to survive beyond 1–2 months of age because of this snake (Wiles 1987a). With regard to human consumption, Lemke (1992) observed that consumption of flying foxes occurred at social events and religious holidays, not as a dietary staple. Generally, one bat was ingested in a meal for two people, but given their scarcity, a single animal may be employed in a meal for several individuals.

Borenstein et al. (2007) studied flying fox and cycad consumption as possible causal factors for ALS/PDC. A positive association of the disease with the consumption of flying foxes as a child, young adult, or adult was not found. Borenstein et al. (2007) also listed the number of times/year that individuals estimated flying foxes were ingested, and in keeping with the idea of its use in special occasions, the average number of times/ year flying foxes were consumed by subjects exhibiting dementia symptoms were 1.2 ± 7.3 (as an adult), 7.5 ± 14.6 (as a young adult), and 10.9 ± 18.1 (as a child) as compared to 1 ± 6.4, 1.9 ± 8.1, and 8.2 ± 15.3, respectively, for those without dementia. Evidence indicated significantly elevated odds ratios for ALS/PDC in young adults that had contact with cycads either in agriculture or as flour in the diet.

Dosing paradigms and data presentation in animal studies, and environmental exposures

Given the proposed impact of BMAA on human health, both dose levels and routes of administration need to be considered. Studies using routes of exposure that are not relevant to environmental exposures may be reliable strategies to answer mechanistic or relative tissue deposition questions, but extremely difficult or impossible to reach firm conclusions on applicability of such data to the oral route that is the primary means of BMAA environmental exposure. Studies by Karlsson and colleagues (Hanrieder et al. 2014; Karlsson, Roman, and Brittebo 2009b, 2011, 2012, 2013, 2014a, 2014b, 2015) present

special challenges since the investigators utilized both sc exposures and extremely high-dose levels, generally 600 mg/ kg BMAA•HCl (460 mg/kg of BMAA) administered in a large volume of buffer (20 μl/g). The effects noted with this treatment regimen need to be replicated, if possible, with doses using similar chemical dose levels given by the oral route. Until this is done, these investigations remain intriguing but of little use in determining the potential health threat of environmental levels of BMAA.

The presentation of data by Cox et al. (2016) consists of a bar graph and two tables in the supplementary materials that summarize results on levels of BMAA in the regions of the brain. The bar graph and Table 2 present the median density of NFT and tau inclusions in 14 regions of the brain. Table 1 shows median BMAA concentrations in plasma, brain tissue, and cerebrospinal fluid (CSF). Neither the graph nor the tables include any information on the variability of values although the use of the Kruskal-Wallis and Jonckheere-Terpstra trend tests would indicate that results did not exhibit normal distributions. The continued use of tissue level values without any information on variability does not allow the reader to take inter-individual variability into account, and this may be a critical factor. The Kruskal-Wallis test may indicate some difference (s) among the treatment groups, but there is no indication of which groups are significantly different. Jonckheere's test is employed to indicate that NFT density increased with BMAA dose; however, there is no indication if, or how, the Serine + BMAA group was included in this analysis. There are many statements in this study that are difficult to understand given the lack of adequate data presentation. The statement that a median NFT density count of 124 in the Serine + BMAA group is significantly less than a count of 136 in the BMAA group, is not possible to evaluate with data as presented. The finding that BMAA significantly elevated the likelihood for a vervet monkey to develop β-amyloid deposits is difficult to understand given the data presented. Both the abstract and text state that a reduction in NFTs with co-administration of serine occurred in the brain but these statements cannot be assessed with data in Tables 1 and 2. In the supplementary information, Table 1, BMAA-protein concentrations in the brain and plasma were higher in the serine + BMAA group than the high dose BMAA. In Table 2, the density of NFTs was higher in 2/14 areas of the brain in high-dose BMAA + serine groups as compared to high dose BMAA tissues. The authors' (Cox et al. 2016) conclusion that Koch's postulates were met in establishing that chronic dietary exposure is a cause of neurodegenerative illness is questionable on several grounds including the application of the postulates to a toxin as opposed to a microbe, and the lack of the necessary supporting data. Additional questions that the data raise, concern the presence of NFTs in control animals, and differences in behavior of these animals and BMAA-exposed macaques (Spencer, Ohta, and Palmer 1987a). Lemere et al. (2004) examined the effects of a vaccine on β-amyloid deposits in vervet monkeys and detected no NFTs in animals that were 20 + years old, which is inconsistent with findings in Cox et al. (2016) that found NFTs in the control animals. The behavior of the vervet monkeys remained normal, and animals did not exhibit the Parkinsonism behavioral and muscular symptomology observed in macaques. The vervet monkeys received 210 mg/kg BMAA for 140 days, while the macaques showed overt toxicity approximately 30 days after being exposed to 200–250 mg/kg.

A comparison of levels in the environment with potential human exposures is an essential component of any discussion of potential adverse effects. Data concerning levels of BMAA

in air, water, cyanobacteria, plants, and various foods need to be examined for comparisons of this nature to be made with confidence. Methods that are prone to over- or under-estimations of BMAA, and inconsistencies in collected data within and across studies are frequent and have been discussed by Cohen (2012); Faassen et al. (2012) (who also tested different methods on the same samples); Jiang et al. (2014b); Faassen (2014) and Lage et al. (2016). The use of fluorescence detection as a primary means of identification has been questioned (Cohen 2012), and many of the earlier studies have used this approach. There is not complete agreement with techniques involving derivatization with recent observations on some methods' accuracy because of recovery concerns (Faassen et al. 2016; Lage et al. 2015). The variability of environmental BMAA levels across studies makes it extremely difficult to formulate general environmental exposure scenarios. This difficulty in reaching a consensus on potential exposures may be illustrated by the following comparisons: There are 10 studies examined levels of BMAA in fish (Scott et al. 2009; Brand et al. 2010; Jonasson et al. 2010; Spacil et al. 2010; Mondo et al. 2012; Al-Sammak et al. 2014; Jiao et al. 2014; Jiang et al. 2014a; Banack et al. 2015; Lage et al. 2015) (Table 2). These investigations all provide BMAA data on a μg/g basis with the exception of Spacil et al. (2010) which are omitted from the calculations immediately below. An average BMAA level in the 560 analyzed fish samples from the 9 studies where levels are given as μg/g may be used to determine the average BMAA content in a dinner of 227 g (0.5 lb.) of fish. This calculation results in a total consumed 16.6 mg, assuming a value of 0 for "non-detected" BMAA. The exposure for a 68 kg (150 lb) individual would therefore be 0.24 mg/kg. Contrasting this level with the intake of 210 mg/kg/day of BMAA in Cox et al. (2016) primate study indicates that the monkeys were exposed to 875-fold more BMAA. Two of the nine investigations, however, reported much higher BMAA levels (Brand et al. (2010) with average BMAA content of 653.2 μg/g in fish species, and Mondo et al. (2012) with 440.3 μg/g). If these two studies, that both used fluorescence detection for BMAA quantification, are removed and the remaining 7 with 488 samples (87% of the total analyzed samples) are utilized, one arrives at an average BMAA intake of 0.001 mg/kg. Using this value, the 210 mg/kg BMAA daily dose to vervets is 210,000-fold greater than human daily intake from fish. These calculated factors are presented to illustrate the enormous inter-study differences in BMAA levels from fish that may lead to different environmental exposure scenarios. A comprehensive analysis of analytical shortcomings involved in studies that have analyzed BMAA levels in a variety of tissues and environments may be found in Faassen (2014). The analytical methodologies for determining BMAA levels in fish are listed in Table 2.

The important issue of optimal strategies for obtaining accurate and replicable BMAA levels was addressed in an inter-person lab comparison of different analytical approaches (Faassen et al. 2016). A study of this nature is a comprehensive approach to reaching some agreement on preferable analytical methods for BMAA. Until this is accomplished and the same samples are analyzed in different labs, the situation with regard to an assessment of environmental levels of BMAA will continue to be chaotic, and it will not be possible to reach any valid estimation of risk.

Another issue is the paucity of definitive studies indicating an association of exposures of BMAA in diets and disease outside of Guam. There has been a series of exploratory investigations that have postulated an association between non-Guam BMAA exposures and

the occurrences of neurodegenerative disease. Caller et al. (2009) reported a possible association between an ALS cluster and proximity to a lake, although a correlation between geographical disparities in disease occurrence and any obvious environmental factors in a subsequent paper (Caller et al. 2012) was not demonstrated. Results used by Torbick et al. (2014) to link water quality to ALS incidence relies upon chlorophyll-a levels (a surrogate for cyanobacteria) and total nitrogen to estimate water quality. Chlorophyll-a is used by both cyanobacteria and eukaryotic algae and therefore may overestimate the presence of cyanobacteria. The use of satellite data to evaluate total nitrogen is problematic since there is no validated optical signature for nutrients. Banack et al. (2015) found levels of BMAA in tissues of three fish collected from this lake but noted that “Although cause and effect have not been demonstrated, our observations and measurement strengthen this association.” Cox et al. (2009) proposed that a reported increase in ALS among Gulf War veterans may have been due to aerosolized BMAA from desert dwelling cyanobacteria, but did not provide adequate evidence of BMAA in the environment or any evidence of the chemical in military personnel (Faassen 2014, Appendix 4 for detailed information). This hypothesis has been followed by reports that identified two isomers of BMAA (DAB and AEG), but not BMAA itself in the desert soil of Qatar (Richer et al. 2015) and other reports that identified BMAA at different soil depths at levels ranging from 0.0004 to 0.0014 μg/g, along with trace amounts of AEG and levels of DAB as high as 0.187 μg/g. (Chatziefthimiou et al. 2016; Metcalf et al. 2015). Possible associations between BMAA and neurodegenerative disease have also been made for the consumption of blue crabs on the basis of a small number of cases (Field et al. 2013); proximity to cooling towers (Stommel, Field, and Caller 2013); and ingestion of lobsters in the diet of a single ALS patient (Banack et al. 2014). These investigators concluded that these findings support a relationship of BMAA exposure and neurodegenerative disease outside of Guam, but these observations are case histories at best, and lack sufficient power to support such associations. It should not be surprising to find BMAA in the food and environments of populations and areas with ALS since it is also postulated (Cox et al. 2005) that environmental BMAA is ubiquitous. A definitive association of BMAA exposure levels and disease is necessary before conclusions may be reached concerning BMAA and human neurodegenerative diseases.

The presence of BMAA in AD and ALS brains

The analysis of BMAA in tissues of various types has been a difficult analytical problem and may be reflected in the general lack of replication noted in the experiments done to date. Both Murch, Cox, and Banack (2004a, 2004b) and Pablo et al. (2009), using FD for quantitation, demonstrated the presence of BMAA in the brains of ALS and AD cases. Brain tissues of Chamorros, Canadians, and Floridians with ALS, AD, Huntington’s disease, or non-neurological diseases were analyzed for the presence of BMAA. In 39/40 ALS or AD patients (98% incidence), BMAA was identified as compared to only 4/36 (11%) without either disease. However, there are five studies that did not find BMAA at similar levels and/or incidence. Perry et al. (1990) were unable to identify BMAA in the brains of five Chamorro patients, two who died with ALS and three with PDC. The brains of eight former PDC patients from Guam, five AD patients from the northwestern United States, and seven controls without diagnosed neurodegenerative disease were tested for the presence of BMAA in their brains by Montine et al. (2005) and Snyder et al. (2009, 2010), and BMAA

was not detected in any of the tissues. In the course of developing and validating a method for analysis of underivatized BMAA, Combes et al. (2014) failed to detect BMAA in the brains of two people from Europe who had ALS. Berntzon et al. (2015) examined BMAA in CSF of 12 subjects from Sweden with ALS and 13 without the disease. BMAA was identified in CSF of 1/12 ALS and 2/13 without. A wide variety of analytical strategies were used in all of these studies (summarized in Table 3) and accuracy of data generated almost certainly varies across investigations.

A general issue that needs to be addressed concerning studies that showed an association of CNS levels of BMAA with ALS/PDC, AD, and/or ALS concerns the virtually complete association of BMAA in affected people (98%) as contrasted to a lower correlation in control cases (11%) in Murch, Cox, and Banack (2004a, 2004b) and Pablo et al. (2009) studies. If BMAA is a globally occurring compound that is associated with cyanobacteria and other organisms, one might assume that it would be present to some extent in all tissue samples as the case for other chemicals or elements with global distribution, such as polychlorinated biphenyls (Safe 1993) and perfluorooctanoic acid, perfluorooctanesulfonic acid (Lau 2012). In these universally distributed chemicals, the inter-individual and inter-population levels vary depending on degree of exposure, but trace levels are generally found in all tissues. BMAA appears to be rarely found in normal tissues although BMAA sources are postulated to be universal. These data allow for three possible interpretations for this pattern:

(1) BMAA is present in control CNS tissues, but levels are below detectable limits.

(2) BMAA is a causal factor in development of neurodegenerative disease.

(3) The neurodegenerative diseases precede BMAA, possibly because the mechanisms responsible for the diseases either generate or prevent the removal of BMAA, as would be the case in normal brain tissues. All of these interpretations might explain this discrepancy, and should be considered.

The presence of BMAA in cyanobacterial species

The presence of BMAA in cyanobacteria was investigated by numerous scientists and results markedly differ between studies with many finding strong evidence of an association, while a similar number of observations concluded that this was not the case. BMAA was detected in cyanobacterial species and/or strains and in planktonic samples by Cox et al. (2005); Banack et al. (2007); Esterhuizen and Downing (2008); Johnson et al. (2008); Metcalf et al. (2008); Faassen et al. (2008); Jonasson et al. (2010); Spacil et al. (2010); Cervantes Cianca et al. (2012); Jiang et al. (2013); Jiao et al. (2014); Scott et al. (2014); Baptista et al. (2015); and Lage et al. (2016). These 14 studies detected BMAA in a total of 130/144 tested. In contrast to these 14 studies, there are eight that did not identify BMAA in cyanobacteria; Kubo et al. (2008); Rosen and Hellenas (2008); Papageorgiou et al. (2009); Kruger et al. (2010); Faassen et al. (2012); Li et al. (2012); Fan et al. (2014); and Reveillon et al. (2014). These investigators detected BMAA in 0/121 samples examined. Many investigations also reported the presence of DAB in the samples and, interestingly, although Jiao et al. (2014) found levels of BMAA in a *M. aeruginosa* bloom in Lake Taihu, China, BMAA was not detected in this, or 17 additional species from 10 genera present in the same lake, although

levels of DAB in the ng/g wet weight range occurred in 13 strains (Fan et al. 2014). Based upon these data, it would appear that the degree of BMAA in cyanobacteria is, at this time, unresolved. These data are summarized in Table 4.

An interesting area that has not been extensively examined is the possibility that BMAA may be produced *in situ.* Both diatoms and dinoflagellates are thought to produce BMAA (Jiang et al. 2014b; Lage et al. 2014; Reveillon et al. 2015), and these species have no direct association with cyanobacteria. Marler, Snyder, and Shaw (2010) demonstrated production of BMAA in *Cycas micronesica* grown in the absence of symbiotic cyanobacteria. Abraham and Newton (1960) showed that DAB was present in the cell walls of *Bacillus polymyxa* and Perkins and Cummins (1964) reported that DAB was found in the cell walls of *Corynebacteria* indicating that this isomer of BMAA may be present more generally in bacteria. These findings raise the possibility that DAB, and possibly BMAA, is produced in many diverse organisms including intestinal flora of humans.

BMAA misincorporation into proteins

The misincorporation of BMAA in proteins, the resultant misfolding and consequent production of protein tangles, has been postulated as the mechanism involved in the induction of BMAA-induced neurodegenerative diseases (Cox et al. 2016) but there is little evidence for this mechanism. Xie, Basile, and Mash (2013) assumed that the long residence time in brain tissue of the acid-insoluble “protein-bound form” of BMAA was evidence of protein incorporation, but no direct results were provided. Karlsson et al. (2014b) used ultra-high pressure LC-MS/MS and noted protein association of BMAA in the liver and brain following administration in rats. Data demonstrated that BMAA may become attached to proteins in liver and areas of brain not fully protected by the BBB following exposure in neonatal rats. Karlsson et al. (2014b) also demonstrated that protein-associated BMAA cleared over time, and none of the liver or brain samples from adult rats displayed any detectable free or protein-associated BMAA. Similar to Xie, Basile, and Mash (2013), the possibility of protein incorporation by BMAA was raised but no data that would specifically support this interpretation were given. The title of a paper by Dunlop et al. (2013) states that BMAA “is misincorporated into human proteins in place of L-serine causing protein misfolding and aggregation”, but “incorporation into human proteins” or “misfolding” are not apparent from the data shown. The degree of association of BMAA with proteins was dependent on protein synthesis and was reduced in the presence of cycloheximide as expected. The conclusion that levels of BMAA associated with proteins are dependent on the concentration of serine cannot be evaluated since no data were provided for other comparably tested amino acids. In addition, there is no way to calculate an absolute quantity of BMAA per μg protein because the calibration standard curve, DPM vs an amount of spiked ^{3}H-BMAA per μg protein hydrolysate, was not reported.

Glover, Mash, and Murch (2014) provided data on protein synthesis after co-incubation of BMAA in a cell-free system (PURExpress) where individual essential amino acids were omitted. Although the interaction of BMAA and serine is highlighted, results indicate that BMAA substitution for alanine occurred to a greater extent. BMAA is listed as having been significantly incorporated into proteins when presented as a substitute for 6/9 amino acids

omitted; threonine, phenylalanine, proline, alanine, glutamate, and serine. BMAA is also incorporated to a higher degree in the presence of all amino acids than in any situation where one amino acid is omitted, implying that BMAA is being incorporated as a substitute for more than one amino acid. Similar data on BMAA, “incorporation” is presented using this cell-free system for DNA templates derived from brain tissues of three people who are normal or suffered from ALS or AD. These observations are also difficult to interpret because Glover, Mash, and Murch (2014) stated that the ALS patient had the highest rate of BMAA “incorporation” although data presented in their Figure 3 appear to indicate that the AD patient did. These findings are additionally difficult to interpret since only the DNA from the patients was used, and there is no evidence that AD changes DNA in affected tissues. The results presented in this study may primarily be a reflection of the relaxed fidelity of translation of the PURExpress *in vitro* construct. This possibility is supported by the high-yield of incorporation of other amino acid analogs into proteins using PURExpress (Hong, Kwon, and Jewett 2014; Singh-Blom, Hughes, and Ellington 2014). Moreover, intra-study comparisons of BMAA studies have been confounded by the fact that none of the investigations demonstrated incorporation of BMAA into protein structure by a proteomic analysis on a protein and peptide level based on a LC-MS/MS data set and protein sequence database-matching software. Rosen et al. (2016) examined the extraction of BMAA from mussel tissue under different times and concentrations of TCA and HCl. The formation and release of BMAA were not due to breakage of peptide bonds, a further indication of lack of protein incorporation. Of possible interest in the serine-neurodegenerative disease, relationship is the study of Madeira et al. (2015) that found higher levels of serine in the brains of people who suffered from AD. This association would appear to be cautionary in terms of treatment regimens involving large doses of serine as in the Cox et al. (2016) primate study.

Van Onselen et al. (2015) examined the potential of BMAA to be misincorporated in five bacterial species belonging to different genera. Growth of cultures in the presence of BMAA was compared to presence of L-canavanine, a non-essential amino acid known to be misincorporated into proteins. Protein incorporation was evaluated with an *E. coli.* expression system using a fragment of the recombinant human protein, sCD23. In contrast to canavanine, BMAA did not markedly affect growth, nor did BMAA exposure initiate a stress response as determined by the normal reactive oxygen species (ROS) levels after exposure. BMAA was detected in both free cellular and protein-associated fractions extracted from the cultures, but not in the 25 kDa protein band corresponding to the sCD23 protein fragment purified by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and IMAC (immobilized metal ion affinity chromatography). The removal of BMAA from bacterial proteins was not accomplished by washing with detergent-containing acid hydrolysis and TCA precipitation although misincorporation did not occur, indicating the probability of a strong association with protein surfaces. In contrast, canavinine was still detected in the protein fraction after SDS-PAGE purification indicating incorporation into protein. Van Onselen et al. (2015) concluded that the association of BMAA with protein did not represent incorporation. Similar findings were noted by Okle et al. (2013) who used human SH-SY5Y neuroblastoma cell cultures and demonstrated BMAA association with proteins after TCA protein precipitation, but not after protein denaturing

SDS gel electrophoresis. Spencer et al. (2016) did not find evidence to support the incorporation of BMAA into proteins in the brains of macaques. Cerebral protein lysates of BMAA-treated animals were analyzed after Soxhlet extraction to remove BMAA from denatured proteins; BMAA was derivatized, and detection was performed with LC- MS/MS, and no incorporation was found.

Definitive proof of misincorporation needs to involve LC-MS/MS evidence that it is part of a protein amino acid backbone in peptides derived from partial enzymatic protein hydrolysis. Currently, there have been no such proteomics reports available supported by precise, high-resolution analytical techniques of nano-LC-MS/MS or 2D-gel-MS/MS and protein database *in-silico* datamatching. This type of evidence is necessary because it is known that the specificity of t-RNAs for specific amino acids is strong. The substitution rate of valine for the structurally similar leucine or isoleucine, for example, is approximately 1/3000 residues (Loftfield 1963). In addition to this specificity, an additional proofreading mechanism ensuring increased fidelity in amino acid choice is active at the level of the aminoacyl-tRNA synthetase (Cramer et al. 1991). In a normal physiological environment where a full complement of amino acids are present, one would therefore expect extremely few errors to be made—estimated to be one in 10^4-10^5 reactions. In a general review of amino acid misincorporation into proteins, Rodgers and Shiozawa (2008) concluded that much of the existing evidence for amino acid misincorporation indicates that it is a rare phenomenon, especially in normal systems where essential amino acids are present in higher levels than structurally similar non-essential amino acids.

Alternate hypotheses of causal factors related to ALS/PDC

Since the observation and documentation of ALS/ PDC, a number of hypotheses have been advanced concerning the underlying causal factors of neurodegeneration. While some of these, such as infectious disease, have not remained viable (Gibbs and Gajdusek 1972), others need to be considered, either singly or in combination with other factors. The following is a brief summary of some of the current major alternate hypotheses.

Genetic susceptibility

The relationship of ALS/PDC and genetic influences has been an integral part of the discussions of factors contributing to both Guam and Kii ALS/ PDC (McGeer and Steele 2011). In some of the earlier studies in Guam ALS, Koerner (1952) and Kurland and Mulder (1954a, 1954b) postulated a possible genetic influence in the incidence of the disease based on strong familial distribution. Plato, Cruz, and Kurland (1969) analyzed the genealogy of populations in the village and concluded that data suggested a dominant gene that is completely penetrant in males and approximately 50% penetrant in females. Reed, Torres, and Brody (1975) reported on the familial incidence of ALS in Guam and found that the risk of the disease was higher in siblings of affected subjects than in more distant relatives. It was also noted that the offspring, ≥ 20 years old, of parents who were both affected did not exhibit a disease incidence that could be explained by simple Mendelian genetics. This conclusion was strengthened in a larger study by Bailey-Wilson et al. (1993) that examined data on 2026 Guam individuals, either with or without neurodegenerative disease. Results

demonstrated that the tendency of individuals to display either ALS or PDC was not dependent on type of disease in relatives, indicating that ALS/PDC was a single disease. There was a high % of ALS/PDC patients in which the disease might be attributed to major gene segregation indicating that genetics may play an important role; but as age increases, the % of cases that may be attributed to environmental causes also rises, indicating that the majority of ALS/PDC cases in cohorts born between 1895 and 1913 might be due to environmental factors. The data did not indicate a single explanation and evidence showed that the incidence of ALS/PDC might be due to a combination of genetic susceptibility and environmental exposure. Plato et al. (2002) noted a significantly higher incidence of new ALS/PDC patients among the relatives of individuals who had the disease than the remainder of the population but this finding can be interpreted as evidence of a genetic basis, an environmental factor, or a combination of both. This concept of a critical ALS/PDC causal factor being environmental was reinforced by the neuroepidemiologic studies of Roman (1996) who identified 6 Filipino and three US men who arrived in Guam between the late 1940's and early 1960's and developed MND. Although Roman (1996) did not identify the specific type of MND (sporadic ALS as opposed to ALS/PDC), it was found that after this period, there have been no similar diseases in the large Filipino population that migrated to Guam. Based on data from the Chamorro population, it was postulated that the height of susceptibility to ALS/PDC occurred during adolescence, a similar finding to that of Borenstein et al. (2007). Kokubo and Kuzuhara (2001) also noted a strong familial factor in the occurrence of ALS/PDC on the Kii Peninsula. A family history of the disease was found in over 70% of the ALS and PDC cases examined. Reiff et al. (2011) observed that different mitochondrial DNA haplotypes present in Chamorros were associated with significant differences in the odds ratios for PD in Guam and concluded that ALS/PDC may be a disease with both environmental and genetic causal factors.

Several genes linked to AD, Parkinsonism, and ALS were examined for possible roles in ALS/PDC disease. Chen et al. (1996) found no significant relationship with CYP2D6 and apolipoprotein 4 (APOE4) genes and PDC in Chamorros. In addition, Galasko et al. (2007) reported no marked association between APOE in ALS/PDC and nonsymptomatic cases in Guam. The *tau* gene associated with Parkinsonism in families with frontotemporal dementia also does not appear to be a primary cause of the PDC cases in Guam (Perez-Tur et al. 1999). Sundar et al. (2007) studied the relationship of specific areas of the microtubule associated protein tau (MAPT) gene that is thought to produce tau proteins on the basis of alternate splicing of the protein it generates that was shown to be a risk factor for supranuclear palsy. Sundar et al. (2007) noted multiple singlenucleotide polymorphism (SNP) sites that were associated with elevated risks ranging from 3- to 6-fold greater incidence for both ALS and PDC compared to asymptomatic individuals. Sieh et al. (2009) employed a different approach to identifying a genetic link(s) to ALS/PDC—a whole genome linkage and association analysis of tissues collected from cases of ALS/PDC in the village of Umetac and others in Guam. Three markers on two regions on chromosome 12 exhibited significant associations with ALS/PDC. Two regions on chromosome 17 were identified that showed a significant association with ALS/PDC, one of the regions containing the MAPT gene. Data indicated that ALS/PDC may be associated with three loci and called for additional research to determine whether these loci play roles in AD,

frontotemporal dementia, Parkinsonism, or ALS. Morris et al. (2004) conducted a genomic analysis of 41 people in Guam, 22 ALS/PDC, and 19 asymptomatic and noted that there was no identified gene locus for the PDC. A genetic link in the ALS/PDC on the Kii Peninsula was examined by looking for mutations of SOD1, a-synuclein, tau, or TDP-43 genes, and none were found although a familial association similar to the one that was detected in Guam was present (Kuzuhara 2007; Kuzuhara et al. 2001).

Environmental trace element levels

Numerous research efforts have explored the possibility that differences in the distribution of minerals in the environment resulted in ALS/ PDC due to their abnormal deposition or uptake levels in individuals. The discrete locations that experienced the high incidence of ALS/PDC were surrounded by other villages with a lower occurrence of the disease. Scientists examined local environments associated with these disease foci in an effort to identify factors that might play significant roles in these hotspots. Yase (1970, 1972) initiated mineral and metal surveys measuring manganese (Mn), iron (Fe), Mg, and Ca of ALS/PDC focal areas in soil, rivers, and drinking water sources in Guam and the Kii Peninsula. Results demonstrated that Mn values in Guam were higher than Kii, but still fell within the normal range for locations where there was no increased incidence of ALS/PDC (Williams et al. 2012). Soil samples were varied, although several in Guam exceeded the ATSDR expected range of 40–900 ppm in soil. Soil taken from riverbanks on the East and West Umatac, and Merizo rivers, as well as soil from Inarajan and Yona contained Mn levels from 1000 up to 6200 ppm compared to the Mn levels of 123 and 306 ppm in the soil of control areas. Ca was reported to have lower levels in the focal area rivers and drinking water on Kii Peninsula (0–3.3 mg/L) versus control areas which had 6.16–8.8 mg/L. Garruto et al. (1984b) conducted a survey of heavy metals in focal areas of MND in Guam, and compared to levels in control areas. The results showed a correlation between areas of ALS/PDC and increased iron (Fe) in the water. Areas in New Guinea with an elevated incidence of ALS/PDC were also assessed (Gajdusek and Salazar 1982) and a “remarkable deficiency of Ca and Mg in soil and drinking water” was noted although results were compared to continental US norms and not to local areas with a low incidence of MND. Two general surveys of Guam were published (Carroll and Hathaway 1963; Zolan and Ellis-Neill 1986). In contrast to the Yase studies (1970, 1972), the 1963 survey did not show unusual levels of aluminum (Al), Mg, or Ca. The 1986 survey was concentrated in four southern rivers and analyzed levels of Al, Ca, Mn, and Fe. Mn levels were within the WHO drinking water guidelines (WHO 2011). Aluminum (Al) was variable, although in some areas exceeded the continental US Al values by 27–285%. Spencer (1989) concluded that the Zolan and Ellis-Neill (1986) survey provided evidence that the mineral imbalance hypothesis was not correct. Zhang, Anderson, and Mantel (1990) compared the incidence of PDC in the Guam ALC/PDC case registry to survey results of Garruto et al. (1984b). Their findings did not demonstrate a significant relationship between PDC and the Ca, Mg, Al, or Mn levels in the water or soil.

Levels of minerals and metals have also been determined in CNS tissues of ALS/PDC patients and controls. Four studies on patients from the Kii Peninsula of Japan tested a total of 17 ALS, three Alzheimer’s disease, and 9 controls. Yoshimasu et al. (1976, 1980) found

increased CNS Ca and Mg in 9/10 of the ALS cases and 2/3 of the Alzheimer cases, but not in the controls. Yasui et al. (1991a, 1992) found increased Ca and/or Al and decreased Mg in the CNS of 7 ALS Kii patients. Yase (1972) cites a 1969 Yoshimasu publication which reported testing the Mn content in the CNS of three ALS patients, and three controls. Mn was detected in both controls and patients in multiple CNS areas, but Mn levels in the spinal cord of ALS patients were 50–100-fold higher than controls. Additional studies examining mineral and heavy metals in the CNS tissue of ALS/PDC patients on Guam assessed tissues from 24 ALS, 24 PD, and 28 controls. Yoshimasu et al. (1980, 1982) measured Ca, Mn, Al, and copper (Cu) in CNS tissue and found increased Al in the CNS of the ALS/PDC patients and also elevated Ca in the 1980 study. Perl et al. (1982) and Garruto et al. (1984a, 1985a, 1986) assessed NFTs (a neuropathological finding in the tauopathies of MND) and Al content which was increased in all test subjects with NFTs which included one control in the Perl et al. (1982) study. Garruto et al. (1984a, 1985a, 1986) also measured Ca and found elevated levels in NFTs. Piccardo et al. (1988) used a histochemical stain to identify Al instead of the x-ray neutron activation employed in all other studies. Piccardo et al. (1988) verified increased Al in CNS tissue of 6 ALS/PDC patients versus five controls. Gellein et al. (2003) determined heavy metals, including Mn, in the CNS of eight ALS, four PDC, and five controls and did not observe an increase in Mn.

Three experiments performed antemortem tests on ALS and PDC patients to evaluate for potential Ca metabolism disorders. Yanagihara et al. (1984) examined Ca and vitamin D metabolism in Chamorros to investigate a possible connection between ALS/PDC and a secondary hyperparathyroidism initiated by a mineral and metal imbalance. Yanagihara et al. (1984) tested 16 ALS patients, and 33 PDC subjects, and found elevated parathyroid hormone (PTH) levels in 35% of ALS patients and 15% of patients with PDC. All individuals exhibited decreased bone mass which suggested evidence of previous Ca deficiency. It was postulated that an elevated PTH was only found in a portion of the patients because the imbalance may have occurred too long before testing and the serum Ca balance had been corrected. Ahlskog et al. (1995) examined 12 Guam ALS patients and 12 controls for indications of Ca and Mg deficiency. Testing samples included urine, nails, hair, and blood which revealed no abnormalities with Ca metabolism or heavy metal contamination. Kihira et al. (2013) reported on the rising incidence of ALS on an island, Oshima, which sits directly offshore from the Kozagawa area of the Kii Peninsula and between 2000 and 2009, identified the first three cases of ALS in the township with a population of just over 1200. These occurrences happened after Oshima began using a new water source in 1975, the Kozagawa River which flowed through the focal area of ALS/ PDC, and noted that drinking water from the Kozagawa River displayed significantly lower Ca than control areas.

Several animal studies explored the influence of reduced dietary levels of Ca and Mg, and whether these deficiencies interact with environmental heavy metals. Kumamoto et al. (1975) fed rats for 6 weeks on a diet that contained 0.4% of the normal Ca and 20% of the normal Mg. Kumamoto et al. (1975) found increased Ca in spinal cord, kidney, and muscle tissues and decreased Ca in blood serum, cerebellum, liver and intestines. Nakagawa et al. (1977) measured Ca levels in spinal cords of these rats by electron probe microanalysis and demonstrated a 6-fold rise in Ca in the Ca and Mg deficient rat spinal cords as well as a 6-fold elevation in number of motor neurons affected when compared with controls. Garruto et

al. (1989) fed 6 cynomolgus monkeys variations of a base diet containing one-third the normal amount of Ca. Two of these monkeys also received daily Al and Mn supplemented in their diet, and two received the Al and Mn supplement plus 2 g daily of unwashed cycad seed flour. There were two monkeys on normal monkey chow diet as controls. The monkeys were maintained on these diets for 41–46 months. Results demonstrated similar motor neuron pathology in all monkeys on the Ca deficient diet regardless of supplementation with the Al, Mn, or cycad flour although the animals were clinically normal at the end of the study. Evidence indicated that the Ca deficiency led to an abnormal absorption of toxic metals and neuropathology (Kumamoto et al. 1975; Yase 1979; Yasui et al. 1991b; Yoshimasu et al. 1976). Yasui et al. (1991b, 1997) evaluated the influence of a 90-day Ca-deficient diet in rats, where the treatment groups were either Ca deficient (0.24% of control), Ca and Mg deficient (0.67% of control for Mg), or Ca and Mg deficient and supplemented with Al (19.4-fold higher than controls). Similar to the findings of Garruto et al. (1989), animals appeared clinically normal at the end of the study, although calcium tissue levels were higher in all treatment groups when compared with the controls. The highest uptake of Ca in the CNS tissue was in the spinal cord of rats fed the Ca and Mg deficient diet with Al. Other tissues and organs that retained significantly higher levels of Ca in the Ca and Mg deficient diets were the heart, liver, abdominal aorta, and kidney. In addition, all treatment groups on the Ca deficient diets had significantly less Ca and Mg in the lumbar spine and femur when compared with controls. Garruto et al. (1989) compared their findings and the Ca and Mg measurements in human CNS tissue found by Yasui, Yase, and Ota (1991a,1992) which were similar for Ca, but not Mg. Garruto et al. (1989) further compared decreased bone density of rats with similar observations in Guam ALS patients and osteoporosis and aortic calcification seen in the population of the focus areas in Kii (Fujita et al. 1984). Calcification of spinal ligaments is another condition related to Ca-Mg imbalance and in 1997, 120 cases were reported in the U.S., Europe, and Japan, and of those, 28 were residents from the Kii Peninsula (Yasui, Ota, and Yoshida 1997). Kihira et al. (2002) exposed mice for 11–24 months to low Ca/Mg diets with and without Al, and also normal diet with Al. The groups fed a Ca/Mg deficient diet with Al as well as a control diet with Al showed a significant increase in the number of tau-positive cells in the cortex, hippocampus, and periaqueductal areas, but in contrast to other studies discussed above, mice fed low Ca and Mg diet alone displayed no increase in tau-positive neurons over controls.

Abnormal deposition of metals and minerals was confirmed in the CNS of Guam and Kii Peninsula ALS/PDC patients although data on mineral and metal content in soil and water in areas of high ALS/PDC incidence are variable. Direct comparisons of these mineral and metal accumulations in ALS/PDC to levels in CNS of patients with MND in other geographical locations have not apparently been published. Aluminum is a common finding in the CNS of Alzheimer’s patients and is thought to accumulate in the cytoplasm and nuclei of neurons which developed NFTs. Aluminum’s role in this is not completely understood, but it was proposed that Al either contributes to formation of NFTs or accumulates in the NFTs passively (Walton 2006,2010). While the totality of data may support a connection between mineral/metal imbalance and ALS/PDC, it should be noted that there are several investigations that dispute this. Reed et al. (1987) noted that diet in villages most affected by ALS/PDC in southern Guam, the Kii Peninsula, and Irian Jaya, utilized fish as a major food

source. Consumption of fish, which have high levels of Ca, may possibly compensate for low levels of Ca from other sources such as water and/or foods grown in Ca- deficient soils. Durlach et al. (1997) indicated that low levels of Ca and Mg as factors in ALS/PDC are derived from data on levels in soil and waters, not from direct dietary intake. Spencer, Palmer, and Ludolph (2005) concluded that reductions in the incidence of ALS/PDC in Guam, Kii Peninsula, and Irian Jaya have not been accompanied by significant changes in the waters used for drinking and cooking or the soils.

Other potential toxins found in cycads

There are two additional groups of toxins that have been advanced as causal factors in ALS/PDC in Guam:

Cycasin and methylazoxymethanol

The glucoside cycasin was first isolated from *Cycas revoluta*, and its structure determined by Nishida et al. (1955). Subsequent studies indicated that it was a potent hepatic and renal carcinogen in mammals (Laqueur et al. 1963). Its metabolite, methylazoxymethanol (MAM) was found to be the proximate carcinogen (Laqueur and Matsumoto 1966). Cycasin was also found to be neurotoxic in late-term fetuses (Spatz and Laqueur 1967), neonates (Hirono and Shibuya 1967), and adults (Hirono, Shibuya, and Hayashi 1969). The reported effects include permanent hind limb paralysis and attendant ataxia and cell death in the outer layers of the cerebral cortex. Lee and Rabe (1992) noted long-term effects and found reduced memory and cognitive functions in older rats with MAM-induced microcephaly.

An early discussion on the possibility of Guam ALS being due to cycasin and/or MAM was made by Kurland (1972) who noted that although acute neurotoxicity of MAM was evident, relationship to the ALS remained equivocal since exposure of animals to prepared cycad flour did not display any signs of toxicity. Kisby, Ellison, and Spencer (1992) analyzed cycasin and BMAA levels in prepared flour in Guam and noted that the levels of cycasin were approximately 10-fold greater than the levels of BMAA and were highest in areas with the largest ALS/PDC prevalence. Zhang et al. (1996) performed a correlation analysis between incidence of ALS/PDC in different Guam election districts and environmental levels of trace elements in water and soil, and concentrations of both BMAA and cycasin in flour samples prepared from cycads. The only significant correlation reported was between levels of cycasin in flour and the incidence of neurodegenerative disease. MAM was administered by mid-scapular injection to neonatal mice by Kisby et al. (2005) and histological analysis revealed disorganization of neurons in cerebellum and DNA strand breaks accompanied by a wide variety of gene expression changes in all aspects of CNS function. A subsequent study by Kisby et al. (2011) using adult mice found that MAM exposure produced a robust gene expression response in liver, but relatively few changes in the brain. Evidence indicated that these differences between brain and liver tissues might reflect variations between organs in which cells are rapidly replicating (liver) and those where this is not the case (brain). The human brain does respond to MAM with some alterations in cell signaling pathways that are associated with cancer in the liver, but in an organ with non-cycling cells, the response might be linked to long-term development of neurodegenerative disease. Kisby and Spencer (2011) postulated that CNS tissue exposures

to genotoxins like MAM during early life may result in persistent changes due to DNA damage that may initiate the types of neurodegenerative diseases later in life that are seen in populations suffering from ALS/PDC. This hypothesis suggests further experiments need to be undertaken with adult and neonatal administration of genotoxins such as nitrosamines and streptozoto-cin that are known to induce tau pathology similar to that prevalent in ALS/PDC. A study on the influence of cycasin in primates was conducted by Sieber et al. (1980), who fed primates *(Macaca mulatta, M. fasicularis,* and *Cercopithicus aethiops)* crude cycad meal for 7–10 months at doses of 0.5–1.0 g/day, 3 times/week and cycasin (oral) or MAM (i.p.) at doses of 50–75 mg/kg 5 times per week for periods of 7 months to >7 years. The studies were designed to evaluate the carcinogenic potential of cycasin and MAM but neurological function was not assessed. There was, however, no comment regarding altered behaviors, and this is difficult to reconcile with those of Dastur (1964) and Spencer, Ohta, and Palmer (1987a) because it is unlikely that pronounced behavioral alterations would be missed by animal caretakers or the experimenters.

Sterol glucosides

The second series of compounds that have been postulated to be linked to cycad-associated neurodegenerative diseases are sterol-β-D-glucosides that are characterized by a carbohydrate attached to a tetracyclic carbon unit. Many of these are highly toxic and include ouabain, digitalin, and scillirosidin that induce paralysis after ingestion (Ly, Singh, and Shaw 2007). Khabazian et al. (2002) and Wilson et al. (2002) searched for higher molecular weight, lipophilic toxins in cycad seeds that would remain stable at cooking temperatures and, unlike BMAA and cycasin-MAM, would not be removed by standard cycad seed washing process. Rats were fed washed cycad seed extracts and a neurotoxic fraction identified that contained a variety of sterol glucosides, the largest component being β-sitosterol β-D-glucoside (BSSG). In parallel investigations, adult mice were fed processed cycad flour for 30 days and a series of motor and behavioral tests including rotarod, Morris water maze, and radial arm maze initiated 2 days after beginning exposure. Results showed tremors in limbs, significant changes in leg extension and gait length, impairment of memory, and cognitive dysfunction. The investigators used TUNEL to investigate cell death and found increased cell death in hippocampus, substantia nigra, and spinal cord. DNA breakage was noted in the cerebral cortex, hippocampus, substantia nigra, and spinal cord. Motor deficits worsened after cessation of treatment, and this response was interpreted as either representing a long CNS half-life or a long metabolic cascade leading to the observed damage. Increases in tau protein, a central component in NFTs, were found in hippocampus, substantia nigra, and cerebral cortex. Data indicated that these effects were not generated by cycasin-MAM since no characteristic alterations in caspase labeling or liver morphology were observed. This lack of apparent liver pathology was consistent with non-detectable levels of cycasin in the processed cycad seeds as determined by HPLC/MS. Levels of BMAA were also determined and found to be extremely low (0.003 μg/g). In contrast, the levels of sterol glucosides were 21–155 μg/g. Tabata et al. (2008) exposed 5–7 months old CD-1 male mice to food pellets that contained 1,000 μg of BSSG per day for 15 weeks (approximately 28–35 mg/kg for mice weighing 29–33 g.). The treated animals had a- motor neuron loss in the spinal cord and displayed progressive weakness. Pathology of the spinal cord indicated profound Nissl staining and indications of chromatolysis, similar to the

effects seen in monkeys that displayed symptoms of Parkinsonism (Spencer, Ohta, and Palmer (1987a). Shen et al. (2010) undertook similar studies using rats and found that, unlike mice, processed cycad flour did not induce tau protein similar to that seen in ALS/PDC, but enhanced α-synuclein deposition in the substantia nigra, a common finding in Parkinsonism, and protein aggregates that appeared 5 months after cessation of exposure.

Discussion

There have been numerous studies investigating the neurotoxicity of BMAA in mammals. A body of evidence from *in vivo* studies demonstrates that BMAA is a neurotoxicant when administered by various routes. Pre-weaning exposure to high concentrations of the BMAA appears to produce neurochemical and behavioral changes in mice and rats but the levels of BMAA, and often the routes of administration, are not consistent with possible human exposures. In primates, high doses elicit symptoms that resemble those of Parkinsonism, but these effects that were seen in macaque monkeys were not seen in vervets. The totality of these data is not sufficient to indicate that the BMAA-neurological disease hypothesis is valid. Many important fundamental issues and inconsistencies with the BMAA hypothesis have been raised and are discussed in the sections above. These issues include:

(1) The nature of the relationships of ALS/PDC to sporadic ALS, classic Parkinsonism, and AD.

(2) Validity of the hypothesis that neurodegenerative diseases are mediated by dietary exposures to BMAA.

(3) Conflicting evidence for BMAA levels in vertebrates including humans or in cyanobacteria.

(4) Fundamental problems with evidence used to support the concept of BMAA misincor-poration into proteins as a basic mechanism for neurotoxicity.

The hypothesis that BMAA plays a decisive role as a causal factor for four important, irreversible, neurodegenerative diseases is appealing for its universality and simplicity. As discussed in this review, however, there is a lack of evidence in support of this idea. The relationship of BMAA and ALS/PDC has never been proven, and the neurotoxic effects of BMAA seen in animals have only been noted after large doses that would involve unrealistic human exposures. The flying fox hypothesis attempted to answer this question by postulating human consumption of large bioaccumulated quantities of BMAA found in flying foxes. The causal relationship of BMAA to the three non-ALS/PDC diseases appears to have arisen after studies were interpreted as showing that BMAA was found in human brains of both ALS and AD patients, and in most species of cyanobacteria. The reliance on high exposure levels inducing ALS/PDC appears to have become a belief that any exposure to BMAA is a causal factor in the induction of ALS, Parkinsonism, and AD. The preponderance of evidence, however, indicates that these are separate diseases, and that ALS/PDC is another neurodegenerative disease, possibly associated with cycad exposure through food or poultices. The differences between ALS/PDC and the separate neurodegenerative diseases include many characteristics as listed in Table 1. ALS/PDC can be characterized by the presence of tau-protein neurofibrillary filaments that are widespread

throughout the brain and spinal cord with the expressed symptomology varying with the portions of the brain that are most affected. Equating ALS/PDC with the separate diseases based simply on symptoms is analogous to assuming that all headaches have a single cause. The symptoms of Parkinsonism may be exhibited in individuals with damage to the neurons within the substantia nigra, regardless of whether the damage results from genetic mutations (including MAPT, PARK2, and LRRK2 genes), disease (postencephalitic Parkinsonism, Pick's disease, AIDS, progressive supranuclear palsy (Ludolph et al. 2009), brain iron accumulation (Schneider 2016), neuroinflammation (Surendranathan, Rowe, and O'Brien 2015), or the herbicide, paraquat (Dinis-Oliveira et al. 2006). The assumption that BMAA produces four separate diseases is not supported by scientific evidence.

In support of a BMAA-neurodegenerative disease connection, a series of case history papers with extremely small sample sizes and inadequate or absent controls were published, and despite the caveats, the implication is that these constitute additional "evidence" of a BMAA-neurotoxicity link. These contentions generally do not satisfy Bradford Hill criteria that have been accepted as general guidance for determining causal relationships between an environmental factor and incidence of an adverse health effect (Hill 1965; Lucas and McMichael 2005). The strength of the BMAA effect has not been adequately determined; specificity has not been shown for global populations; temporal factors differ significantly between localities; and reproducibility of the neurotoxicity, incidence and environmental exposures have not been demonstrated.

The flying fox hypothesis assumes that flying foxes contained large amounts of BMAA and were ingested in sufficient quantities to yield toxic levels of BMAA. The rarity of these animals during the period of time when the critical exposure was supposed to have taken place precludes flying foxes as a dietary staple. This is indicated by observers who found that flying foxes were consumed only sporadically at weddings, religious holidays, and other celebratory events. The use of guns that made the harvesting of bats easier was not allowed during the WWII Japanese occupation. The limited nature of their consumption was also shown by data indicating that this occurred an average of less than once/month in the population surveyed (Borenstein et al. 2007). The evidence in support of flying fox consumption as the cause of ALS/PDC is therefore insufficient to support this hypothesis. The original examples of high BMAA levels in flying foxes were based on a small number of preserved, dehydrated animal skin samples (Banack and Cox 2003b) that cannot be used to extrapolate to fresh, hydrated tissues. The magnitude of these BMAA levels has not been replicated, and this is also true of similar levels reported in fish (Brand et al. 2010). It should also be noted that glutamate, which was found to be more toxic than BMAA in cell cultures (Chiu et al. 2012; Staton and Bristow 1997), was found in higher concentrations (molar ratios of 23 and 26) in two fish species analyzed (Brand et al. 2010).

There are data that support assumptions of deposition of BMAA in the brains of ALS/PDC, ALS, and Alzheimer's sufferers as well as the universality of BMAA in the environment, but there are equal numbers of investigations that have failed to find BMAA in brains or cyanobacteria. There are considerable issues with the relative sensitivity and specificity of the analytical strategies that have been employed in all aspects of BMAA research. Rosen and Hellenas (2008), Cohen (2012), Faassen, Gillissen, and Lurling (2012), Faassen (2014),

and Lage et al. (2016) indicated shortcomings with analytical approaches that relied upon as support for BMAA occurring universally.

The neurodegenerative diseases suggested to be associated with BMAA involve abnormal protein aggregations (tangles). The different neurodegenerative diseases are characterized, in large part, by type of proteins and/or positions of these aggregations. ALS/PDC is characterized by widespread tau proteins throughout the CNS, and other diseases are characterized by different types of proteins and the position of these proteins within the CNS. The role of protein misfolding in the formation of these tangles is well accepted. It has been assumed that BMAA can be, and is, misincorporated into normal proteins, that then form tangles that initiate CNS dysfunction. There is, however, no definitive proof that BMAA is misincorporated into proteins in *in vivo* systems and well-designed studies investigating BMAA incorporation concluded that it is not present (Okle et al. 2013; Van Onselen et al. 2015).

Conclusions

Possible relationship(s) of presence of ALS/PDC in Guam, Irian Jaya, and Kii Peninsula, remain unknown. BMAA presence in desert air or isolated food samples as causative factors for ALS has been presented but simply relating this presence to neurodegenerative diseases involves “correlation-causality fallacy.” The cause(s) of ALS/PDC has not been determined, and research to date has been focused on single possible factors. No conclusive evidence that any single agent hypotheses for neurodegenerative diseases has been found, and a more complex causation should be considered. The combination of mineral imbalance, genetic background, stress-induced physiological alterations, any of several other toxins present in cycads, and the stress of WWII occupation in Guam may have all contributed to inception of disease.

The BMAA hypothesis is built on 4 major contentions:

1. BMAA produces ALS/PDC
2. ALS/PDC is similar to ALS, Parkinsonism, and AD, and therefore BMAA produces those diseases.
3. BMAA acts by incorporation into proteins as a substitute for serine.
4. Environmental/dietary exposure levels are sufficient to produce disease(s).

The first contention has not been proven; the second disregards the general conclusion that ALS/PDC is a separate disease, and there is no reason to assume that BMAA is a causal factor in all of these diseases; the third is not supported by conclusive evidence that BMAA is incorporated into proteins in normal biological systems, and the fourth is impossible to evaluate at this time due to lack of validated methods for analysis, lack of replicated studies in multiple labs, and sampling strategies. A hypothesis this important needs considerable evidence of all its facets, and replication by scientists in multiple labs who are not connected with the originators of the hypothesis. Proper controls (including other amino acids) need to be used in all studies. All conflicting data need to be considered and evaluated. If BMAA toxicity requires high dose levels, the possibility of relevant environmental exposures must

be conclusively demonstrated using appropriate analytical techniques. The human health implications of this hypothesis are too important to act on erroneous assumptions and inadequate data, and by ignoring or discounting conflicting findings and alternate hypotheses of ALS/PDC, ALS, Parkinsonism, and AD causal factors.

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Glossary of disease names and acronyms

There are several neurodegenerative diseases that play prominent roles in the BMAA-disease hypothesis. Their definitions have altered over time as the definitions of the key diseases on Guam have changed as a result of new research. The authors have decided that the terminology used by researchers in their published work should be faithfully reproduced in this review but this can lead to some confusion, especially when the symptoms of individual diseases are studied although they are part of others that have broader symptoms. Although definitions of diseases will be given as they occur in the text, the following simple glossary may be useful:

ALS/PDC (Amyotrophic Lateral Sclerosis/ Parkinsonism Dementia Complex)
A disease that occurs on Guam, the Kii Peninsula of Japan, and the Irian Jaya region of New Guinea. ALS/PDC has symptoms associated with ALS, Parkinsonism, and Dementia, and these may occur separately or together to varying degrees in the same individuals. There is a strong familial component to the disease and ocular effects not found in other forms of neurodegenerative diseases.

ALS (sporadic ALS)
A disease that involves the death of nerves that innervate voluntary musculature. Symptoms include muscle weakness, wasting and eventually, paralysis.

PDC or PD
Parkinsonism Dementia Complex. A component of ALS/PDC characterized by Parkinsonism symptomology and dementia, the loss of memory and cognitive function.

Parkinsonism
A disease characterized by muscle tremors and/or rigidity and difficulty in voluntary movements including walking.

Dementia
A condition caused by many diseases that involves loss of memory and cognitive function. Causes of senility include advanced Parkinson's disease, Lewy body dementia, vascular dementia, Huntington's disease, and CreutzfeldtJacob disease.

Alzheimer's disease

The most common disease causing dementia.

References

Abdel-Salam OME 2014 The paths to neurodegeneration in genetic Parkinson's disease. CNS Neurological Disorders Drug Targets 13:1485–512. doi:10.2174/1871527313666140806142955. [PubMed: 25106632]

Abraham EP, and Newton GG. 1960 Chemistry and classification of antibiotics. British Medical Bulletin 16:3–10. doi:10.1093/oxfordjournals.bmb.a069788. [PubMed: 13791527]

Ahlskog JE, Waring SC, Kurland LT, Petersen RC, Moyer TP, Harmsen WS, Maraganore DM, O'Brien PC, Esteban-Santillan C, and Bush V. 1995 Guamanian neurodegenerative disease: Investigation of the calcium metabolism/heavy metal hypothesis. Neurology 45:1340–44. doi: 10.1212/WNL.45.7.1340. [PubMed: 7617195]

Al-Sammak MA, Hoagland KD, Cassada D, and Snow DD. 2014 Co-occurrence of the cyanotoxins BMAA, DABA and anatoxin-a in Nebraska reservoirs, fish, and aquatic plants. Toxins (Basel) 6:488–508. doi:10.3390/toxins6020488. [PubMed: 24476710]

Anderson FH, Richardson EP Jr.Okazaki H, and Brody JA. 1979 Neurofibrillary degeneration on Guam: Frequency in Chamorros and non Chamorros with no known neurological disease. Brain 102:65–77. doi:10.1093/brain/102.1.65. [PubMed: 427533]

Appenzeller BM, and Tsatsakis AM. 2012 Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: State of the art, critical review and future needs. Toxicology Letters 210:119–40. doi: 10.1016/j.toxlet.2011.10.021. [PubMed: 22079616]

Araki S, Iwahashi Y, and Kuroiwa Y. 1967 Epidemiological study of amyotrophic lateral sclerosis and allied disorders in the Kii Peninsula (Japan). Journal of the Neurological Sciences 4:279–87. doi: 10.1016/0022-510X(67)90106-2. [PubMed: 6037097]

Arnold A, Edgren DC, and Palladino VS. 1953 Amyotrophic lateral sclerosis: Fifty cases observed on Guam. The Journal of Nervous and Mental Disease 117:135–39. doi: 10.1097/00005053-195302000-00005. [PubMed: 13061952]

Ashraf GM, Greig NH, Khan TA, Hassan I, Tabrez S, Shakil S, et al. 2014 Protein misfolding and aggregation in Alzheimer's disease and type 2 diabetes mellitus. CNS & Neurological Disorders Drug Targets 13:1280–93. doi: 10.2174/1871527313666140917095514. [PubMed: 25230234]

Bailey-Wilson JE, Plato CC, Elston RC, and Garruto RM. 1993 Potential role of an additive genetic component in the cause of amyotrophic lateral sclerosis and Parkinsonism-Dementia in the western Pacific. American Journal of Medical Genetics 45:68–76. doi:10.1002/(ISSN)1096-8628. [PubMed: 8418664]

Banack SA 1998 Diet selection and resource use by flying foxes (Genus Pteropus). Ecology 79:1949–67. doi:10.1890/0012-9658(1998)079[1949:DSARUB]2.0.CO;2.

Banack SA, Caller T, Henegan P, Haney J, Murby A, Metcalf JS, Powell J, Cox PA, and Stommel E. 2015 Detection of cyanotoxins, beta-N-methylamino-L-alanine and microcystins, from a lake surrounded by cases of amyotrophic lateral sclerosis. Toxins 7:322–36. doi:10.3390/toxins7020322. [PubMed: 25643180]

Banack SA, and Cox PA. 2003a Biomagnification of cycad neurotoxins in flying foxes: Implications for ALS-PDC in Guam. Neurology 61:387–89. doi:10.1212/01.WNL.0000078320.18564.9F. [PubMed: 12913204]

Banack SA, and Cox PA. 2003b Distribution of the neurotoxic nonprotein amino acid BMAA in Cycas micronesica. Botanic Journal Linnean Social 143:165–68. doi:10.1046/j.1095-8339.2003.00217.x.

Banack SA, Johnson HE, Cheng R, and Cox PA. 2007 Production of the neurotoxin BMAA by a marine cyanobacterium. Marine Drugs 5:180–96. doi:10.3390/md504180. [PubMed: 18463731]

Banack SA, Metcalf JS, Bradley WG, and Cox PA. 2014 Detection of cyanobacterial neurotoxin beta-N-methylamino-L-alanine within shellfish in the diet of an ALS patient in Florida. Toxicon 90:167–73. doi:10.1016/j.toxicon.2014.07.018. [PubMed: 25123936]

Banack SA, and Murch SJ. 2009 Multiple neurotoxic items in the Chamorro diet link BMAA with ALS/PDC. Amyotrophic Lateral Sclerosis 10 (Suppl 2):34–40. doi:10.3109/17482960903278451. [PubMed: 19929729]

Banack SA, Murch SJ, and Cox PA. 2006 Neurotoxic flying foxes as dietary items for the Chamorro people, Marianas Islands. Journal of Ethnopharmacology 106:97–104. doi:10.1016/j.jep.2005.12.032. [PubMed: 16457975]

Banos G, Daniel PM, and Pratt OE. 1978 The effect of age upon the entry of some amino acids into the brain, and their incorporation into cerebral protein. Developmental Medicine and Child Neurology 20:335–46. doi:10.1111/j.1469-8749.1978.tb15223.x. [PubMed: 669065]

Baptista MS, Vasconcelos RG, Ferreira PC, Almeida CM, and Vasconcelos VM. 2015 Assessment of the non-protein amino acid BMAA in Mediterranean mussel Mytilus galloprovincialis after feeding with estuarine cyanobacteria. Environmental Science and Pollution Research International 22:12501–10. doi:10.1007/s11356-015-4516-5. [PubMed: 25903181]

Beard JD, and Kamel F. 2015 Military service, deployments, and exposures in relation to Amyotrophic lateral sclerosis etiology and survival. Epidemiologic Reviews 37:55–70. doi:10.1093/epirev/mxu001. [PubMed: 25365170]

Bell EA, Vega A, and Nunn PB 1967 A neurotoxic amino acid in seeds of Cycas circinalis. Paper presented at Fifth Cycad Conference, Miami, FL.

Berntzon L, Ronnevi LO, Bergman B, and Eriksson J. 2015 Detection of BMAA in the human central nervous system (Vol 292, Pg 137, 2015). Neuroscience 300:619–619. doi: 10.1016/j.neuroscience.2015.04.008.

Borenstein AR, Mortimer JA, Schofield E, Wu Y, Salmon DP, Gamst A, Olichney J, Thal LF, Silbert L, Kaye J, Craig UL, Schellenberg GD, and Galasko DR. 2007 Cycad exposure and risk of dementia, MCI, and PDC in the Chamorro population of Guam. Neurology 68:1764–71. doi:10.1212/01.wnl.0000262027.31623.b2. [PubMed: 17515538]

Bradley WG, Borenstein AR, Nelson LM, Codd GA, Rosen BH, Stommel EW, and Cox PA. 2013 Is exposure to cyanobacteria an environmental risk factor for amyotrophic lateral sclerosis and other neurodegenerative diseases? Amyotrophic Lateral Sclerosis Frontotemp Degen 14:325–33. doi: 10.3109/21678421.2012.750364.

Bradley WG, and Mash DC. 2009 Beyond Guam: The cyanobacteria/BMAA hypothesis of the cause of ALS and other neurodegenerative diseases. Amyotrophic Lateral Sclerosis 10 (Suppl. 2):7–20. doi:10.3109/17482960903286009.

Brand LE 2009 Human exposure to cyanobacteria and BMAA. 2009. Amyotrophic Lateral Sclerosis 10 (Suppl. 2):85–95. doi:10.3109/17482960903273585.

Brand LE, Pablo J, Compton A, Hammerschlag N, and Mash DC. 2010 Cyanobacterial blooms and the occurrence of the neurotoxin beta-N-methylamino-L-alanine (BMAA) in south Florida aquatic food webs. Harmful Algae 9:620–35. doi:10.1016/j.hal.2010.05.002. [PubMed: 21057660]

Briand JF, Leboulanger C, Humbert JF, Bernard C, and Dufour P. 2004 Cylindrospermopsis raciborskii (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, or global warming? Journal of Phycology 40:231–38. doi:10.1111/j.1529-8817.2004.03118.x.

Brion JP 1998 Neurofibrillary tangles and Alzheimer's disease. European Neurology 40:130–40. doi: 10.1159/000007969. [PubMed: 9748670]

Brody JA, Edgar AH, and Gillespie MM. 1978 Amyotrophic lateral sclerosis. No increase among US construction workers in Guam. Journal of the American Medical Association 240:551–60. doi: 10.1001/jama.1978.03290060053013. [PubMed: 671666]

Brody JA, Hirano A, and Scott RM. 1971 Recent neuropathologic observations in Amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. Neurology 21:528–36. doi:10.1212/WNL.21.5.528. [PubMed: 5104096]

Buee-Scherrer V, Buee L, Hof PR, Leveugle B, Gilles C, Loerzel AJ, et al. 1995 Neurofibrillary degeneration in Amyotrophic lateral sclerosis/Parkinsonism dementia complex of Guam. Immunochemical characterization of tau proteins. The American Journal of Pathology 146:924–32. [PubMed: 7717459]

Caller TA, Doolin JW, Haney JF, Murby AJ, West KG, Farrar HE, Ball A, Harris BT, and Stommel EW. 2009 A cluster of Amyotrophic lateral sclerosis in New Hampshire: A possible role for toxic

cyanobacteria blooms. Amyotrophic Lateral Sclerosis 10:101–08. doi: 10.3109/17482960903278485. [PubMed: 19929741]

Caller TA, Field NC, Chipman JW, Shi X, Harris BT, and Stommel EW. 2012 Spatial clustering of Amyotrophic lateral sclerosis and the potential role of BMAA. Amyotrophic Lateral Sclerosis 13:25–32. doi: 10.3109/17482968.2011.621436. [PubMed: 22214351]

Campbell RJ, Steele JC, Cox TA, Loerzel AJ, Belli M, and Belli DD. 1993 Pathologic findings in the retinal pigment epitheliopathy associated with the Amyotrophic lateral sclerosis/Parkinsonism dementia complex of Guam. Ophthalmology 100:37–42. doi:10.1016/S0161-6420(93)31696-9. [PubMed: 8433825]

Carr DJ, and Carr SGM, ed. 1981 The botany of the first Australians People and plants in Australia. Sydney: Academic Press.

Carroll D, and Hathaway JC. 1963 Mineralogy of selected soils from Guam Geological Survey Professional Paper 403-F; Department of the Interior. Washington, DC: United States Government Printing Office.

Cervantes Cianca RC, Baptista MS, Lopes VR, and Vasconcelos VM. 2012 The non-protein amino acid beta- N-methylamino-L-alanine in Portuguese cyanobacterial isolates. Amino Acids 42:2473–79. doi:10.1007/s00726-011-1057-1. [PubMed: 21863230]

Chatziefthimiou AD, Metcalf JS, Glover WB, Banack SA, Dargham SR, and Richer RA. 2016 Cyanobacteria and cyanotoxins are present in drinking water impoundments and groundwater wells in desert environments. Toxicon 114:75–84. doi:10.1016/j.toxicon.2016.02.016. [PubMed: 26921462]

Chen X, Xia Y, Gresham LS, Molgaard CA, Thomas RG, Galasko D, Wiederholt WC, and Saitoh T. 1996 ApoE and CYP2D6 polymorphism with and without Parkinsonism dementia complex in the people of Chamorro, Guam. Neurology 47:779–84. doi:10.1212/WNL.47.3.779. [PubMed: 8797479]

Chiu AS, Gehringer MM, Braidy N, Guillemin GJ, Welch JH, and Neilan BA. 2012 Excitotoxic potential of the cyanotoxin beta-methyl-amino-L-alanine (BMAA) in primary human neurons. Toxicon 60:1159–65. doi:10.1016/j.toxicon.2012.07.169. [PubMed: 22885173]

Cohen SA 2012 Analytical techniques for the detection of alpha-amino-beta-methylaminopropionic acid. The Analyst 137:1991–2005. doi:10.1039/c2an16250d. [PubMed: 22421821]

Combes A, El Abdellaoui S, Vial J, Lagrange E, Pichon V, and Grp B. 2014 Development of an analytical procedure for quantifying the underivatized neurotoxin beta-N-methylamino-L-alanine in brain tissues. Analytical and Bioanalytical Chemistry 406:4627–36. doi:10.1007/s00216-014-7872-y. [PubMed: 24858470]

Contardo-Jara V, Schwanemann T, and Pflugmacher S. 2014 Uptake of a cyanotoxin, beta-N-methylamino-L-ala-nine, by wheat (Triticum aestivum). Ecotoxicology and Environmental Safety 104:127–31. doi:10.1016/j.ecoenv.2014.01.039. [PubMed: 24675440]

Cox PA 2004 Indigenous horticulturists and human health: An ethnobotanical approach. Horticulture: Art Sciences Life 642:173–85.

Cox PA, Banack SA, and Murch SJ. 2003 Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proceedings of the National Academy of Sciences of the United States of America 100:13380–83. doi:10.1073/pnas.2235808100. [PubMed: 14612559]

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, and Bergman B. 2005 Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. Proceedings of the National Academy of Sciences of the United States of America 102:5074–78. doi:10.1073/pnas.0501526102. [PubMed: 15809446]

Cox PA, Davis DA, Mash DC, Metcalf JS, and Banack SA. 2016 Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid deposits in the brain. Proceedings R Social B-Biological Sciences 283:20152397. doi:10.1098/rspb.2015.2397.

Cox PA, Richer R, Metcalf JS, Banack SA, Codd GA, and Bradley WG. 2009 Cyanobacteria and BMAA exposure from desert dust: A possible link to sporadic ALS among Gulf War veterans. Amyotrophic Lateral Sclerosis 10 (Suppl 2):109–17. doi:10.3109/17482960903286066. [PubMed: 19929742]

Cox PA, and Sacks OW. 2002 Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. Neurology 58:956–59. doi:10.1212/WNL.58.6.956. [PubMed: 11914415]

Cox TA, McDarby JV, Lavine L, Steele JC, and Calne DB. 1989 A retinopathy on Guam with high prevalence in Lytico-Bodig. Ophthalmology 96:1731–35. doi:10.1016/S0161-6420(89)32656-X. [PubMed: 2622619]

Cramer F, Englisch U, Freist W, and Sternbach H. 1991 Aminoacylation of tRNAs as critical step of protein biosynthesis. Biochimie 73:1027–35. doi:10.1016/0300-9084(91)90144-P. [PubMed: 1720662]

Cruz-Aguado R, Winkler D, and Shaw CA. 2006 Lack of behavioral and neuropathological effects of dietary beta-methylamino-L-alanine (BMAA) in mice. Pharmacology, Biochemistry, and Behavior 84:294–99. doi:10.1016/j.pbb.2006.05.012.

Dastur DK 1964 Cycad toxicity in monkeys: Clinical, pathological, and biochemical aspects. Federation Proceedings 23:1368–69. [PubMed: 14236153]

Dawson R Jr.Marschall EG, Chan KC, Millard WJ, Eppler B, and Patterson TA. 1998 Neurochemical and neurobehavioral effects of neonatal administration of beta-N-methylamino-L-alanine and 3,3-iminodipropioni-trile. Neurotoxicology and Teratology 20:181–92. doi:10.1016/S0892-0362(97)00078-0. [PubMed: 9536463]

De Munck E, Munoz-Saez E, Antonio MT, Pineda J, Herrera A, Miguel BG, and Arahuetes RM. 2013a Effect of beta-N-methylamino-L-alanine on oxidative stress of liver and kidney in rat. Environmental Toxicology and Pharmacology 35:193–99. doi:10.1016/j.etap.2012.12.004. [PubMed: 23328118]

De Munck E, Munoz-Saez E, Miguel BG, Solas MT, Martinez A, and Arahuetes RM. 2015 Morphometric and neurochemical alterations found in l-BMAA treated rats. Environmental Toxicology and Pharmacology 39:1232–45. doi:10.1016/j.etap.2015.04.022. [PubMed: 26002186]

De Munck E, Munoz-Saez E, Miguel BG, Solas MT, Ojeda I, Martinez A, Gil C, and Arahuetes RM. 2013b Beta-N-methylamino-L-alanine causes neurological and pathological phenotypes mimicking amyotrophic lateral sclerosis (ALS): The first step towards an experimental model for sporadic ALS. Environmental Toxicology and Pharmacology 36:243–55. doi:10.1016/j.etap.2013.04.007. [PubMed: 23688553]

Dinis-Oliveira RJ, Remiao F, Carmo H, Duarte JA, Navarro AS, Bastos ML, and Carvalho F. 2006 Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology 27:1110–22. doi:10.1016/j.neuro.2006.05.012. [PubMed: 16815551]

Dobson CM 2001 The structural basis of protein folding and its links with human disease. Philosophical Transactions of the Royal Society of London 356:133–45. doi:10.1098/rstb.2000.0758. [PubMed: 11260793]

Downing S, Contardo-Jara V, Pflugmacher S, and Downing TG. 2014 The fate of the cyanobacterial toxin beta-N-methylamino-L-alanine in freshwater mussels. Ecotoxicology and Environmental Safety 101:51–58. doi: 10.1016/j.ecoenv.2013.11.028. [PubMed: 24507126]

Duncan MW, Kopin IJ, Garruto RM, Lavine L, and Markey SP. 1988 2-Amino-3 (methylamino)-propionic acid in cycad-derived foods is an unlikely cause of amyotrophic lateral sclerosis/Parkinsonism. Lancet 2:631–32. doi:10.1016/S0140-6736(88)90671-X.

Duncan MW, Markey SP, Weick BG, Pearson PG, Ziffer H, Hu Y, and Kopin IJ. 1992 2-Amino-3-(methylamino)propanoic acid (BMAA) bioavailability in the primate. Neurobiology of Aging 13:333–37. doi:10.1016/0197-4580(92)90047-2. [PubMed: 1522948]

Duncan MW, Steele JC, Kopin IJ, and Markey SP. 1990 2-Amino-3-(methylamino)-propanoic acid (BMAA) in cycad flour: An unlikely cause of amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. Neurology 40:767–72. doi:10.1212/WNL.40.5.767. [PubMed: 2330104]

Duncan MW, Villacreses NE, Pearson PG, Wyatt L, Rapoport SI, Kopin IJ, Markey SP, and Smith QR. 1991 2-amino-3-(methylamino)-propanoic acid (BMAA) pharmacokinetics and blood-brain barrier permeability in the rat. The Journal of Pharmacology and Experimental Therapeutics 258:27–35. [PubMed: 2072299]

Dunlop RA, Cox PA, Banack SA, and Rodgers KJ. 2013 The non-protein amino acid BMAA Is misincorporated into human proteins in place of L-serine causing protein misfolding and aggregation. PLoS One 8:e75376. doi:10.1371/journal.pone.0075376. [PubMed: 24086518]

Durlach J, Bac P, Durlach V, Durlach A, Bara M, and Guiet-Bara A. 1997 Are age-related neurodegenerative diseases linked with various types of magnesium depletion? Magnesium Researcher 10:339–53.

Eldridge R, Ryan E, Rosario J, and Brody JA. 1969 Amyotrophic lateral sclerosis and Parkinsonism dementia in a migrant population from Guam. Neurology 19:1029–37. doi:10.1212/WNL.19.11.1029. [PubMed: 5388071]

Elizan TS, Hirano A, Abrams BM, Need RL, Van Nuis C, and Kurland LT. 1966 Amyotrophic lateral sclerosis and Parkinsonism dementia complex of Guam. Neurological Reevaluation. Archives of Neurology 14:356–68. doi: 10.1001/archneur.1966.00470100012002. [PubMed: 5906460]

Ellisdon AM, and Bottomley SP. 2004 The role of protein misfolding in the pathogenesis of human diseases. IUBMB Life 56:119–23. doi:10.1080/15216540410001674003. [PubMed: 15185744]

Eplett L 2015 Fin (the end): Putting a stop to the consumption of shark fin soup. Scientific American, 7 8; https://blogs.scientificamerican.com/food-matters/fin-the-end-putting-a-stop-to-the-consumption-of-shark-fin-soup/.

Esterhuizen M, and Downing TG. 2008 Beta-N-methylamino-L-alanine (BMAA) in novel South African cyanobacterial isolates. Ecotoxicology and Environmental Safety 71:309–13. doi:10.1016/j.ecoenv.2008.04.010. [PubMed: 18538391]

Esterhuizen-Londt M, and Downing TG. 2011 Solid phase extraction of β-N-methylamino-L-alanine (BMAA) from South African water supplies. Water SA 37:523–27.

Faassen EJ 2014 Presence of the neurotoxin BMAA in aquatic ecosystems: What do we really know? Toxins (Basel) 6:1109–38. doi:10.3390/toxins6031109. [PubMed: 24662480]

Faassen EJ, Antoniou MG, Beekman-Lukassen W, Blahova L, Chernova E, Christophoridis C, Combes A, Edwards C, Fastner J, Harmsen J, Hiskia A, Ilag LL, Kaloudis T, Lopicic S, Lurling M, Mazur-Marzec H, Meriluoto J, Porojan C, Viner-Mozzini Y, and Zguna N. 2016 A collaborative evaluation of LC-MS/MS based methods for BMAA analysis: Soluble bound BMAA found to be an important fraction. Marine Drugs 14:45. doi:10.3390/md14030045.

Faassen EJ, Gillissen F, and Lurling M. 2012 A comparative study on three analytical methods for the determination of the neurotoxin BMAA in cyanobacteria. PLoS One 7:e36667. doi:10.1371/journal.pone.0036667. [PubMed: 22570736]

Faassen EJ, Gillissen F, Zweers HA, and Lurling M. 2009 Determination of the neurotoxins BMAA (beta-N-methylamino-L-alanine) and DAB (alpha-,gamma-diami-nobutyric acid) by LC-MSMS in Dutch urban waters with cyanobacterial blooms. Amyotrophic Lateral Sclerosis 10 (Suppl 2):79–84. doi:10.3109/17482960903272967.

Falconer IR, and Humpage AR. 2005 Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. International Journal of Environmental Research and Public Health 2:43–50. doi:10.3390/ijerph2005010043. [PubMed: 16705800]

Fan H, Qiu J, Fan L, and Li A. 2014 Effects of growth conditions on the production of neurotoxin 2,4-diamino-butyric acid (DAB) in Microcystis aeruginosa and its universal presence in diverse cyanobacteria isolated from freshwater in China. Environmental Science and Pollution Research International 22:5943–51. doi:10.1007/s11356-014-3766-y. [PubMed: 25354443]

Field NC, Metcalf JS, Caller TA, Banack SA, Cox PA, and Stommel EW. 2013 Linking beta-methylamino-L-alanine exposure to sporadic amyotrophic lateral sclerosis in Annapolis, MD. Toxicon 70:179–83. doi:10.1016/j.toxicon.2013.04.010. [PubMed: 23660330]

Fritts TH, and Rodda GH. 1998 The role of introduced species in the degradation of island ecosystems: A case history of Guam1. Annual Reviews Ecology System 29:113–40. doi:10.1146/annurev.ecolsys.29.1.113.

Fujita T, Okamoto Y, Sakagami Y, Ota K, and Ohata M. 1984 Bone changes and aortic calcification in aging inhabitants of mountain versus seacoast communities in the Kii Peninsula. Journal of the American Geriatrics Society 32:124–28. doi:10.1111/j.1532-5415.1984.tb05852.x. [PubMed: 6693698]

Gajdusek DC 1963 Motor-neuron disease in natives of New Guinea. The New England Journal of Medicine 268:474–76. doi:10.1056/NEJM196302282680906. [PubMed: 13946175]

Gajdusek DC, and Salazar AM. 1982 Amyotrophic lateral sclerosis and Parkinsonian syndromes in high incidence among the Auyu and Jakai people of West New Guinea. Neurology 32:107–26. doi: 10.1212/WNL.32.2.107. [PubMed: 7198738]

Galasko D, Salmon D, Gamst A, Olichney J, Thal LJ, Silbert L, et al. 2007 Prevalence of Dementia in Chamorros on Guam: Relationship to age, gender, education, and APOE. Neurology 68:1772–81. doi:10.1212/01.wnl.0000262028.16738.64. [PubMed: 17515539]

Galasko D, Salmon DP, Craig UK, Thal LJ, Schellenberg G, and Wiederholt W. 2002 Clinical features and changing patterns of neurodegenerative disorders on Guam, 1997–2000. Neurology 58:90–97. doi:10.1212/WNL.58.1.90. [PubMed: 11781411]

Garruto RM, Fukatsu R, Yanagihara R, Gajdusek DC, Hook G, and Fiori CE. 1984a Imaging of calcium and aluminum in neurofibrillary tangle-bearing neurons in Parkinsonism-dementia of Guam. Proceedings of the National Academy of Sciences of the United States of America 81:1875–79. doi:10.1073/pnas.81.6.1875. [PubMed: 6584922]

Garruto RM, Gajdusek C, and Chen KM. 1980 Amyotrophic lateral sclerosis among Chamorro migrants from Guam. Annals of Neurology 8:612–19. doi:10.1002/ana.410080612. [PubMed: 7212649]

Garruto RM, Shankar SK, Yanagihara R, Salazar AM, Amyx HL, and Gajdusek DC. 1989 Low-calcium, high-aluminum diet-induced motor neuron pathology in cynomolgus monkeys. Acta Neuropathologica 78:210–19. doi: 10.1007/BF00688211. [PubMed: 2750490]

Garruto RM, Swyt C, Fiori CE, Yanagihara R, and Gajdusek DC. 1985a Intraneuronal deposition of calcium and aluminum in amyotrophic lateral sclerosis of Guam. Lancet 2:1353. doi:10.1016/S0140-6736(85)92642-X.

Garruto RM, Swyt C, Yanagihara R, Fiori CE, and Gajdusek DC. 1986 Intraneuronal co-localization of silicon with calcium and aluminum in amyotrophic lateral sclerosis and Parkinsonism with dementia of Guam. The New England Journal of Medicine 315:711–12. doi:10.1056/NEJM198609113151115. [PubMed: 3748078]

Garruto RM, Yanagihara R, and Gajdusek DC. 1985b Disappearance of high-incidence amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam. Neurology 35:193–98. doi:10.1212/WNL.35.2.193. [PubMed: 3969206]

Garruto RM, Yanagihara R, Gajdusek DC, and Arion DM. 1984b Concentrations of heavy metals and essential minerals in garden soil and drinking water in the western Pacific In Amyotrophic lateral sclerosis in Asia and Oceania, eds. K-M C, and Yase Y, 343–53. Taipei, Taiwan: National Taiwan University.

Garruto RM, and Yase Y. 1986 Neurodegenerative disorders of the Western Pacific - The search for mechanisms of pathogenesis. Trend Neuroscience 9:368–74. doi:10.1016/0166-2236(86)90116-5.

Gellein K, Garruto RM, Syversen T, Sjobakk TE, and Flaten TP. 2003 Concentrations of Cd, Co, Cu, Fe, Mn, Rb, V, and Zn in formalin-fixed brain tissue in amyotrophic lateral sclerosis and Parkinsonism-dementia complex of Guam determined by high-resolution ICP-MS. Biological Trace Element Research 96:39–60. doi:10.1385/BTER:96:1-3:39. [PubMed: 14716085]

Geser F, Winton MJ, Kwong LK, Xu Y, Xie SX, Igaz LM, Garruto RM, Perl DP, Galsko D, Lee VM, and Trojanowski JQ. 2008 Pathological TDP-43 in Parkinsonism-demetia complex and amyotrophic lateral sclerosis of Guam. Acta Neuropathologica 115:133–45. doi: 10.1007/s00401-007-0257-y. [PubMed: 17713769]

Gibbs CJ Jr., and Gajdusek DC. 1972 Amyotrophic lateral sclerosis, Parkinson's disease, and the Amyotrophic lateral sclerosis-Parkinsonism dementia Complex on Guam: A review and summary of attempts to demonstrate infection as the etiology. Journal Clinical Pathologists 25 Supplement (R College Pathol) 132–40. doi:10.1136/jcp.s3-6.1.132.

Glover WB, Cohen SA, and Murch SJ. 2015 Liquid chromatography and mass spectrometry for the analysis of n-beta-methylamino-l-alanine with 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate. Advancement Protocols Oxidative Stress Iii 1208:379–91.

Glover WB, Mash DC, and Murch SJ. 2014 The natural non-protein amino acid N-beta-methylamino-L-alanine (BMAA) is incorporated into protein during synthesis. Amino Acids 46:2553–59. doi: 10.1007/s00726-014-1812-1. [PubMed: 25096519]

Griapon Y, and Togodly A. 2008 Research report on neurodegenerative diseases in Bade, Edera district, Mappi, Papua, Indonesia: Case studies on Auyu and Jakay people, vol. 9, 153–58. Kyoto University Bhutan Friendship Program. Kyoto: Field Institute of Southeast Asian Studies, Kyoto University.

Hanlon SD, and Steele JC. 1993 An unusual retinal pigment epitheliopathy endemic to the island of Guam. Opthomol Vision Sciences 70:854–59. doi:10.1097/00006324-199310000-00014.

Hanrieder J, Gerber L, Persson Sandelius A, Brittebo EB, Ewing AG, and Karlsson O. 2014 High resolution metabolite imaging in the hippocampus following neonatal exposure to the environmental toxin BMAA using ToF-SIMS. ACS Chemical Neuroscience 5:568–75. doi: 10.1021/cn500039b. [PubMed: 24779349]

Hill AB 1965 The environment and disease: Association or causation? Proceedings of the Royal Society of Medicine 58:295–300. [PubMed: 14283879]

Hirano A, Kurland LT, Krooth RS, and Lessell S. 1961b Parkinsonism dementia complex, an endemic disease on the island of Guam. I. Clinical features. Brain 84:642–61. doi: 10.1093/brain/84.4.642. [PubMed: 13907609]

Hirano A, Malamud N, Elizan TS, and Kurland LT. 1966 Amyotrophic lateral sclerosis and Parkinsonism dementia complex on Guam. Further pathologic studies. Archives of Neurology 15:35–51. doi:10.1001/archneur.1966.00470130039004. [PubMed: 5939450]

Hirano A, Malamud N, and Kurland LT. 1961a Parkinsonism dementia complex, an endemic disease on the island of Guam. II. Pathological features. Brain 84:662–79. doi: 10.1093/brain/84.4.662. [PubMed: 13907610]

Hirano A, and Zimmerman HM. 1962 Alzheimer's neurofibrillary changes. A topographic study. Archives of Neurology 7:227–42. doi:10.1001/archneur.1962.04210030065009. [PubMed: 13907611]

Hirata Y 2008 Walking in coastal jungle of West New Guinea-landscape, neurodegenerative disease, totem spirits and medical science. Report No. 9, Kyoto University Bhutan Friendship Program, Kyoto.

Hirono I, and Shibuya C. 1967 Induction of a neurological disorder by cycasin in mice. Nature 216:1311–12. doi:10.1038/2161311a0. [PubMed: 6080057]

Hirono I, Shibuya C, and Hayashi K. 1969 Induction of a cerebellar disorder with cycasin in newborn mice and hamsters. Proceedings of the Society for Experimental Biology and Medicine 131:593–99. doi:10.3181/00379727-131-33933. [PubMed: 5815077]

Hof PR, Perl DP, Loerzel AJ, and Morrison JH. 1991 Neurofibrillary tangle distribution in the cerebral cortex of Parkinsonism-dementia cases from Guam: Differences with Alzheimer's disease. Brain Research 564:306–13. doi:10.1016/0006-8993(91)91467-F. [PubMed: 1810629]

Hof PR, Perl DP, Loerzel AJ, Steele JC, and Morrison JH. 1994 Amyotrophic lateral sclerosis and Parkinsonism-dementia from Guam: Differences in neurofibrillary tangle distribution and density in the hippocampal formation and neocortex. Brain Research 650:107–16. doi: 10.1016/0006-8993(94)90212-7. [PubMed: 7953661]

Hong SH, Kwon YC, and Jewett MC. 2014 Nonstandard amino acid incorporation into proteins using Escherichia coli cell-free protein synthesis. Frontiers Chemical 2:34. doi:10.3389/fchem. 2014.00034.

Hynd MR, Scott HL, and Dodd PR. 2004 Glutamatemediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochemistry International 45:583–95. doi: 10.1016/j.neuint. 2004.03.007. [PubMed: 15234100]

Ikemoto A, Hirano A, and Akiguchi I. 2000 Neuropathology of Amyotrophic lateral sclerosis with extra-motor system degeneration: Characteristics and differences in the molecular pathology between ALS with dementia and Guamanian ALS. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders 1:97–104. doi:10.1080/14660820050515395. [PubMed: 11467056]

Jiang L, Eriksson J, Lage S, Jonasson S, Shams S, Mehine M, et al. 2014a Diatoms: A novel source for the neurotoxin BMAA in aquatic environments. PLoS One 9: e84578. doi:10.1371/journal.pone. 0084578. [PubMed: 24392143]

Jiang L, Johnston E, Aberg KM, Nilsson U, and Ilag LL. 2013 Strategy for quantifying trace levels of BMAA in cyanobacteria by LC/MS/MS. Analytical and Bioanalytical Chemistry 405:1283–92. doi:10.1007/s00216-012-6550-1. [PubMed: 23180086]

Jiang L, Kiselova N, Rosen J, and Ilag LL. 2014b Quantification of neurotoxin BMAA (beta-N-methyla-mino-L-alanine) in seafood from Swedish markets. Scientific Reports 4:6931. doi: 10.1038/srep06931. [PubMed: 25373604]

Jiao Y, Chen Q, Chen X, Wang X, Liao X, Jiang L, et al. 2014 Occurrence and transfer of a cyanobacterial neurotoxin beta-methylamino-L-alanine within the aquatic food webs of Gonghu Bay (Lake Taihu, China) to evaluate the potential human health risk. The Science ofthe Total Environment 468–469:457–63. doi:10.1016/j.scitotenv.2013.08.064.

Johnson HE, King SR, Banack SA, Webster C, Callanaupa WJ, and Cox PA. 2008 Cyanobacteria (Nostoc commune) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. Journal of Ethnopharmacology 118:159–65. doi:10.1016/j.jep.2008.04.008. [PubMed: 18495396]

Jonasson S, Eriksson J, Berntzon L, Spacil Z, Ilag LL, Ronnevi LO, et al. 2010 Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. Proceedings of the National Academy of Sciences of the United States of America 107:9252–57. doi:10.1073/pnas.0914417107. [PubMed: 20439734]

Karlsson O, Berg AL, Hanrieder J, Arnerup G, Lindstrom AK, and Brittebo EB. 2015 Intracellular fibril formation, calcification, and enrichment of chaperones, cytoskeletal, and intermediate filament proteins in the adult hippocampus CA1 following neonatal exposure to the non-protein amino acid BMAA. Archives of Toxicology 89:423–36. doi:10.1007/s00204-014-1262-2. [PubMed: 24798087]

Karlsson O, Berg AL, Lindstrom AK, Hanrieder J, Arnerup G, Roman E, et al. 2012 Neonatal exposure to the cyanobacterial toxin BMAA induces changes in protein expression and neurodegeneration in adult hippocampus. Toxicological Sciences 130:391–404. doi:10.1093/toxsci/kfs241. [PubMed: 22872059]

Karlsson O, Bergquist J, and Andersson M. 2014a Quality measures of imaging mass spectrometry aids in revealing long-term striatal protein changes induced by neonatal exposure to the cyanobacterial toxin beta-N-methyla- mino-L-alanine (BMAA). Molecular & Cellular Proteomics 13:93–104. doi:10.1074/mcp.M113.031435. [PubMed: 24126143]

Karlsson O, Jiang L, Andersson M, Ilag LL, and Brittebo EB. 2014b Protein association of the neurotoxin and non-protein amino acid BMAA (beta-N-methylamino-L-alanine) in the liver and brain following neonatal administration in rats. Toxicology Letters 226:1–5. doi:10.1016/j.toxlet.2014.01.027. [PubMed: 24472610]

Karlsson O, Kultima K, Wadensten H, Nilsson A, Roman E, Andren PE, et al. 2013 Neurotoxin-induced neuropeptide perturbations in striatum of neonatal rats. Journal of Proteome Research 12:1678–90. doi:10.1021/pr3010265. [PubMed: 23410195]

Karlsson O, Lindquist NG, Brittebo EB, and Roman E. 2009a Selective brain uptake and behavioral effects of the cyanobacterial toxin BMAA (beta-N-methylamino-L-ala-nine) following neonatal administration to rodents. Toxicological Sciences 109:286–95. doi:10.1093/toxsci/kfp062. [PubMed: 19321797]

Karlsson O, Roman E, Berg AL, and Brittebo EB. 2011 Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (beta-N-methylamino-L-alanine) during the neonatal period. Behavioural Brain Research 219:310–20. doi: 10.1016/j.bbr.2011.01.056. [PubMed: 21315110]

Karlsson O, Roman E, and Brittebo EB. 2009b Long-term cognitive impairments in adult rats treated neonatally with beta-N-methylamino-L-alanine. Toxicological Sciences 112:185–95. doi: 10.1093/toxsci/kfp196. [PubMed: 19692667]

Kato S, Hirano A, Llena JF, Ito H, and Yen SH. 1992 Ultrastructural identification of neurofibrillary tangles in the spinal cords in Guamanian amyotrophic lateral sclerosis and Parkinsonism dementia complex on Guam. Acta Neuropathologica 83:277–82. doi:10.1007/BF00296790. [PubMed: 1557958]

Khabazian I, Bains JS, Williams DE, Cheung J, Wilson JM, Pasqualotto BA, et al. 2002 Isolation of various forms of sterol beta-D-glucoside from the seed of Cycas circinalis: Neurotoxicity and

implications for ALS-Parkinsonism dementia complex. Journal of Neurochemistry 82:516–28. doi:10.1046/j.1471-4159.2002.00976.x. [PubMed: 12153476]

Kihira T, Okamoto K, Yoshida S, Kondo T, Iwai K, Wada S, Kajimoto Y, Kondo T, Kokubo Y, and Kuzuhara. 2013 Environmental characteristics and oxidative stress of inhabitants and patients with Amyotrophic lateral sclerosis in a high-incidence area on the Kii Peninsula, Japan. Internal Medicine 52:1479–86. doi:10.2169/internalmedicine.52.9521. [PubMed: 23812195]

Kihira T, Yoshida S, Yase Y, Ono S, and Kondo T. 2002 Chronic low-Ca/Mg high-Al diet induces neuronal loss. Neuropathology 22:171–79. doi:10.1046/j.1440-1789.2002.00441.x. [PubMed: 12416556]

Kimura K, Yase Y, and Higashi Y. 1963 Epidemiological and geomedical studies on amyotrophic lateral sclerosis. Diseases of the Nervous System 24:155–59.

Kisby GE, Ellison M, and Spencer PS. 1992 Content of the neurotoxins cycasin (methylazoxymethanol beta-D- glucoside) and BMAA (beta-N-methylamino-L-alanine) in cycad flour prepared by Guam Chamorros. Neurology 42:1336–40. doi:10.1212/WNL.42.7.1336. [PubMed: 1620343]

Kisby GE, Fry RC, Lasarev MR, Bammler TK, Beyer RP, Churchwell M, Doerge DR, Meira LB, Palmer VS, Ramos-Crawford AL, Ren X, Sullivan RC, Kavanagh TJ, Samson LD, Zarbl H, and Spencer PS. 2011 The cycad genotoxin MAM modulates brain cellular pathways involved in neurodegenerative disease and cancer in a DNA damage-linked manner. PLoS One 6:e20911. doi: 10.1371/journal.pone.0020911. [PubMed: 21731631]

Kisby GE, and Spencer PS. 2011 Is neurodegenerative disease a long-latency response to early-life genotoxin exposure? International Journal of Environmental Research and Public Health 8:3889–921. doi:10.3390/ijerph8103889. [PubMed: 22073019]

Koerner DR 1952 Amyotrophic lateral sclerosis on Guam. Annals of Internal Medicine 37:1204–20. doi:10.7326/0003-4819-37-6-1204. [PubMed: 13008298]

Kokubo Y, Ito K, Fukunaga T, Matsubara H, and Kuzuhara S. 2006 Pigmentary retinopathy of ALS/PDC in Kii. Ophthalmology 113:e2111–e2112. doi:10.1016/j.ophtha.2006.07.002.

Kokubo Y, Ito K, and Kuzuhara S. 2003 Ophthalmomyiasislike pigmentary retinopathy in ALS/PDC in the Kii Peninsula of Japan. Neurology 60:1725–26. doi:10.1212/01.WNL.0000061487.16841.72. [PubMed: 12771286]

Kokubo Y, and Kuzuhara S. 2001 Neurological and neuro-pathological studies of Amyotrophic lateral sclerosis/ Parkinsonism dementia complex in the Kii Peninsula of Japan. Rinsho Shinkeigaku 41:769–74. [PubMed: 12080607]

Kokubo Y, and Kuzuhara S. 2003 Neuroradiological study of patients with amyotrophic lateral sclerosis and Parkinsonism-dementia complex on the Kii Peninsula of Japan. Archives of Neurology 60:1257–61. doi:10.1001/archneur.60.9.1257. [PubMed: 12975292]

Kruger T, Monch B, Oppenhauser S, and Luckas B. 2010 LC-MS/MS determination of the isomeric neurotoxins BMAA (beta-N-methylamino-L-alanine) and DAB (2,4- diaminobutyric acid) in cyanobacteria and seeds of Cycas revoluta and Lathyrus latifolius. Toxicon 55:547–57. doi: 10.1016/j.toxicon.2009.10.009. [PubMed: 19825383]

Kubo T, Kato N, Hosoya K, and Kaya K. 2008 Effective determination method for a cyanobacterial neurotoxin, beta-N-methylamino-L-alanine. Toxicon 51:1264–68. doi: 10.1016/j.toxicon. 2008.02.015. [PubMed: 18407310]

Kumamoto T, Nakagawa S, Suematsu C, Shimizu E, Yata Y, and Hirohata T. 1975 Experimental calcium-magnesium deficiency in rats - Chemical and histochemical study. Acta Histochemica et Cytochemica 8:294–303. doi:10.1267/ahc.8.294.

Kurland LT 1961 Parkinsonism-dementia complex, an endemic disease on the island of Guam. Transactions of the American Neurological Association 86:115–20. [PubMed: 14460747]

Kurland LT 1972 An appraisal of the neurotoxicity of cycad and the etiology of amyotrophic lateral sclerosis on Guam. Federation Proceedings 31:1540–42. [PubMed: 4561643]

Kurland LT, and Mulder DW 1954a Epidemiologic investigations of amyotrophic lateral sclerosis. II. Preliminary report on geographic distribution and special reference to the Mariana Islands, including clinical and pathologic observations. Neurology 4: 438–48. doi:10.1212/WNL.4.6.438. [PubMed: 13165883]

Kurland LT, and Mulder DW. 1954b Epidemiologic investigations of amyotrophic lateral sclerosis. I. Preliminary report on geographic distribution, with special reference to the Mariana Islands, including clinical and pathologic observations. Neurology 4:355–78. doi:10.1212/WNL.4.5.355. [PubMed: 13185376]

Kuzuhara S 2007 Revisit to Kii ALS-the innovated concept of ALS-Parkinsonism dementia complex, clinicopathological features, epidemiology and etiology. Brain and Nerve 59:1065–74. [PubMed: 17969346]

Kuzuhara S, Kokubo Y, Sasaki R, Narita Y, Yabana T, Hasegawa M, and Iwatsubo T. 2001 Familial amyotrophic lateral sclerosis and Parkinsonism dementia complex of the Kii Peninsula of Japan: Clinical and neuropathological study and tau analysis. Annals of Neurology 49:501–11. doi: 10.1002/(ISSN)1531-8249. [PubMed: 11310628]

Lage S, Annadotter H, Rasmussen U, and Rydberg S. 2015 Biotransfer of beta-N-methylamino-l-alanine (BMAA) in a eutrophicated freshwater lake. Marine Drugs 13:1185–201. doi:10.3390/md13031185. [PubMed: 25738330]

Lage S, Burian A, Rasmussen U, Costa PR, Annadotter H, and Godhe A. 2016 BMAA extraction of cyanobacteria samples: Which method to choose? Environmental Science and Pollution Research International 23:338–50. doi:10.1007/s11356-015-5266-0. [PubMed: 26304815]

Lage S, Costa PR, Moita T, Eriksson J, Rasmussen U, and Rydberg SJ. 2014 BMAA in shellfish from two Portuguese transitional water bodies suggests the marine dinoflagellate Gymnodinium catenatum as a potential BMAA source. Aquatic Toxicology 152:131–38. doi:10.1016/j.aquatox.2014.03.029. [PubMed: 24747603]

Laqueur GL, and Matsumoto H. 1966 Neoplasms in female Fischer rats following intraperitoneal injection of methylazoxy-methanol. Journal of the National Cancer Institute 37:217–32. [PubMed: 5912614]

Laqueur GL, Mickelsen O, Whiting MG, and Kurland LT. 1963 Carcinogenic properties of nuts from Cycas circinalis L. indigenous to Guam. Journal of the National Cancer Institute 31:919–51. [PubMed: 14069834]

Laqueur GL, and Spatz M. 1968 Toxicology of cycasin. Cancer Research 28:2262–67. [PubMed: 4881504]

Lau C 2012 Perfluorinated compounds. EXS 101:47–86. [PubMed: 22945566]

Leblanc B, Jezequel S, Davies T, Hanton G, and Taradach C. 1998 Binding of drugs to eye melanin is not predictive of ocular toxicity. Regulatory Toxicology and Pharmacology 28:124–32. doi: 10.1006/rtph.1998.1243. [PubMed: 9927562]

Lee MH, and Rabe A. 1992 Premature decline in Morris water maze performance of aging micrencephalic rats. Neurotoxicology and Teratology 14:383–92. doi: 10.1016/0892-0362(92)90048-F. [PubMed: 1488032]

Lemere CA, Beierschmitt A, Iglesias M, Spooner ET, Bloom JK, Leverone JF, Zheng JB, Seabrook TJ, Louard D, Li D, Selkoe DJ, Palmour RM, and Ervin FR. 2004 Alzheimer's disease A beta vaccine reduces central nervous system A beta levels in a non-human primate, the Caribbean vervet. The American Journal of Pathology 165:283–97. doi:10.1016/S0002-9440(10)63296-8. [PubMed: 15215183]

Lemke TO 1992 History of fruit bat use, research, and, protection in the Northern Mariana Islands. Biologic Reports, United States Fish and Wildlife Service 90:135–42.

Lepore FE, Steele JC, Cox TA, Tillson G, Calne DB, Duvoisin RC, Lavine L, and McDarby JV. 1988 Supranuclear disturbances of ocular motility in Lytico-Bodig. Neurology 38:1849–53. doi: 10.1212/WNL.38.12.1849. [PubMed: 3194062]

Li A, Fan H, Ma F, McCarron P, Thomas K, Tang X, et al. 2012 Elucidation of matrix effects and performance of solid-phase extraction for LC-MS/MS analysis of beta-N-methylamino-L-alanine (BMAA) and 2,4-diaminobutyric acid (DAB) neurotoxins in cyanobacteria. The Analyst 137:1210–19. doi:10.1039/c2an15887f. [PubMed: 22249403]

Lim R 2012 BMAA neurotoxin found in shark fins linked to Alzheimer's Disease. Asian Scientist.

Lindblad P 1990 Nitrogen and carbon metabolism in coralloid roots of cycads. Memoirs of the New York Botanical Garden 57:104–13.

Loftfield RB 1963 The frequency of errors in protein biosynthesis. The Biochemical Journal 89:82–92. doi:10.1042/bj0890082. [PubMed: 14097371]

Lucas RB, and McMichael AJ. 2005 Association and causation: Evaluating risks between "environment and disease. Bulletin WHO 83:792–95.

Ludolph AC, Kassubek J, Landwehrmeyer BG, Mandelkow E, Mandelkow EM, and Burn DJ. 2009 Tauopathies with Parkinsonism: Clinical spectrum, neuropathologic basis, biological markers, and treatment options. European Journal of Neurology 16:297–309. doi: 10.1111/j.l468-1331.2008.02513.x. [PubMed: 19364361]

Ly PT, Singh S, and Shaw CA. 2007 Novel environmental toxins: Steryl glycosides as a potential etiological factor for age-related neurodegenerative diseases. Journal of Neuroscience Research 85:231–37. doi:10.1002/(ISSN)1097-4547. [PubMed: 17149752]

Madeira C, Lourenco MV, Vargas-Lopes C, Suemoto CK, Brandao CO, Reis T, Leite REP, Laks J, Jacob-Filho W, Pasqualucci CA, Grinberg LT, Ferreira ST, and Panizzutti R. 2015 D-serine levels in Alzheimer's disease: Implications for novel biomarker development. Translational Psychiatry 5:e561. doi:10.1038/tp.2015.52. [PubMed: 25942042]

Malamud N, Hirano A, and Kurland LT. 1961 Pathoanatomic changes in amyotrophic lateral sclerosis on Guam. Special reference to the occurrence of neurofibrillary changes. Archives of Neurology 5:401–15. doi: 10.1001/archneur.1961.00450160051003. [PubMed: 14468803]

Marler TE, Snyder LR, and Shaw CA. 2010 Cycas micronesica (Cycadales) plants devoid of endophytic cyanobacteria increase in beta-methylamino-L-alanine. Toxicon 56:563–68. doi: 10.1016/j.toxicon.2010.05.015. [PubMed: 20570592]

Matsumoto S, Hirano A, and Goto S. 1990 Spinal-cord neurofibrillary tangles of Guamanian amyotrophic lateral sclerosis and Parkinsonism dementia complex - An immunohistochemical study. Neurology 40:975–79. doi:10.1212/WNL.40.6.975. [PubMed: 2161095]

McAuliffe K 2011 There's something in the water. Discover 30:41–46.

McCarron P, Logan AC, Giddings SD, and Quilliam MA. 2014 Analysis of beta-N-methylamino-L-alanine (BMAA) in spirulina-containing supplements by liquid chromatography-tandem mass spectrometry. Aquatic Biosystems 10:5. doi:10.1186/2046-9063-10-5. [PubMed: 25120905]

McGeer PL, Schwab C, McGeer EG, Haddock RL, and Steele JC. 1997 Familial nature and continuing morbidity of the amyotrophic lateral sclerosis-Parkinsonism dementia complex of Guam. Neurology 49:400–09. doi:10.1212/WNL.49.2.400. [PubMed: 9270568]

McGeer PL, and Steele JC. 2011 The ALS/PDC syndrome of Guam: Potential biomarkers for an enigmatic disorder. Progress in Neurobiology 95:663–69. doi:10.1016/j.pneurobio.2011.04.001. [PubMed: 21527311]

McGorum BC, Pirie RS, Glendinning L, McLachlan G, Metcalf JS, Banack SA, Cox PA, and Codd GA. 2015 Grazing livestock are exposed to terrestrial cyanobacteria. Veterinary Research 46:16. [PubMed: 25828258]

Metcalf JS, Banack SA, Kotut K, Krienitz L, and Codd GA. 2013 Amino acid neurotoxins in feathers of the Lesser Flamingo. Phoeniconaias Minority Chemosphere 90:835–39. doi:10.1016/j.chemosphere.2012.09.094.

Metcalf JS, Banack SA, Lindsay J, Morrison LF, Cox PA, and Codd GA. 2008 Co-occurrence of beta-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990–2004. Environmental Microbiology 10:702–08. doi:10.1111/j.1462-2920.2007.01492.x. [PubMed: 18237305]

Metcalf JS, Banack SA, Richer R, and Cox PA. 2015 Neurotoxic amino acids and their isomers in desert environments. Journal of Arid Environments 112:140–44. doi:10.1016/j.jaridenv.2014.08.002.

Miklossy J, Steele JC, Yu S, McCall S, Sandberg G, McGeer EG, and McGeer PL. 2008 Enduring involvement of tau, beta-amyloid, alpha-synuclein, ubiquitin and TDP-43 pathology in the amyotrophic lateral sclerosis/ Parkinsonism dementia complex of Guam (ALS/PDC). Acta Neuropathologica 116:625–37. doi:10.1007/s00401-008-0439-2. [PubMed: 18843496]

Mimuro M, Kokubo Y, and Kuzuhara S. 2007 Similar topographical distribution of neurofibrillary tangles in Amyotrophic lateral sclerosis and Parkinsonism dementia complex in people living in

the Kii Peninsula of Japan suggests a single tauopathy. Acta Neuropathologica 113:653–58. doi: 10.1007/s00401-007-0197-6. [PubMed: 17277950]

Mondo K, Broc Glover W, Murch SJ, Liu G, Cai Y, Davis DA, and Mash DC. 2014 Environmental neurotoxins beta-N-methylamino-L-alanine (BMAA) and mercury in shark cartilage dietary supplements. Food and Chemical Toxicology 70:26–32. doi:10.1016/j.fct.2014.04.015. [PubMed: 24755394]

Mondo K, Hammerschlag N, Basile M, Pablo J, Banack SA, and Mash DC. 2012 Cyanobacterial neurotoxin beta-N-methylamino-L-alanine (BMAA) in shark fins. Marine Drugs 10:509–20. doi: 10.3390/md10020509. [PubMed: 22412816]

Monson CS, Banack SA, and Cox PA. 2003 Conservation implications of Chamorro consumption of flying foxes as a possible cause of amyotrophic lateral sclerosis-Parkinsonism dementia complex in Guam. Conservation Biology 17:678–86. doi:10.1046/j.1523-1739.2003.02049.x.

Montine TJ, Li K, Perl DP, and Galasko D. 2005 Lack of beta-methylamino-l-alanine in brain from controls, AD, or Chamorros with PDC. Neurology 65:768–69. doi:10.1212/01.wnl. 0000174523.62022.52. [PubMed: 16157919]

Morris HR, Al-Sarraj S, Schwab C, Gwinn-Hardy K, Perez-Tur J, Wood NW, Hardy J, Lees AJ, McGeer PL, Daniel SE, and Steele JC. 2001 A clinical and pathological study of motor neurone disease on Guam. Brain 124:2215–22. doi:10.1093/brain/124.11.2215. [PubMed: 11673323]

Morris HR, Steele JC, Crook R, Wavrant-De Vrieze F, Onstead-Cardinale L, Gwinn-Hardy K, Wood NW, Farrer M, Lees AJ, McGeer PL, Siddique T, Hardy J, and Perez-Tur J. 2004 Genome-wide analysis of the Parkinsonism dementia complex of Guam. Archives of Neurology 61:1889–97. doi:10.1001/archneur.61.12.1889. [PubMed: 15596609]

Murakami N 1999 Parkinsonism dementia complex on Guam - Overview of clinical aspects. Journal of Neurology 246:16–18. doi:10.1007/BF03161077. [PubMed: 9987709]

Murch SJ, Cox PA, and Banack SA. 2004a A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. Proceedings of the National Academy of Sciences of the United States of America 101:12228–31. doi:10.1073/pnas. 0404926101. [PubMed: 15295100]

Murch SJ, Cox PA, Banack SA, Steele JC, and Sacks OW. 2004b Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurologica Scandinavica 110:267–69. doi:10.1111/ane.2004.110.issue-4. [PubMed: 15355492]

Nakagawa S, Yoshida S, Suematsu C, Shimizu E, Hirohata T, Kumamoto T, Yase Y, Kawai K, and Iwata S. 1977 The calcium-magnesium-deficient rat: A study on the distribution of calcium in the spinal cord using the electron probe microanalyser. Experientia 33:1225–26. doi:10.1007/ BF01922343. [PubMed: 891886]

Nakao T, Aozasa O, Ohta S, and Miyata H. 2002 Assessment of human exposure to PCDDs, PCDFs and Co-PCBs using hair as a human pollution indicator sample I: Development of analytical method for human hair and evaluation for exposure assessment. Chemosphere 48:885–96. doi: 10.1016/S0045-6535(02)00156-X. [PubMed: 12222782]

Nishida K, Kobayashi A, and Nagahama T. 1955 A new toxic glycoside of Cycas revoluta Thunb. Part I. Isolation and the structure of cycasin. Bulletin Agricultural Chemical Social Japan 19:77–83.

Obendorf P, and Spencer PS. 2000 Medicinal use of cycads (Cycas sp.) in New Guinea and neighbouring islands. Perspectives in Human Biology 5:31–41.

Okle O, Stemmer K, Deschl U, and Dietrich DR. 2013 LBMAA induced ER stress and enhanced caspase 12 cleavage in human neuroblastoma SH-SY5Y cells at low nonexcitotoxic concentrations. Toxicological Sciences 131:217–24. doi:10.1093/toxsci/kfs291. [PubMed: 23047912]

Okumiya K, Fujisawa M, Ishine M, Tada T, Sakamoto R, Hirata Y, Del Saz EG, Griapon Y, Sanggenafa N, Togodly A, Rantetampang AL, Kokubo Y, Kuzuhara S, and Matsubayashi K. 2008 Neurodegenerative diseases in West New-Guinea. Himalayan Study Monographs 9:141–45.

Oyanagi K, Makifuchi T, Ohtoh T, Chen KM, van der Schaaf T, Gajdusek DC, Chase TN, and Ikuta F. 1994 Amyotrophic lateral sclerosis of Guam: The nature of the neuropathological findings. Acta Neuropathologica 88:405–12. doi:10.1007/BF00389491. [PubMed: 7847068]

Pablo J, Banack SA, Cox PA, Johnson TE, Papapetropoulos S, Bradley WG, Buck A, and Mash DC. 2009 Cyanobacterial neurotoxin BMAA in ALS and Alzheimer's Disease. Acta Neurologica Scandinavica 120:216–25. doi:10.1111/j.1600-0404.2008.01150.x. [PubMed: 19254284]

Papageorgiou J, Nicholson B, Wickramasinghe W, and Kapralos C. 2009 BMAA in potable waters: An international assessment of exposure levels WQRA Research Report 65. Adelaide: Water Quality Research Limited.

Papapetropoulos S 2007 Is there a role for naturally occurring cyanobacterial toxins in neurodegeneration? The beta-N-methylamino-L-alanine (BMAA) paradigm. Neurochemistry International 50:998–1003. doi:10.1016/j.neuint.2006.12.011. [PubMed: 17296249]

Partensky F, Hess WR, and Vaulot D. 1999 Prochlorococcus, a marine photosynthetic prokaryote of global significance. Microbiology and Molecular Biology Reviews 63:106–27. [PubMed: 10066832]

Perez-Tur J, Buee L, Morris HR, Waring SC, Onstead L, Wavrant-De Vrieze F, Crook R, Buee-Scherrer V, Hof PR, Petersen RC, McGeer PL, Delacourte A, Hutton M, Siddique T, Ahlskog JE, Hardy J, and Steele JC. 1999 Neurodegenerative diseases of Guam: Analysis of Tau. Neurology 53:411–13. doi:10.1212/WNL.53.2.411. [PubMed: 10430438]

Perkins HR, and Cummins CS. 1964 Chemical structure of bacterial cell walls. Ornithine and 2,4-diaminobutyric acid as components of the cell walls of plant pathogenic corynebacteria. Nature 201:1105–07. doi:10.1038/2011105a0. [PubMed: 14152785]

Perl DP, Gajdusek DC, Garruto RM, Yanagihara RT, and Gibbs CJ. 1982 Intraneuronal aluminum accumulation in Amyotrophic lateral sclerosis and Parkinsonismdementia of Guam. Science 217:1053–55. doi:10.1126/science.7112111. [PubMed: 7112111]

Perry TL, Bergeron C, Biro AJ, and Hansen S. 1989 Beta-N-methylamino-L-alanine. Chronic oral administration is not neurotoxic to mice. Journal of the Neurological Sciences 94:173–80. doi: 10.1016/0022-510X(89)90227-X. [PubMed: 2614465]

Perry TL, Bergeron C, Steele JC, McLachlan DR, and Hansen S. 1990 Brain amino acid contents are dissimilar in sporadic and Guamanian amyotrophic lateral sclerosis. Journal of the Neurological Sciences 99:3–8. doi:10.1016/0022-510X(90)90194-R. [PubMed: 2250169]

Piccardo P, Yanagihara R, Garruto RM, Gibbs CJ Jr., and Gajdusek DC. 1988 Histochemical and X-ray microanalytical localization of aluminum in Amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. Acta Neuropathologica 77:1–4. doi:10.1007/BF00688235. [PubMed: 2467502]

Plato CC, Cruz MT, and Kurland LT. 1969 Amyotrophic lateral sclerosis-Parkinsonism dementia complex of Guam: Further genetic investigations. American Journal Human Genetics 21:133–41.

Plato CC, Galasko D, Garruto RM, Plato M, Gamst A, Craig UK, Torres JM, and Wiederholt W. 2002 ALS and PDC of Guam: Forty-year follow-up. Neurology 58:765–73. doi:10.1212/WNL.58.5.765. [PubMed: 11889241]

Plato CC, Garruto RM, Galasko D, Craig UK, Plato M, Gamst A, Torres JM, and Wiederholt W. 2003 Amyotrophic lateral sclerosis and Parkinsonism dementia complex of Guam: Changing incidence rates during the past 60 years. American Journal of Epidemiology 157:149–57. doi:10.1093/aje/kwf175. [PubMed: 12522022]

Pragst F, and Balikova MA. 2006 State of the art in hair analysis for detection of drug and alcohol abuse. Chimica Acta 370:17–49. doi:10.1016/j.cca.2006.02.019.

Rao SL, Adiga PR, and Sarma PS. 1964 The isolation and characterization of beta-N-oxalyl-L-alpha,beta-diami-nopropionic Acid: A neurotoxin from the seeds of Lathyrus sativus. Biochemistry 3:432–36. doi:10.1021/bi00891a022. [PubMed: 14155110]

Reed D, Labarthe D, Chen KM, and Stallones R. 1987 A cohort study of Amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam and Rota. American Journal of Epidemiology 125:92–100. doi:10.1093/oxfordjournals.aje.a114515. [PubMed: 3788958]

Reed DM, and Brody JA. 1975 Amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam, 1945–1972. I. Descriptive epidemiology. American Journal of Epidemiology 101:287–301. doi: 10.1093/oxfordjournals.aje.a112097. [PubMed: 1124759]

Reed DM, Torres JM, and Brody JA. 1975 Amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam, 1945–1972. II. Familial and genetic studies. American Journal of Epidemiology 101:302–10. doi:10.1093/oxfordjournals.aje.a112098. [PubMed: 1124760]

Reiff DM, Spathis R, Chan CW, Vilar MG, Sankaranarayanan K, Lynch D, Ehrlich E, Kerath S, Chowdhury R, Robinowitz L, Koji Lum J, and Garruto RM. 2011 Inherited and somatic mitochondrial DNA mutations in Guam amyotrophic lateral sclerosis and Parkinsonism-dementia. Neurological Sciences 32:883–92. doi:10.1007/s10072-011-0735-9. [PubMed: 21822691]

Reveillon D, Abadie E, Sechet V, Brient L, Savar V, Bardouil M, Hess P, and Amzil Z. 2014 Beta-N-methylamino-L-alanine: LC-MS/MS optimization, screening of cyanobacterial strains and occurrence in shellfish from Thau, a French Mediterranean lagoon. Marine Drugs 12:5441–67. doi:10.3390/md12115441. [PubMed: 25405857]

Reveillon D, Abadie E, Sechet V, Masseret E, Hess P, and Amzil Z. 2015 β-N-methylamono-L-alanine (BMAA) and its isomers: Distribution in different food web compartments of Thau lagoon, French Mediterranean Sea. Marine Environmental Research 110:8–18. doi:10.1016/j.marenvres.2015.07.015. [PubMed: 26254582]

Reveillon D, Sechet V, Hess P, and Amzil Z. 2016 Production of BMAA and DAB by diatoms (Phaeodactylum tricornutum, Chaetoceros sp., Chaetoceros calcitrans and Thalassiosira pseudonana) and bacteria isolated from a diatom. Harmful Algae 58:45–50. doi:10.1016/j.toxicon.2015.11.011. [PubMed: 28073457]

Richer R, Banack SA, Metcalf JS, and Cox PA. 2015 The persistence of cyanobacterial toxins in desert soils. Journal of Arid Environments 112:134–39. doi:10.1016/j.jaridenv.2014.01.023.

Rodgers KJ, and Shiozawa N. 2008 Misincorporation of amino acid analogues into proteins by biosynthesis. The International Journal of Biochemistry & Cell Biology 40:1452–66. doi:10.1016/j.biocel.2008.01.009. [PubMed: 18329946]

Rodgers-Johnson P, Garruto RM, Yanagihara R, Chen KM, Gajdusek DC, and GibbsJr CJ. 1986 Amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam: A 30-year evaluation of clinical and neuro- pathologic trends. Neurology 36:7–13. doi:10.1212/WNL.36.1.7. [PubMed: 3941784]

Rogers RF 1995 Destiny's landfall: A history of Guam. Honolulu, HI: University of Hawaii Press.

Roman GC 1996 Neuroepidemiology of amyotrophic lateral sclerosis: Clues to aetiology and pathogenesis. Journal of Neurology, Neurosurgery 61:131–37. doi:10.1136/jnnp.61.2.131.

Roney BR, Li RH, Banack SA, Murch S, Honegger R, and Cox PA. 2009 Consumption of fa cai Nostoc soup: A potential for BMAA exposure from Nostoc cyanobacteria in China? Amyotrophic Lateral Sclerosis 10:44–49. doi:10.3109/17482960903273031. [PubMed: 19929731]

Rosen J, and Hellenas KE. 2008 Determination of the neurotoxin BMAA (beta-N-methylamino-L-alanine) in cycad seed and cyanobacteria by LC-MS/MS (liquid chromatography tandem mass spectrometry). The Analyst 133:1785–89. doi:10.1039/b809231a. [PubMed: 19082084]

Rosen J, Westerberg E, Schmiedt S, and Hellenas KE. 2016 BMAA detected as neither free nor protein bound amino acid in blue mussels. Toxicon 109:45–50. doi: 10.1016/j.toxicon.2015.11.008. [PubMed: 26577502]

Safe S 1993 Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environmental Health Perspectives 100:259–68. [PubMed: 8354174]

Schneider SA 2016 Neurodegeneration with brain iron accumulation. Current Neurology and Neuroscience Reports 16:9. doi:10.1007/s11910-015-0608-3. [PubMed: 26739693]

Scott LL, Downing S, Phelan RR, and Downing TG. 2014 Environmental modulation of microcystin and beta- N-methylamino-L-alanine as a function of nitrogen availability. Toxicon 87:1–5. doi: 10.1016/j.toxicon.2014.05.001. [PubMed: 24878376]

Scott PM, Niedzwiadek B, Rawn DF, and Lau BP. 2009 Liquid chromatographic determination of the cyanobacter- ial toxin beta-N-methylamino-L-alanine in algae food supplements, freshwater fish, and bottled water. Journal of Food Protection 72:1769–73. doi:10.4315/0362-028X-72.8.1769. [PubMed: 19722418]

Seawright AA, Brown AW, Nolan CC, and Cavanagh JB. 1990 Selective degeneration of cerebellar cortical neurons caused by cycad neurotoxin, L-beta-methyla- minoalanine (L-BMAA), in rats. Neuropathology and Applied Neurobiology 16:153–69. doi:10.1111/j.1365-2990.1990.tb00944.x. [PubMed: 2345599]

Shankar SK, Yanagihara R, Garruto RM, Grundke- Iqbal I, Kosik KS, and Gajdusek DC. 1989 Immunocytochemical characterization of neurofibrillary tangles in amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. Annals of Neurology 25:146–51. doi:10.1002/(ISSN)1531-8249. [PubMed: 2493213]

Shen WB, McDowell KA, Siebert AA, Clark SM, Dugger NV, Valentino KM, Jinnah HA, Sztalryd C, Fishman PS, Shaw CA, Jafri MS, and Yarowsky PJ. 2010 Environmental neurotoxin-induced progressive model of Parkinsonism in rats. Annals of Neurology 68:70–80. doi:10.1002/ana.22018. [PubMed: 20582986]

Shiraki H 1969 The neuropathology of Amyotrophic lateral sclerosis (ALS) in the Kii Peninsula and other areas of Japan In Motor neuron diseases: Research on amyotrophic lateral sclerosis and related disorders, eds. NorrisJr FH, and Kurland LT, 80–84. New York, NY: Grune and Stratton.

Sieber SM, Correa P, Dalgard DW, McIntire KR, and Adamson RH. 1980 Carcinogenicity and hepatotoxicity of cycasin and its aglycone methylazoxymethanol acetate in nonhuman primates. Journal of the National Cancer Institute 65:177–89. [PubMed: 6248673]

Sieh W, Choi Y, Chapman NH, Craig UK, Steinbart EJ, Rothstein JH, Oyanagi K, Garruto RM, Bird TD, Galasko DR, Schellenberg GD, and Wijsman EM. 2009 Identification of novel susceptibility loci for Guam neurodegenerative disease: Challenges of genome scans in genetic isolates. Human Molecular Genetics 18:3725–38. doi:10.1093/hmg/ddp300. [PubMed: 19567404]

Singh-Blom A, Hughes RA, and Ellington AD. 2014 An amino acid depleted cell-free protein synthesis system for the incorporation of non-canonical amino acid analogs into proteins. Journal of Biotechnology 178:12–22. doi: 10.1016/j.jbiotec.2014.02.009. [PubMed: 24631721]

Smith QR, Nagura H, Takada Y, and Duncan MW. 1992 Facilitated transport of the neurotoxin, beta-N-methylamino-L-alanine, across the blood-brain barrier. Journal of Neurochemistry 58:1330–37. doi:10.1111/j.1471-4159.1992.tb11346.x. [PubMed: 1548467]

Snyder LR, Cruz-Aguado R, Sadilek M, Galasko D, Shaw CA, and Montine TJ. 2009 Lack of cerebral BMAA in human cerebral cortex. Neurology 72:1360–61. doi: 10.1212/WNL.0b013e3181a0fed1. [PubMed: 19365059]

Snyder LR, Hoggard JC, Montine TJ, and Synovec RE. 2010 Development and application of a comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry method for analysis of L-β-methyla-mino-alanine in human tissue. Journal of Chromatography A 1217:4639–47. doi:10.1016/j.chroma.2010.04.065. [PubMed: 20483417]

Soto C 2003 Unfolding the role of protein misfolding in neurodegenerative diseases. Nature Reviews. Neuroscience 4:49–60. doi:10.1038/nrn1007. [PubMed: 12511861]

Spacil Z, Eriksson J, Jonasson S, Rasmussen U, Ilag LL, and Bergman B. 2010 Analytical protocol for identification of BMAA and DAB in biological samples. The Analyst 135:127–32. doi:10.1039/B921048B. [PubMed: 20024192]

Spatz M, and Laqueur GL. 1967 Transplacental induction of tumors in Sprague-Dawley rats with crude cycad material. Journal of the National Cancer Institute 38:233–39. [PubMed: 6066949]

Spencer P 1989 Western pacific ALS-Parkinsonism-dementia: A model of neuronal aging triggered by environmental toxins In Parkinsonism and aging, ed. Calne DB, 133–44. New York, NY: Raven Press.

Spencer PS, Allen RG, Kisby GE, and Ludolph AC. 1990 Excitotoxic disorders. Science 248:144. doi:10.1126/science.2326627. [PubMed: 2326627]

Spencer PS, Garner CE, Palmer VS, and Kisby GE. 2016 Vervets and macaques: Similarities and differences in their responses. Neurotoxicology 56:284–286. doi:10.1016/j.neuro.2016.03.018. [PubMed: 27032904]

Spencer PS, Kisby GE, and Ludolph AC. 1991 Slow toxins, biologic markers, and long-latency neurodegenerative disease in the western Pacific region. Neurology Supplement 2 41:62–66.

Spencer PS, Nunn PB, Hugon J, Ludolph AC, Ross SM, Roy DN, and Robertson RC. 1987c Guam amyotrophic lateral sclerosis-Parkinsonism-dementia linked to a plant excitant neurotoxin. Science 237:517–22. doi:10.1126/science.3603037. [PubMed: 3603037]

Spencer PS, Ohta M, and Palmer VS. 1987a Cycad use and motor neurone disease in Kii Peninsula of Japan. Lancet 2:1462–63. doi:10.1016/S0140-6736(87)91159-7. [PubMed: 2892020]

Spencer PS, Palmer VS, Herman A, and Asmedi A. 1987b Cycad use and motor neurone disease in Irian Jaya. Lancet 2:1273–74. doi:10.1016/S0140-6736(87)91883-6.

Spencer PS, Palmer VS, and Ludolph AC. 2005 On the decline and etiology of high-incidence motor system disease in West Papua (Southwest New Guinea). Movement Disorders 20 Supplement 12:S119–S126. doi:10.1002/mds.20552.

Stanhope JM, Brody JA, and Morris CE. 1972 Epidemiologic features of amyotropic lateral sclerosis and Parkinsonism-dementia in Guam, Mariana Islands. International Journal of Epidemiology 1:199–210. doi:10.1093/ije/1.3.199. [PubMed: 4669196]

Staton PC, and Bristow DR. 1997 The dietary excitotoxins beta-N-methylamino-L-alanine and beta-N-oxalyla-mino-L-alanine induce necrotic- and apoptotic-like death of rat cerebellar granule cells. Journal of Neurochemistry 69:1508–18. doi:10.1046/j.1471-4159.1997.69041508.x. [PubMed: 9326280]

Steahly LP, and Peterson CA. 1982 Ophthalmomyiasis. Annals of Ophthalmology 14:137–39. [PubMed: 7092021]

Steele JC, Richardson JC, and Olszewski J. 1964 Progressive Supranuclear Palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, Nuchal dystonia and dementia. Archives of Neurology 10:333–59. doi: 10.1001/archneur.1964.00460160003001. [PubMed: 14107684]

Steele JC, Wresch R, Hanlon SD, Keystone J, and Ben-Shlomo Y. 2015 A unique retinal epitheliopathy is associated with amyotrophic lateral sclerosis/Parkinsonism dementia complex of Guam. Movement Disorders 30:1271–75. doi:10.1002/mds.v30.9. [PubMed: 26153661]

Stommel EW, Field NC, and Caller TA. 2013 Aerosolization of cyanobacteria as a risk factor for amyotrophic lateral sclerosis. Medical Hypotheses 80:142–45. doi: 10.1016/j.mehy.2012.11.012. [PubMed: 23246360]

Stone BC 1970 The flora of Guam. Micronesica 6:1–659.

Sundar PD, Yu CE, Sieh W, Steinbart E, Garruto RM, Oyanagi K, Craig UK, Bird TD, Wijsman EM, Galasko DR, and Schellenberg GD. 2007 Two sites in the MAPT region confer genetic risk for Guam ALS/PDC and dementia. Human Molecular Genetics 16:295–306. doi: 10.1093/hmg/ddl463. [PubMed: 17185385]

Surendranathan A, Rowe JB, and O'Brien JT. 2015 Neuroinflammation in Lewy Body dementia. Parkinsonism & Related Disorders 21:1398–406. doi:10.1016/j.parkreldis.2015.10.009. [PubMed: 26493111]

Tabata RC, Wilson JM, Ly P, Zwiegers P, Kwok D, Van Kampen JM, Cashman N, and Shaw CA. 2008 Chronic exposure to dietary sterol glucosides is neurotoxic to motor neurons and induces an ALS-PDC phenotype. Neuromolecular Medicine 10:24–39. doi:10.1007/s12017-007-8020-z. [PubMed: 18196479]

Torbick N, Hession S, Stommel E, and Caller T. 2014 Mapping Amyotrophic lateral sclerosis lake risk factors across northern New England. International Journal of Health Geographics 13:1. doi: 10.1186/1476-072X-13-1. [PubMed: 24383521]

Torres J, Iriarte LL, and Kurlund LT. 1957 Amyotrophic lateral sclerosis in Guamanian in Californians. California Medicine 86:385–88. [PubMed: 13426814]

Troost BT, and Daroff RB. 1977 Ocular motor defects in progressive Supranuclear Palsy. Annals of Neurology 2:397–403. doi:10.1002/ana.410020509. [PubMed: 617579]

Umahara T, Hirano A, Kato S, Shibata N, and Yen SH. 1994 Demonstration of neurofibrillary tangles and neuropil thread-like structures in spinal cord white matter in Parkinsonism dementia complex on Guam and in Guamanian amyotrophic lateral sclerosis. Acta Neuropathologica 88:180–84. doi:10.1007/BF00294512. [PubMed: 7985499]

Van Onselen R, Cook NA, Phelan RR, and Downing TG. 2015 Bacteria do not incorporate beta-N-methylamino-L-alanine into their proteins. Toxicon 102:55–61. doi:10.1016/j.toxicon.2015.05.014. [PubMed: 26051985]

Vega A, and Bell EA. 1967 a-Amino-β-methylaminopro- pionic acid, a new amino acid from seeds of Cycas circi- nalis. Phytochemistry 6:759–62. doi:10.1016/S0031-9422(00)86018-5.

Vega A, Bell EA, and Nunn PB. 1968 The preparation of L-and D-alpha-aminobeta-methylaminopropionic acids and the identification of the compound isolated from Cycas circinalis as the L-isomer. Phytochemistry 7:1885–87. doi:10.1016/S0031-9422(00)86667-4.

Walton JR 2006 Aluminum in hippocampal neurons from humans with Alzheimer's disease. Neurotoxicology 27:385–94. doi:10.1016/j.neuro.2005.11.007. [PubMed: 16458972]

Walton JR 2010 Evidence for participation of aluminum in neurofibrillary tangle formation and growth in Alzheimer's disease. Journal of Alzheimer's Disease 22:65–72.

Whiting MG 1963 Toxicity of cycads. Economic Botany 17:271–302. doi: 10.1007/BF02860136.

Whiting MG 1964 Food practices in Als Foci in Japan, the Marianas, and New Guinea. Federation Proceedings 23:1343–45. [PubMed: 14236144]

WHO. 2011 Guidelines for drinking-water quality 4th edn Chapter 12: Chemical fact sheet. 386–87. Geneva: WHO.

Wiles GJ 1987a Current research and future management of marianas fruit bats (Chiroptera pteropodidae) on Guam. Australian Mammol 10:93–95.

Wiles GJ 1987b The status of fruits on Guam. Pacific Science 41:148–57.

Williams M, Todd GD, Roney N, Crawford J, Coles C, McClure PR, Garey JD, Zaccaria K, and Citra M. 2012 Toxicological profile for manganese. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

Wilson JM, Khabazian I, Wong MC, Seyedalikhani A, Bains JS, Pasqualotto BA, Williams DE, Andersen RJ, Simpson RJ, Smith R, Craig UK, Kurland LT, and Shaw CA. 2002 Behavioral and neurological correlates of ALS-Parkinsonism dementia complex in adult mice fed washed cycad flour. Neuromolecular Medicine 1:207–21. doi: 10.1385/NMM:1:3:207. [PubMed: 12095162]

Xie X, Basile M, and Mash DC. 2013 Cerebral uptake and protein incorporation of cyanobacterial toxin beta-N- methylamino-L-alanine. Neuroreport 24:779–84. doi:10.1097/WNR.0b013e328363fd89. [PubMed: 23979257]

Yanagihara R, Garruto RM, Gajdusek DC, Tomita A, Uchikawa T, Konagaya Y, Km C, Sobue I, Cc P, and GibbsJr CJ . 1984 Calcium and vitamin D metabolism in Guamanian Chamorros with amyotrophic lateral sclerosis and Parkinsonism-dementia. Annals of Neurology 15:42–48. doi: 10.1002/ana.410150108. [PubMed: 6546847]

Yase Y 1970 Neurologic disease in the western Pacific islands, with a report on the focus of amyotrophic lateral sclerosis found in the Kii peninsula, Japan. The American Journal of Tropical Medicine and Hygiene 19: 155–66. [PubMed: 5416288]

Yase Y 1972 The pathogenesis of amyotrophic lateral sclerosis. Lancet 2:292–96. doi:10.1016/S0140-6736(72)92903-0. [PubMed: 4115029]

Yase Y 1978 [Motor neuron disease]. Nihon Rinsho 35 (Suppl):1716–17.

Yase Y 1979 ALS in the Kii Peninsula: One possible etiological hypothesis In Amyotrophic lateral sclerosis, eds. Tsubaki T, and Toyokwa Y, 307–18. Tokyo: University of Tokyo Press.

Yasui M, Ota K, and Yoshida M. 1997 Effects of low calcium and magnesium dietary intake on the central nervous system tissues of rats and calcium-magnesium related disorders in the Amyotrophic lateral sclerosis focus in the Kii Peninsula of Japan. Magnesium Research 10:39–50. [PubMed: 9339837]

Yasui M, Yase Y, Kihira T, Adachi K, and Suzuki Y. 1992 Magnesium and calcium contents in CNS tissues of amyotrophic lateral sclerosis patients from the Kii peninsula, Japan. European Neurology 32:95–98. doi:10.1159/000116800. [PubMed: 1314186]

Yasui M, Yase Y, and Ota K. 1991a Distribution of calcium in central nervous system tissues and bones of rats maintained on calcium-deficient diets. Journal of the Neurological Sciences 105:206–10. doi:10.1016/0022-510X(91)90146-X. [PubMed: 1757797]

Yasui M, Yase Y, Ota K, Mukoyama M, and Adachi K. 1991b High aluminum deposition in the central nervous system of patients with amyotrophic lateral sclerosis from the Kii Peninsula, Japan: Two case reports. Neurotoxicology 12:277–83. [PubMed: 1956586]

Yoshimasu F, Uebayashi Y, Yase Y, Iwata S, and Sasajima K. 1976 Studies on amyotrophic lateral sclerosis by neutron activation analysis. Folia Psychiatrica et Neurologica Japonica 30:49–55. [PubMed: 955523]

Yoshimasu F, Yasui M, Yase Y, Iwata S, Gajdusek DC, GibbsJr CJ., and Chen K. 1980 Studies on amyotrophic lateral sclerosis by neutron activation analysis-2. Comparative study of analytical results on Guam PD, Japanese ALS and Alzheimer disease cases. Folia Psychiatrica et Neurologica Japonica 34:75–82. [PubMed: 7390331]

Yoshimasu F, Yasui M, Yase Y, Uebayashi Y, Tanaka S, Iwata S, Sasajima K, Gajdusek DC, Gibbs CJ, and Chen K. 1982 Studies on amyotrophic lateral sclerosis by neutron activation analysis. 3. Systematic analysis of metals on Guamanian ALS and PD cases. Folia Psychiatrica et Neurologica Japonica 36:173–79. [PubMed: 7129262]

Zhang ZX, Anderson DW, and Mantel N. 1990 Geographic patterns of Parkinsonism-dementia complex on Guam. 1956 through 1985. Archives of Neurology 47:1069–74. doi:10.1001/archneur.1990.00530100031010. [PubMed: 2222238]

Zhang ZX, Anderson DW, Mantel N, and Roman GC. 1996 Motor neuron disease on Guam: Geographic and familial occurrence, 1956–85. Acta Neurologica Scandinavica 94:51–59. doi:10.1111/j.1600-0404.1996.tb00039.x. [PubMed: 8874594]

Zolan WJ, and Ellis-Neill L. 1986 Concentrations of aluminum, magnesium, iron, and calcium in four southern Guam rivers Technical Report No. 64: 1–68. Guam: University of Guam; Water and Energy Institute of the Western Pacific.

Zurawell RW, Chen H, Burke JM, and Prepas EE. 2005 Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. Journal Toxicogical Environment Health B 8:1–37. doi:10.1080/10937400590889412.

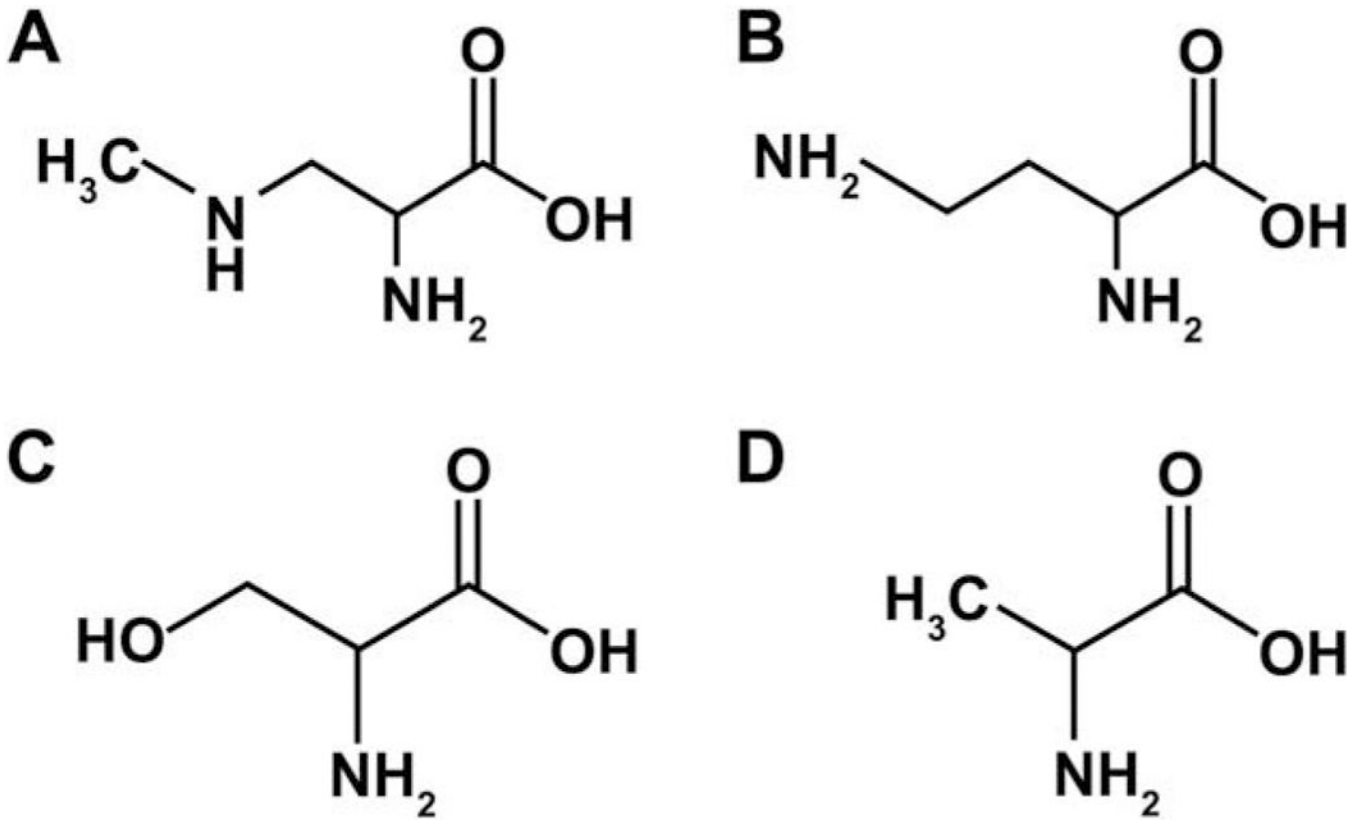

Figure 1.

Chemical structures for BMAA and similar amino acids A. β-*N*-methylamino-L-alanine (BMAA); B. 2,4-diamino butyric acid (DAB); C. serine; D. alanine.

Table 1.

Comparison of ALS/PDC relationships to amyotropic lateral sclerosis, Parkinsonism, and Alzheimer's disease

Characteristic	ALS/PDC	ALS	Parkinsonism	Alzheimer's	References
Geographical Component	Strong	Absent	Absent	Absent	McGeer and Steele (2011); Shiraki (1969); Gajdusek and Salazar (1982)
Environmental Factors Implicated	Strong	Absent	Absent	Absent	Whiting (1964); Kurland (1972)
Mixed Syndromes	Common	Absent	Absent	Absent	Koerner (1952); Malamud, Hirano, and Kurland (1961); Murakami (1999)
Familial Occurrence	Common	Absent[1]	Absent	Absent	Kurland and Mulder (1954a, 1954b); Malamud, Hirano, and Kurland (1961)
Age of Onset	44 yrs in pre-1980's period	59 yrs	62 yrs	72 yrs	Elizan et al. (1966); Hirano et al. (1966)
Presence/location of tau protein NFTs	Cerebral cortex layers II and III, hypothalamus, and spinal cord	Absent	Absent[2]	Cerebral cortex and cortical layers V and VI	Hof et al. (1991, 1994); Ludolph et al. (2009)
Substantia Nigra Effects	Yes	Absent	Yes	Absent	Hirano et al. (1966)
Presence of Ubiquitin Proteins (e.g. TDP43)	Present	Present	Rare or absent	Absent	Geser et al. (2008)
Progressive Paralysis	Present	Present	Absent	Absent	
Presence of Synucleopathy	Rare or absent	Rare or absent	Present as Lewy bodies	Rare or absent	Hirano et al. (1966); Mimuro, Kokubo and Kuzuhara (2007)
Retinal Pigment Epitheliopathy	Present	Absent	Absent	Absent	Hanlon and Steele (1993); Campbell et al. (1993);Kokubo et al. (2006)
Pill Rolling	Absent	Absent	Present	Absent	Brody et al. (1971)
Senile Plaques	Absent	Absent	Absent	Present	Hirano et al. (1961b); Hof et al. (1994)

[1]Familial associations do occur in ALS (FALS—mutation in superoxide dismutase gene (SOD1) they are rare and only account for approximately 10% of the cases); and parkinsonism (mutation in alpha synuclein gene (SNCA)), but these are relatively uncommon as compared to the sporadic forms of the diseases.

[2]Neurofibrillary tangles.

[3]There are uncommon tauopathies associated parkinsonism (frontotemporal dementia with parkinsonism; progressive supranuclear palsy; Pick disease; postencephalitic parkinsonism).

Table 2.

Presence of BMAA in fish

Year	Author	Quantification method[7]	BMAA Type	LOD[1]	LOQ[2]	Derivatization	Total fish sampled (pos/neg)	Avg per sample (μg/g)	Range (μg/g)
2009	Scott et al.	FD	free, protein bound	0.000034 nmol/inj.	0.00011 nmol/inj.	FMOC[3]	2 (0/2)	ND[6]	ND
2010	Brand et al.	FD	total	0.019 nmol/inj.	0.084 nmol/inj	AQC[4]	43 (22/21)	653.2	ND-6937
2010	Jonasson et al.	HPLC/MS	total	0.65 nmol/mL	6.5 nmol/mL	AQC	7 (5/2)	0.23	ND-1.29
2010	Spacil et al.	HPLC/MS	total	0.0014 nmol/mL	NR[5]	AQC	1 (1/1)	NR	
2012	Mondo et al.	FD	total	23 nmol/mL	590 nmol/mL	AQC	29 (23/6)	440.3	ND-1836
2014	Al-Sammak et al.	HPLC/FD LC/MS	free, protein bound	0.04 nmol/mL 0.007 nmol/mL	NR NR	AQC	246 (31/215)	0.122	ND-2.57 ND-4.13
2014	Jiao et al.	HPLC/MS	free, protein bound	NR	NR	AQC	30 (30/0)	5.702	0.07–35.91
2014	Jiang et al.	UHPLC/MS	total	0.84 nmol/g	0.84 nmol/g	AQC	21 (5/16)	0.003	ND-0.02
2015	Banack et al.	LC/MS	free, protein bound	0.000048 nmol/inj.	0.00048 nmol/inj.	AQC	1 (1/0)	1.27	0.43–1.28
2015	Lage et al.	UPLC/MS	total	0.006 nmol/mL	0.006 nmol/mL	AQC	181 (27/154)	0.0007	ND-0.42

[1] Limit of detection.

[2] Limit of quantification.

[3] 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate.

[4] 9-Fluorenylmethyl chloroformate.

[5] Not recorded.

[6] Not detected.

[7] FD is fluorescence detection; HPLC is high performance liquid chromatography; MS is mass spectrophotometry; LC is liquid chromatography; UHPLC is ultra high performance liquid chromatography.

Table 3.

Presence of BMAA in human brains and cerebrospinal fluid

DATE	AUTHOR	Quantification method[11]	BMAA type	LOD1	LOQ2	Derivatized	Disease present; BMAA Present (yes/no)	Disease absent; BMAA Present (yes/no)	Range (μg/g)
1990	Perry et al.	amino acid analyzer	total	NR[4]	NR	No	0/5	NA[9]	ND[10]
2004a	Murch et al.	FD	protein bound	0.1 nmol/inj.	13 nmol/inj.	AQC[5]	8/8	1/15	ND-1190
2004b							6/7	0/1	ND-235.6
2005	Montine et al.[3]	FD	free	0.001 nmol/inj.	NR	FMOC[6]	0/13	0/7	ND
2009	Pablo et al.	FD	protein bound	16.9 nmol/mL	85 nmol/mL	AQC	25/25	3/20	ND-256.0
2009	Snyder et al.[3]	HPLC/MS	free, protein bound	free: 0.85 nmol/g bound: 42 nmol/g	free: 2 nmol/g bound: 85 nmol/g	EOC[7]	0/13	0/7	ND
2010	Snyder et al.[3]	GC/MS	free, protein bound	0.005 nmol/g	NR	TMS[8]	0/13	0/7	ND
2014	Combes et al.	LC/MS	free	NR	0.17 nmol/g	No	0/2	NA	ND
2015	Berntzon et al.	UHPLC/MS	free, protein bound	0.12 nmol/mL	0.12 nmol/mL	AQC	1/12	2/13	ND-0.0005 ug/ml

[1] Limit of Detection.

[2] Limit of Quantification.

[3] Identical tissues examined in Montine et al. and Snyder et al. (2009, 2010).

[4] Not Reported.

[5] 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate.

[6] 9-Fluorenylmethylchloroformate.

[7] Ethyl chloroformate.

[8] Trimethylsilation reagent.

[9] Not Applicable.

[10] Not Detected.

[11] FD is fluorescence detection; HPLC is high performance liquid chromatography; MS is mass spectrophotometry; GC is gas chromatography; LC is liquid chromatography; UHPLC is ultra high performance liquid chromatography.

Table 4.

Presence of BMAA in cyanobacterial species

Year	Author	Quantification method[6]	BMAA Type	LOD1	LOQ2	Derivatization	Total samples (pos/neg)	Range (μg/g)
2005	Cox et al.	HPLC/FD	total, free	0.065 nmol/inj.	6.5 nmol/inj	AQC[5]	30 (29/1)	ND[4]-6478.00
2007	Banack et al.	HPLC/FD, UPLC/UV	free, total	0.25 nmol/g	2.8 nmol/g	AQC	1 (1/0)	7.00–25.00
2008	Esterhuizen & Downing	GC/MS	total	2.1 nmol/mL	42 nmol/mL	Phenomonex eZ:faast and proprietary chloroformate derivative	27 (26/1)	ND-2755.60
2008	Johnson et al.	UPLC/MS, LC/MS	total	0.65 nmol/mL	6.5 nmol/mL	AQC	7 (7/0)	2.04–21.51
2008	Kubo et al.	LC/MS	free, total	NR**3**	17 nmol/g	No	5 (0/5)	ND
2008	Metcalf et al.	HPLC/FD	free, bound	0.065 nmol/inj.	NR	AQC	12 (12/0)	ND-276.00
2008	Rosen and Hellenas	LC/MS	total, free, bound	free: <8.5 nmol/g bound: <34 nmol/g	NR	AQC	36 (0/36)	ND
2009	Faassen et al.	LC/MS	free, bound	free: 34 nmol/g bound: 51 nmol/g	NR	No	21 (9/12)	ND-42.00
2009	Papageorgiou	HPLC/FD, HPLC/MS	free	0.850 nmol/g	1.7 nmol/g	AQC	12 (0/12)	ND
2010	Jonasson et al.	UPLC/MS	total	0.0014 nmol/mL	NR	AQC	13 (13/0)	0.001 –0.087
2010	Kruger et al.	LC/MS	free, bound	8.5 nmol/g	NR	No	30 (0/30)	ND
2010	Spacil et al.	UPLC/MS	free	0.0014 nmol/mL	NR	AQC	2 (2/0)	NR
2012	Cervantes Cianca et al.	HPLC/FD	both	free: 0.13 e –6 nmol/mL bound 0.06 e –6 nmol/mL	free: 0.42 e –6 nmol/mL bound: 0.19 e –6 nmol/mL	AQC	15 (15/0)	0.04–63
2012	Faassen et al.	LC/MS	free, bound	Derivated: 8.5 nmol/g Underived: 3.4 nmol/g	Derivated: 8.5 nmol/g Underived: 3.4 nmol/g	AQC	8 (8/0)	ND
		HPLC/FD		NR	NR			6.3–56.2
2012	Li et al.	LC/MS	free, bound	0.03 nmol/g	NR	No	2 (0/2)	ND
2013	Jiang et al.	LC/MS	free	0.84 nmol/g	0.84 nmol/g	AQC	1 (1/0)	0.73

Year	Author	Quantification method[6]	BMAA Type	LOD1	LOQ2	Derivatization	Total samples (pos/neg)	Range (μg/g)
2014	Fan et al.	LC/MS	free, bound	0.003 nmol/g	NR	AQC	17 (0/17)	ND
2014	Jiao et al.	UPLC/MS	total	NR	NR	AQC	10 (10/0)	2.03–7.14
2014	Reveillon et al.	LC/MS	total, free, bound	NR	1.9 nmol/g	No	10 (0/10)	<LOD- 7.2
2014	Scott et al.	LC/MS	bound	NR	NR	Propyl Chloroformate (EzFaast)	1 (1/0)	252
2015	Baptista et al.	LC/MS	total	NR	0.83 nmol/g	Microwave	2 (0/2)	ND
2016	Lage et al.	UPLC/MS	total, free, bound	0.14 nmol/g	0.14 nmol/g	AQC and No	3 (3/0)	0.0080–1.7687

[1] Limit of Detection.

[2] Limit of Quantification.

[3] Not Reported.

[4] Not Detected.

[5] 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate.

[6] FD is fluorescence detection; HPLC is high performance liquid chromatography; MS is mass spectrophotometry; GC is gas chromatography; LC is liquid chromatography; UHPLC is ultra high performance liquid chromatography.