Chemical Information Review Document

for

L-β-Methylaminoalanine
[CAS No. 15920-93-1]

Supporting Nomination for Toxicological Evaluation by the National Toxicology Program

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National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
Research Triangle Park, NC
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Abstract

L-β-Methylaminoalanine (L-BMAA) is a neurotoxic non-protein amino acid that is produced by cyanobacteria, a blue-green algae that is common to many lakes, oceans, and soils, and is found in *Cycas circinalis* seeds. It has previously been associated with the neurological disease amyotrophic lateral sclerosis-Parkinson dementia complex (ALS-PDC) in an indigenous population in Guam. Consumption of tortillas made from cycad-seed flour and of flying foxes (Mariana fruit bats, a "traditional delicacy" in Guam) that feed on cycad fruit has been implicated as the source of L-BMAA exposure. Protein-bound and free L-BMAA have been shown to bioconcentrate in the Guam ecosystem (bacteria, plants, animals, and human tissues) and detectable levels have been measured in brain tissue of people in Guam who suffered from dementia-related disorders. Because it is present in blue-green algae from a variety of water sources around the world and samples of Baltic Sea and oceanic blooms, significant quantities may be released into the world's oceans and water supplies. Studies in primates showed that L-BMAA produced a neurodegenerative syndrome very similar to that of ALS-PDC. Single doses also induced seizures in mice and rats and intracranial injection in mice produced hippocampal damage. In short-term studies, L-BMAA produced no neurotoxic effects in mice but induced chronic convulsions, splay, and rigidity (all reversible) in rats. In rat pups, L-BMAA resulted in an increase in open field behavior, hindlimb splay, and ovarian and anterior pituitary weights and a decrease in spinal cord and cerebellar weights. Neuronal degeneration and cell death were seen in *in vitro* studies. However, these only occurred in the presence of bicarbonate. L-BMAA also induced intracellular Ca^{+2} release and reactive oxygen species generation in spinal cord cultures. Stimulation of both NMDA and non-NMDA glutamate receptors by L-BMAA has been suggested as the mechanisms mediating the neurotoxic effects. Studies in rats showed that L-BMAA is rapidly taken up by central nervous system tissues and is eliminated at a much slower rate. Although numerous neurotoxicity studies have been conducted with L-BMAA in mice, rats, chickens, primates, and humans *in vivo* and several systems *in vitro*, studies to evaluate other toxicological endpoints, including reproductive, genotoxicity, and carcinogenicity effects are lacking. There is evidence to suggest that a long latency period between exposure and affect may exist and could affect the results of toxicity studies.

Executive Summary

Basis for Nomination

L-β-Methylaminoalanine (L-BMAA) is a neurotoxic non-protein amino acid produced naturally by cyanobacteria found in freshwater, marine and terrestrial ecosystems. L-BMAA was nominated by the National Institute of Environmental Health Sciences for toxicological characterization based on potential human exposure due to localized and possibly widespread occurrence of L-BMAA in the environment; its purported role as a risk factor for neurodegenerative diseases in certain populations, and insufficient toxicological data. L-BMAA has been associated with the neurological disease amyotrophic lateral sclerosis-Parkinson dementia complex (ALS-PDC) in an indigenous population in Guam. Consumption of tortillas made from cycad-seed flour and of flying foxes (Mariana fruit bats) that feed on cycad fruit has been implicated as the source of exposure. L-BMAA has also been detected in brain tissues of individuals in Canada with Alzheimer's disease. It is present in blue-green algae samples from a variety of water sources around the world and in samples from the Baltic Sea and oceanic blooms suggesting that significant quantities may be released into the world's oceans. Although numerous neurotoxicity studies have been conducted with L-BMAA in mice, rats, chickens, primates, and humans *in vivo* and in several systems *in vitro*, studies of other toxicological effects, including reproductive, developmental, and genotoxic, are lacking.

Nontoxicological Data

L-BMAA is a neurotoxic excitatory amino acid found in fresh false sago palm *Cycas circinalis* seeds at a level of 0.015%. It is concentrated in cycad reproductive tissues, with the highest concentrations of L-BMAA in the immature staminate sporangium and the outer layer of the sarcotesta. L-BMAA can be identified in plants and animals (e.g., flying foxes) by high performance liquid chromatography and liquid chromatography with mass spectrometry (MS) and in foods and biological tissues/fluids by gas chromatography with MS. L-BMAA is produced by cyanobacteria, a blue-green algae common to lakes, oceans, and soil, and can be synthesized from the reactions of α-acetamidoacrylic acid and [^{15}N]methylamine in enzyme or α-acetamino-β-methylaminopropionic acid and Acylase I. Cycad seeds are used in food (mainly to make flour for tortillas by the Chamorro people), medicines, and cosmetics, as well as in cultural and religious ceremonies.

L-BMAA is produced by cyanobacteria and therefore found in lakes, oceans, and soil. Additionally, it has been noted that fish and animals may incorporate L-BMAA from their consumption of cyanobacterial blooms in drinking water. Protein-bound and free L-BMAA have been found in the Guam ecosystem (bacteria, plants, animals, and human tissues); it is biomagnified as it travels up the ecosystem. Human populations may potentially be exposed to L-BMAA from water sources containing blue-green algae blooms, consumption of fish or animals that have been exposed to L-BMAA, or ingestion of foods derived from cycad plants or of flying foxes, which are considered a traditional delicacy in Guam. Used as food, the cycad seed flour releases up to 169 μg/g L-BMAA via acid hydrolysis. Exposure may also occur from the use of L-BMAA-containing cycad seeds in medicines and cosmetics or blue-green algae dietary supplements containing L-BMAA.

Toxicological Data

No studies on chronic exposure, carcinogenicity, genotoxicity, or immunotoxicity were available.

<u>Human Data</u>

In 1956 the Guam Research Center reported a high degree of familial occurrence of ALS and PDC, suggesting these are inherited disorders yet there was no demonstrable genetic or infectious origin. More recent studies have linked the high incidence of ALS-PDC among the Chamorro people of Guam to exposure to L-BMAA from consumption of cycad-derived foods. L-BMAA (3-10 μg/g [25-85 nmol/g])

was detected as the free amino acid in brain tissue from the superior frontal gyrus of 5/6 Chamorro people who suffered from dementia-related disorders including ALS-PDC (aged 39-89 at death). It was present in the protein fraction (82-1190 µg/g [0.69-10.07 µmol/g]) of all six patients. Both forms of L-BMAA were also found at comparable levels in two Canadians who died of progressive neurodegenerative disease. L-BMAA was detected at high levels in the frontal cortex (25.9-45.7 µg/g [0.219-0.387 µmol/g]), temporal cortex (33.7-170.8 µg/g [0.285-1.446 µmol/g]), parahippocampal gyrus (34.2 µg/g [0.290 µmol/g]), or caudate (29.6 µg/g [0.251 µmol/g]) of the brain of nine Canadian Alzheimer's patients. In contrast, no free L-BMAA was detected in frozen brain samples from control subjects and Alzheimer's disease patients from the U.S. Pacific Northwest and from Chamorros with and without the PDC.

The neurotoxic effect of L-BMAA appears to be latent and related to the release of free L-BMAA during protein metabolism. Approximately 100,000 Chamorros in California had the same incidence of neurological diseases as those in Guam even though many of them had left Guam 40 years earlier. There is also evidence that a single exposure to L-BMAA from cycad flour can contribute to the development of a neurological disease. In 1978, a Saipanese woman and her son developed Parkinson and ALS, respectively, 19 years after participating in a feast on the northern island of Pagan where foods prepared from four pounds of cycad flour were served.

Acute Exposure

Single doses of L-BMAA (not provided in abstract) induced seizures in neonatal mice, as well as postsynaptic neuronal edema and degeneration in explants of mouse spinal cord and frontal cortex. A single dose of BMAA injected into the striatum of mice induced damage in the CA1 region of the hippocampus; some animals showed intermittent pyknotic neurons in the pyramidal layer while others exhibited significant neuronal death and complete regression of the apical processes. Intraperitoneal (i.p.) injection produced convulsions in neonatal mice (1.68-3.34 mg/g [14.2-28.3 µmol/g] bw). In male Swiss Webster mice, intracerebroventricular (i.c.v.) injection of L-BMAA (0.5-1.5 µmol [59-180 µg]) induced ataxia, ptosis, rolling, unsteady gait, scratching, jumping, myoclonic jerks, forelimb clonus, hyperlocomotion, clonic muscle spasms, tonic seizure, and hypolocomotion; all animals fully recovered. In rats, i.p. injection of L-BMAA (0.7-1.7 mg/g [6-14 µmol/g] bw) caused seizures, as well as unsteadiness, ataxia, slow torsion splasm, and significant reductions in body weights; complete recovery was seen within two to three days with the lower doses. In another rat study, i.c.v. injection of L-BMAA (500 µg [4.23 µmol]) induced clonic convulsions, splay, and rigidity, as well as irritability, forepaw and hindpaw grooming, wet-dog shaking, and deep breathing. In another study, i.c.v. administration of L-BMAA (10 nmol [1.18 µg]) caused slow growth and a typical behavioral pattern which included loss of mobility. BMAA perfused through a microdialysis probe increased basal striatum dopamine levels in a dose-dependent manner. The increase was transient and levels returned to baseline within ~75 minutes after BMAA was removed from the perfusions solution. A significant decrease in the levels of dopamine metabolites was only observed during perfusion at higher concentration (50 mM BMAA). Challenge with 1-methyl-4-phenylpyridinium ion (MPP^+) produced an inverse effect on dopamine levels relative to the BMAA dose used. Decreases in metabolite levels also were observed.

Short-term and Subchronic Exposure

No effects were observed in five different neurobehavioral tests or a memory test in mice fed a diet containing L-BMAA (1 mg [28 mg/kg bw]) for 30 days, when compared to control animals. No signs of brain or spinal damage were noted.

Daily administration of BMAA (totaling 15.5 g/kg [131 mmol/kg] of the *L*-isomer) via gavage to female CD1 mice for 11 weeks produced no neurotoxic effects. There were no changes in weight and no behavioral abnormalities or pathological changes in the brain or spinal cord.

In male Sprague-Dawley rats, daily i.c.v. injection of L-BMAA (500 µg [4.23 µmol]) for ten days induced clonic convulsions, splay, and rigidity, which were reduced four days after initial treatment. With a longer exposure period (60 days), similar behavior changes were observed for at least six days after treatment. At day 10, the behavior of the treated animals was comparable to that of controls.

Repeated daily oral administration of L-BMAA (0.81 mmol/kg [96 mg/kg]) for up to 13 weeks to male cynomolgus monkeys produced signs of behavioral changes, pyramidal dysfunction, limb weakness, atrophy, upper extremity tremor, wrist drop, bradykinesia, conduction deficits in central and peripheral motor pathways, and neuropathological changes within eight weeks.

Synergistic/Antagonistic Effects

In Vivo Assays: In rats, L-BMAA-induced behavioral changes were affected by several antagonists. Duration and severity of clonic convulsions were reduced by 6,7-dinitroquinoxaline-2,3-dione (DNQX), a non-*N*-methyl-*D*-aspartate (NMDA) glutamate receptor antagonist, while latency was significantly prolonged by DNQX, *DL*(±)2-amino-5-phosphonopentanoic acid (AP5), a NMDA receptor antagonist, and [(+)5-methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate (MK-801), a noncompetitive NMDA antagonist. Splay was slightly enhanced by MK-801, while rigidity was destroyed by MK-801 and partially inhibited by DNQX and AP5.

In Vitro Assays: In the presence of MK-801 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), L-BMAA dose-dependently increased inositol phosphate (InsP) formation in murine striatal neuronal cultures. The effects of L-BMAA were additive with carbachol-induced InsP formation but were completely blocked by phorbol ester phorbol-12,13-dibutyrate. In neonatal mouse cortex explants, L-BMAA-induced cellular changes were dose-dependently protected by 2-amino-7-phosphono-heptanoic acid (AP7).

In fetal rat cell cultures, tetrandrine significantly reduced L-BMAA-induced neuronal injury, while in rat cerebellar granule cells, *D*-AP5, (*RS*)-α-methyl-4-carboxyphenylglycine, and (*RS*)-α-methylserine-*O*-phosphate reduced L-BMAA-induced cell death. In hippocampal slices from adult rats, L-BMAA-induced increase in InsP hydrolysis was antagonized by *L*-2-amino-4-phosphonobutanoate (AP4).

In mouse primary cortical cell cultures, L-BMAA potentiated the neurotoxic effects of iron, amyloid-β, MPP+, NMDA, and buthionine sulfoxamine.

Cytotoxicity

In mouse cortical cells, L-BMAA (up to 10 M [1.2 g/mL]) induced dose-dependent neuronal degeneration; neurotoxicity was found to depend on the presence and concentration of bicarbonate. In spinal cord cultures, vacuolar change, pathological change (primarily in synapses), and postsynaptic edematous swelling were observed. In a murine neuroblastoma cell line, L-BMAA (up to 10 µM [1.2 µg/mL]) decreased neurite growth at low doses (≤0.1 µM [0.012 µg/mL]) but increased growth at high doses (≥1 µM). In dissociated spinal cord cultures, L-BMAA induced damage was mainly confined to motor neurons; no injury was reported in astrocytes. L-BMAA also induced intracellular Ca^{+2} release and reactive oxygen species generation in motor neurons. Extracts of cycad seeds caused selective dose-dependent motor neuron injury, with spinal neurons showing less damage.

L-BMAA induced effects in rat CNS monoamine neurons both *in vivo* and *in vitro*. Intracerebral and intracisternal injections of L-BMAA (10 or 400 µg [0.08 or 3.39 µmol]) produced a lesion in substantia nigra and caused loss of tyrosine hydroxylase (TH) immunoreactivity, the occurrence of TH-immunoreactive pyknotic neurons, loss of substance P-immunoreactive terminals, and a decrease in norepinephrine levels in the hypothalamus, while incubations with L-BMAA (1 mM [0.1 mg/mL]) resulted in a decrease in high-affinity norepinephrine uptake in cortical synaptosomes. In newborn rat

brain cells, L-BMAA (5 mM [0.6 mg/mL]) increased intracellular calcium levels in a dose-dependent manner in the presence of bicarbonate ion. In rat cerebellar granule cells and ventral spinal cord cells, cell death was induced. Dose-dependent toxicity was also seen in rat neuronal cells and hippocampal neurons. At >100 µM [11.8 µg/mL], L-BMAA inhibited forskolin-stimulated cAMP formation in rat brain slices and cortical membranes.

In guinea pig synaptosomes, L-BMAA (100 µM or 1 mM [11.8 µg/mL or 0.1 mg/mL]) had no effect on basal cytosolic or potassium chloride-stimulated vesicular release of glutamate or calcium-independent release of glutamate. In baby hamster kidney cells, L-BMAA (up to log 10^{-3} M) stimulated basal InsP hydrolysis and displaced glutamate binding to membranes.

In cortex and cord cultures of macaques, L-BMAA (125-500 µg/mL [1.06-4.23 mM]) produced synaptic edematous vacuolation and degeneration of selected neurons.

L-BMAA (0.1-3 mM [0.01-0.4 mg/mL]) significantly reduced input membrane resistance in leech Retzius nerve cells. The effect on membrane potential was enhanced in the presence of bicarbonate.

Reproductive and Developmental Effects

In Sprague-Dawley rat pups, s.c. injection of L-BMAA (500 mg/kg [4.23 mmol/kg] on postnatal day 5 only [group A], 500 mg/kg [4.23 mmol/kg] on postnatal days 2 and 5 [B], or 100 mg/kg [0.847 mmol/kg] on postnatal days 2 and 5 [C]) produced an increase in open field behavior in males in groups A and B and in hindlimb splay in females in group A. Deaths prior to postnatal day 7 occurred in 1/12 rats in group A, 7/24 in group B, and none in group C. Significant increases were seen in anterior pituitary weight for group B males and in ovarian weight for group A and B females compared to controls. Significant decreases were seen in spinal cord weight for group A males and in cerebellar weight for Group A males and group B females.

Other Data

Endocrine Activity: In cultured fetal rat brain cells, L-BMAA increased thyrotrophin-releasing hormone levels twofold but had no effect on somatostatin. In Sprague-Dawley rat pups, s.c. injection of L-BMAA (500 mg/kg [4.23 mmol/kg] on postnatal day 5 only [group A], 500 mg/kg on postnatal days 2 and 5 [B], or 100 mg/kg [0.847 mmol/kg] on postnatal days 2 and 5 [C]) did not affect the day of vaginal opening. Pituitary luteinizing hormone content was decreased in group A females, while significant increases in growth hormone and free thyroxine levels were seen in group B females compared to controls. Group A males had significant increases in serum thyroid-stimulating hormone levels, while group B males had significantly increased insulin growth factor 1 levels.

In rat prostate tissue or a human prostate adenocarcinomas cell line (LNCaP), L-BMAA (1-100 µM [0.1-11.8 µg/mL]) reduced androgen levels in the nuclear fraction of rat prostate tissue by 23% within 30 minutes at the lowest dose. In LNCaP cells, L-BMAA (3 mM [0.4 mg/mL]) reduced the amount by >20% in the nuclear and cytosol fractions within 18 hours.

Mechanisms of Action: In neonatal mice, L-BMAA was stated to likely exert most of its acute neurotoxic action via the A1 glutamate receptor. L-BMAA was a potent agonist of "metabolotropic" glutamate receptors and a proposed mixed agonist of "metabotropic" NMDA receptors in rats. It also modulated glutamate-induced neurotoxicity in the animals. Stimulation of both NMDA and non-NMDA glutamate receptors by L-BMAA has been suggested as the mechanisms mediating the neurotoxic effects. In dissociated rat brain cells, L-BMAA dose-dependently increased intracellular calcium levels only in the presence of bicarbonate ion, suggesting its neurotoxicity was due to an excitotoxic mechanism. In primary cortical cells, L-BMAA was proposed to exert its neurotoxic effects through three different pathways: activation of NMDA and mGluR5 receptors and induction of oxidative stress.

Effects on Blood: L-BMAA (0.30-5.00 mM [35-591 μg/mL]) produced a significant and dose-dependent change in the physical state of the cytoskeletal proteins of human erythrocyte membranes *in vitro*; the half-maximal effect on protein-protein interactions was ~0.8 mM.

Structure-Activity Relationships

β-Oxalylamino-*L*-alanine (L-BOAA) [5302-45-4; 118267-16-6]

L-BOAA is a chemically related amino acid to L-BMAA present in the seeds of *Lathyrus sativus* at levels of 1-2%. It is associated with lathyrism, and like L-BMAA has caused abnormal neurological signs (e.g., tremor, mild spasms, and bilateral extensor plantar reflexes) in macaques. In well-nourished humans, oral doses of L-BOAA (hundreds of mg/kg bw) caused neurological signs after several weeks, while intake of 100 mg/kg bw produced beginning signs of lathyrism in minimally nourished humans after two to three months.

Single doses of L-BOAA given via systemic administration or intraventricular injection have caused convulsions in newborn mice. An i.p. LD_{50} of 301 mg/kg was reported in the mouse and an LD_{50} value of 1043 mg/kg in mammals [route and species not specified]. Like L-BMAA, seizures and prominent excitotoxic pathology were seen in rodents administered L-BOAA.

L-BOAA caused dose-dependent inhibition of mitochondrial NADH-dehydrogenase in mouse brain slices as well as in isolated mouse brain mitochondria and induced lactate dehydrogenase leakage from the slice into the medium. In rat striatal homogenates, L-BOAA reduced glutamine synthetase activity and high affinity transport of D-^{3}H-asparate. However, L-BOAA and glutamate together had a synergistic stimulation of cGMP synthesis in cultured rat cerebellar granule cells.

Other Analogues

The β-*N*-carboxy adduct of L-BMAA, similar in structure to glutamate, was determined to be the compound probably responsible for the bicarbonate-dependent cytotoxicity of L-BMAA. Similar to the effects of L-BMAA, the neurotoxic excitatory amino acid analog kainate [CAS No. 487-79-6] decreased neurite outgrowth of mouse neuroblastoma cells at low doses (0.001-0.1 μM) but increased growth at higher doses (1-10 μM). In guinea pig cerebellar slices, *N*-methylamino acids (*N*-methyl-γ-aminobutyrate, *N*-methylglycine, *N*-methyltaurine, and *N*-methyl-β-alanine) diminished the frequency of spontaneous spike discharges. Like L-BMAA, the neurotoxic and neuroexcitatory effects of the structural analogs *DL*-2,4-diaminobutyrate and *DL*-2,3-diaminopropionate in isolated membrane patches were potentiated by bicarbonate.

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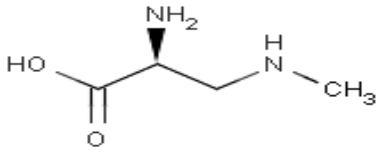
Tables:

1.0 Basis for Nomination

L-β-Methylaminoalanine (L-BMAA) is a neurotoxic non-protein amino acid produced naturally by cyanobacteria found in freshwater, marine and terrestrial ecosystems. L-BMAA was nominated by the National Institute of Environmental Health Sciences for toxicological characterization based on potential human exposure due to localized and possibly widespread occurrence of L-BMAA in the environment; its purported role as a risk factor for neurodegenerative diseases in certain populations, and insufficient toxicological data. L-BMAA has been associated with the neurological disease amyotrophic lateral sclerosis-Parkinson dementia complex (ALS-PDC) in an indigenous population in Guam. Consumption of tortillas made from cycad-seed flour and of flying foxes (Mariana fruit bats) that feed on cycad fruit has been implicated as the source of exposure. L-BMAA has also been detected in brain tissues of individuals in Canada with Alzheimer's disease. It is present in blue-green algae samples from a variety of water sources around the world and in samples from the Baltic Sea and oceanic blooms suggesting that significant quantities may be released into the world's oceans. Although numerous neurotoxicity studies have been conducted with L-BMAA in mice, rats, chickens, primates, and humans *in vivo* and in several systems *in vitro*, studies of other toxicological effects, including reproductive, developmental, and genotoxic, are lacking.

2.0 Introduction

L-β-Methylaminoalanine
[15920-93-1]



L-BMAA is a neurotoxic excitatory amino acid found in fresh false sago palm *Cycas circinalis* seeds at a level of 0.015% (Spencer et al., 1987b [PMID:3107939], 1987c [PMID:3603037]). It is produced naturally by cyanobacteria, a blue-green algae common to lakes, oceans, and soils around the world (Munro, 2005; Vowles, 2004). Cyanobacteria of the genus *Nostoc* are also found in the roots of cycad plants and produce L-BMAA that is concentrated in the plant's reproductive tissues. The highest concentrations are found in the immature seed, especially in the fleshy outer coating, the sarcotesta (Banack and Cox, 2003a). L-BMAA has been linked to the onset of a variant ALS that occurs with high incidence in the western Pacific region (Duncan et al., 1991 [PMID:2072299]). A connection between the consumption of flour from cycad seeds and neurological problems that afflicted the Chamorro people of Guam was first noted over 40 years ago (Armon, 2003; Banack and Cox, 2003a). Numerous studies since then have linked ALS-PDC disease in the Chamorro people to the consumption of cycads and of flying foxes, indigenous bats that also feed on cycads (Anonymous, 1987; Armon, 2003; Cox and Sacks, 2002 [PMID:11914415]; Duncan, 1992; Edwards, 2005; Le Page, 2004; Lieberman, 2004; Vowles, 2004). L-BMAA has been detected in samples of blue-green algae from a variety of water sources throughout the world (Edwards, 2005; Vowles, 2004) and in the Baltic Sea and oceanic blooms (Cox et al., 2005).

Substantial public health resources have been devoted to the study of potential human health effects of harmful algal blooms (HABs) and the potential health impacts of chronic low level exposure to HAB toxins remain largely unknown (Lopez et al., 2008).

2.1 Chemical Identification and Analysis

L-β-Methylaminoalanine (L-BMAA) ($C_4H_{10}N_2O_2$; mol. wt. = 118.13) is also called:

L-Alanine, 3-(methylamino)- (9CI)
L-α-Amino-β-(methylamino)propionic acid
β-(*N*-Methylamino)-*L*-alanine
L-(β-Methylamino)alanine
3-(Methylamino)-*L*-alanine
Propionic acid, 2-amino-3-(methylamino)-, *L*- (8CI)

Source: Registry (2005)

L-BMAA was first isolated and identified from the female gametophyte of *C. circinalis* (now known as *C. micronesica* Hill) from Guam using paper chromatography and high-voltage ionophoresis. The structure was determined by nuclear magnetic resonance spectroscopy (Banack and Cox, 2003a; Reece and Nunn, 1989). L-BMAA in cycad tissues was quantified by high-pressure liquid chromatography (HPLC) and Dual-1 fluorescence detection (Banack and Cox, 2003a). The protein-bound form of L-BMAA appears to be quite stable since it was detected in 55-year-old specimens of flying fox tissues analyzed by HPLC with Dual-1 fluorescence detector at concentrations of 1287-7502 µg/g [10.89-63.51 µmol/g]. If eaten, this would be equivalent to a BMAA dose received by eating 174-1014 kg of processed cycad flour (Banack and Cox, 2003a,b).

L-BMAA content in the leaves and female gametophyte of a variety of cycads was determined by combined gas chromatography/mass spectrometry (GC/MS) and showed that the content in leaves and seeds of the same species were comparable. The concentrations varied between species with the highest concentration being found in the genus Cycas (≤1800 µg/g [15.24 µmol/g]). Direct assessment of L-BMAA content in foods, biological tissues, and fluids were quantified by GC/MS (Charlton et al., 1992; Duncan et al., 1989 [PMID:2490746]). Concentrations of L-BMAA were also determined in biological samples by derivatization with 9-fluorenylmethyl chloroformate (FMOC) prior to HPLC-separation and fluorescence detection (Banack and Cox, 2003b; Kisby et al., 1988a,b [PMID:3199847]).

The L-BMAA content in cycad flour obtained from 17 Chamorro residents in Guam and the adjacent island of Rota analyzed by HPLC ranged from 0-18.39 µg/g [155.7 mmol/g] (Kisby et al., 1992a [PMID:1620343]). Methods were also described for screening L-BMAA in cycad seeds by water extraction of seed-flour samples derivatized by N(O,S)-isobutyloxycarbonylation with subsequent tert-butyldimethylsilylation and GC analysis for 17 derivatives of non-protein amino acids (Oh et al., 1995 [PMID:7700995]).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
Physical State	not provided	
Melting Point (°C)	177 (with decomposition)	Nunn et al. (1987 [PMID:3109690])
Boiling Point (°C)	284.2±30.3 @ 760.0 Torr	Registry (2005)*
Flash Point (°C)	125.7±44.2	Registry (2005)*
Vapor Pressure (Torr)	0.000782779 @ 25.0 °C	Registry (2005)*
Molar Solubility (mol/L)	≥1 @ pH 1, 4, 7, 8, 10	Registry (2005)*
Bioconcentration Factor	1 @ pH 1, 4, 7, 8, 10	Registry (2005)*

*calculated properties using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (©1994-2004 ACD/Labs)

L-BMAA is polar and non-lipophilic and can be released from its protein-bound form by acid hydrolysis (Murch et al., 2004a). L-BMAA that is bound to proteins within the body can slowly be released in the free form when contaminated proteins are metabolized over time (Cox et al., 2005). L-BMAA is a stable adduct with bicarbonate (Nunn and O'Brien, 1989 [PMID:2666171]).

2.3 Commercial Availability

For laboratory purposes, L-BMAA (>99% purity) is available from Sigma Chemical Company (St. Louis, MO) (Butterfield et al., 1993 [PMID:8397004]). It can also be purchased from Cambridge Research Biochemicals (Valley Stream, NY) (Myers and Nelson, 1990). L-BMAA and its hydrochloride form can be custom-synthesized by Research Biochemical Inc. (Natick, MA) (Brownson et al., 2002 [PMID:12241991]; Rakonczay et al., 1991 [PMID:1653366]).

The growth and commercial distribution of cycad plants between 1977 and 2002 reported that the bulk of cycad trade was in artificially propagated specimens. Only 38,500 out of ~30 million plants exported in this 24-year period were of wild origin and the vast majority of the plants came from Australia. Plant trade in tropical and subtropical parts of North America, South America, Central America and the Caribbean, Asia, Africa, and Oceania was also reported and included <1500 wild type plants. Australia has four genera and ~75 species of cycad, Mexico three genera and ~45 species, Thailand 10 species, and Madagascar one species (CITES, 2003).

3.0 Production Processes

L-BMAA is produced naturally by cyanobacteria, a blue-green algae common to lakes, oceans, and soils around the world (Munro, 2005; Vowles, 2004). Synthetic BMAA was produced from α-acetamidoacrylic acid and [^{15}N]-methylamine; enantioselective hydrolysis of the acetamide group, mediated by the enzyme Acylase 1 (EC 3.5.1.14; aminoacylase), from porcine kidney yielded (*R*)-BMAA and the (*S*)-α-acetamido derivative. Acid hydrolysis of the latter compound yielded (*S*)-BMAA (Hu and Ziffer, 1990). L-BMAA was also produced by a similar process using α-acetamino-β-methylaminopropionic acid, Acylase I, and ion-exchange chromatography (Vega et al., 1968).

4.0 Production and Import Volumes

No data were available.

5.0 Uses

Uses of L-BMAA itself were not reported but L-BMAA-containing cycad seeds are used in several Pacific islands for food stuffs and medicinal purposes. In Guam, cycad seeds are used to

make flour for tortillas (Vowles, 2004). Studies showed, however, that 87% of the L-BMAA was removed from the seeds during washing and processing. A single wash after a 24-hour soaking removed 90% L-BMAA (Duncan, 1991; Duncan et al., 1990 [PMID:2330104]).

In west New Guinea, cycad seed poultices are applied to open wounds; in Japan, cycad seeds used to prepare oral steeps are reportedly beneficial in treating a number of disorders (Duncan, 1992; Duncan et al., 1991 abstr.); in China, cycads are used for medicinal (skin complaints, astringent, to regulate energy flow, pain relief, kidney tonic, treat hypertension, rheumatic pain or colds and to cure hepatomegaly/liver cancer and stomach trouble), cultural (cycad in bloom as a sign of luck and happiness; symbolized nobility and authority; folklore to imbue supernatural powers), and religious purposes (planted in graves and doors to expel evil; fronds dipped in water and sprinkled on disciples by priest; in Buddhist temples to reflect austerity and constancy of Buddhism) (Hill, 2004). In Melville Island, cycads are used for first-fruit rites; in Australia, they are used for initiation ceremonies; and in Fiji, they are reserved for use by the chiefs (Lieberman, 2004).

Cycad parts used for food are the leaves, seeds, and the white meat which is comparable to a roasted chestnut in flavor and texture. In Africa, the stem of one species has been used to make cycad beer; seeds of another species are used in the Ryukyu Islands to prepare a kind of sake (Lieberman, 2004).

6.0 Environmental Occurrence and Persistence

L-BMAA was detected in 29/30 samples of blue-green algae tested from a variety of water sources in Scotland, Northern Ireland, the Netherlands, Israel, India, Australia, the United States, and elsewhere (Edwards, 2005; Vowles, 2004). It was also detected in samples from the Baltic Sea and oceanic blooms suggesting that significant quantities may be released into the world's oceans (Cox et al., 2005). Additionally, it has been noted that fish and animals may be exposed to L-BMAA from consumption of cyanobacterial blooms in sources of drinking water (Munro, 2005).

Free and protein-associated L-BMAA was detected in 12 samples (blooms, scums, and mats) collected from bodies of water in the United Kingdom over a 14 year period. Concentrations of free and protein-associated L-BMAA ranged from not detectable to 276 µg/g dry weight and 6 to 48 µg/g dry weight, respectively. Co-occurrence of other cyanobacterial toxins (e.g., microcystins and saxitoxins) were found in all but two of the samples (Metcalf et al., 2008 [PMID:18237305]).

Protein-bound and free L-BMAA have been found in the Guam ecosystem (bacteria, plants, animals, and human tissues). Cyanobacteria-infected roots of *C. micronesica* contained 2 µg/g [17 nmol/g] of the bound form (versus 0.3 µg/g [3 nmol/g] in free form), while cultures of cyanobacteria contained 72 µg/g [0.61 µmol/g] of bound L-BMAA. The latter becomes biomagnified as it travels up the Guam ecosystem from plants, to flying foxes, to humans, yielding a 10-fold increase from cyanobacteria to humans. A 10,000-fold increase from cyanobacteria to flying foxes was reported for levels of free L-BMAA. However, the level of free L-BMAA in human tissue was ~500-fold less than that in flying foxes and 87-fold less than that of bound L-BMAA in human tissue (Cox et al., 2005; Murch et al., 2004a).

7.0 Human Exposure

Human populations may potentially be exposed to L-BMAA from water sources containing blue-green algae blooms, consumption of fish or animals that have been exposed to L-BMAA, or ingestion of foods derived from cycad plants or of flying foxes, which are considered a traditional delicacy in Guam (Gantar and Svir ev, 2008; Munro, 2005). Flour from cycad seeds released up to 169 µg/g [1.43 µmol/g] free L-BMAA via acid hydrolysis (Murch et al., 2004a). L-BMAA has been discovered in the protein fraction of hair (146 µg/g [1.24 µmol/g]) and skin (2 µg/g [0.02 µmol/g] in wing membrane) of flying foxes that are >50 years old (Murch et al., 2004a). A close correlation between the decline of ALS-PDC in the Chamorro people and that of the flying fox population in Guam supports the premise that these bats were a major source of exposure to L-BMAA (Cox and Sacks, 2002 [PMID:11914415]). L-BMAA (2.04-21.51 µg/g [17.3-182.1 nmol/g) also was detected in 21 Peruvian samples of *Nostoc commune*, a cyanobacteria which is consumed in numerous countries including Peru, Java, and China (Johnson et al., 2008 [PMID:18495396]).

It has also been reported that several blue-green algae dietary supplements contained large quantities of L-BMAA (Dietrich et al., 2008). One blue-green algae dietary supplement manufacturer, however, reported its Spirulina supplements contained no L-BMAA (NutraIngredientsUSA, 2005).

8.0 Regulatory Status

No regulations pertaining to L-BMAA were available.

9.0 Toxicological Data

9.1 General Toxicology

Numerous studies have been published on the possible link between exposure to L-BMAA from the use and consumption of cycad-derived food and the high incidence of ALS-PDC in the western Pacific region, in particular Guam (e.g., Cox and Sacks, 2002 [PMID:11914415]; Duncan, 1991, 1992; Ince and Codd, 2005 [PMID:16008818]; Kisby et al., 1992b; Spencer et al., 1987c [PMID:3603037]; Steele and Guzman, 1987 [PMID:3315143]). Studies in primates showed that L-BMAA produced a neurodegenerative syndrome very similar to that of Guam ALS-PDC (e.g., behavioral abnormalities, corticomotonueronal dysfunction, and features of parkinsonism) (Spencer et al., 1987c [PMID:3603037]). A murine model of ALS-PDC has been created (mice are fed processed cycad flour) that allows analysis of the disease in four dimensions—behavioral deficits, biochemical changes, and morphological or pathological results with time (Shaw and Wilson, 2003 [PMID:14599431]; Schulz et al., 2005).

9.1.1 Human Data

The Poison Center in Taiwan reported 21 cases of poisoning from eating cycad seeds that had been washed and cooked. Symptoms included severe vomiting and gastrointestinal disturbance and occurred from 30 minutes to 7 hours after ingesting 1-30 seeds; patients recovered quickly. Blood cyanide or thiocyanate levels in these subjects were reported to be higher than normal (Chang et al., 2004 [PMID:15083936]).

In 1956, the Guam Research Center reported a high degree of familial occurrence of ALS and PDC, suggesting these are inherited disorders yet there was no demonstrable genetic or infectious origin. The disease skipped some generations or may not have affected large numbers of family members in a given generation. Likewise, family members such as spouses who are not genetically related may also develop the disorder (Lieberman, 2004; Mabry, 2001 [PMID:11754626]; Murch et al., 2004a). More recent studies have linked the high incidence of ALS-PDC among the Chamorro people of Guam to exposure to L-BMAA from consumption of cycad-derived foods.

L-BMAA (3-10 µg/g [25-85 nmol/g]) was detected as the free amino acid in brain tissue from the superior frontal gyrus of 5/6 Chamorro people who suffered from dementia-related disorders including ALS-PDC (aged 39-89 at death). It was present in the protein fraction (82-1190 µg/g [0.69-10.07 µmol/g]) of all six patients. Both forms of L-BMAA were also found at comparable levels in two Canadians who died of progressive neurodegenerative disease (Murch et al., 2004a, 2004b [PMID:15355492]). L-BMAA was detected at high levels in the frontal cortex (25.9-45.7 µg/g [0.219-0.387 µmol/g]), temporal cortex (33.7-170.8 µg/g [0.285-1.446 µmol/g]), parahippocampal gyrus (34.2 µg/g [0.290 µmol/g]), or caudate (29.6 µg/g [0.251 µmol/g]) of the brain of nine Canadian Alzheimer's patients (Munro, 2005). There was a 60- to 130-fold greater amount of L-BMAA in the bound form than was recovered from the free amino acid pool (Murch et al., 2004a). In contrast, no free L-BMAA was detected in frozen brain samples from control subjects (n=5) and Alzheimer's disease patients (n=5) from the U.S. Pacific Northwest and from Chamorros with (n=8) and without (n=2) PDC (Montine et al., 2005 [PMID:16157919]). Additionally, no detectable level of L-BMAA was found in the plasma or cerebrospinal fluid of ALS patients (Perry et al., 1990 [PMID:2375629]). Analysis of brain tissues from 13 Canadians who had not died from neurological diseases showed no L-BMAA was present (Le Page, 2004).

The neurotoxic effect of L-BMAA appears to be latent and related to the release of free L-BMAA during protein metabolism. Approximately 100,000 Chamorros in California had the same incidence of neurological diseases as those in Guam even though many of them had left Guam 40 years earlier. Neurological diseases among this population now occur only in older adults and rarely in any individual born after 1960 (ACNR, 2002 interview; Cox and Sacks, 2002 [PMID:11914415]). There is also evidence that a single exposure to L-BMAA from cycad flour can contribute to the development of a neurological disease. In 1978, a Saipanese woman and her son developed Parkinson and ALS, respectively, 19 years after participating in a feast on the northern island of Pagan where foods prepared from four pounds of cycad flour were served. Accurate histories of single cycad exposures were obtained from 21 patients with ALS-PD and two patients reported having a single exposure many years before the onset of their illness (Steele and Guzman, 1987 [PMID:3315143]).

Methods for diagnosing or predicting the likelihood of developing a neurological disorder based on L-BMAA levels in tissue samples have been proposed as a screening for environmental factors associated with these disorders (Cox et al., 2007 pat.).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

A total of 31 g/kg BMAA•HCl (equivalent to 15.5 mg/kg [131 mmol/kg] L-BMAA) was administered to female CD1 mice via gavage over 11 weeks. Tissue concentrations of BMAA 24 hours after the first treatment with 500 mg/kg [4.23 mmol/kg] ranged from 0.1-0.3 mM (11-35 µg/mL] in the liver and 0.1-0.15 mM [11-18 µg/mL] in the brain. The administered dose was increased to 1000 mg/kg [8.465 mmol/kg] every other day starting week eight. Liver and brain tissue concentrations of L-BMAA 48 hours after the first dose were 0.2 and 0.5 mM [24 and 59 µg/mL], respectively (Perry et al., 1989 [PMID:2614465]).

Clearance of L-BMAA from plasma of male Sprague-Dawley rats had a rapid distribution phase [volume of distribution (V_d) ~16 L/kg] followed by a slower elimination phase (half time ($t_{1/2}$) ~1 day) after a single intravenous (i.v.) injection of 25-400 mg/kg [0.21-3.39 mmol/kg]; L-BMAA levels in the brain peaked within eight hours after injection and decreased with a $t_{1/2}$ similar to that of plasma. After two weeks of continuous infusion with L-BMAA (100 mg/kg [0.847 mmol/kg] per day) L-BMAA levels in the brain were 10-30 µg/g [0.08-0.25 µmol/g], moderately exceeding those in plasma. BMAA•HCl (100 mg/kg [0.647 mmol/kg]) administered to rats by gastric intubation was rapidly absorbed and levels of BMAA in the plasma reached a peak five minutes after injection. Eight hours after treatment ~80% of the dose had been absorbed and cortical brain L-BMAA levels averaged 3.6 µg/g [0.03 µmol/g], which was comparable to that reported for i.v. treated rats (4.4 µg/g [0.04 µmol/g]). L-BMAA levels in tissue homogenates prepared from liver, muscle, kidney and brain did not change significantly during *in vitro* incubation suggesting the rate of transformation of L-BMAA is quite slow ($t_{1/2} > 4$ hr) (Duncan et al., 1991 [PMID:2072299]).

The uptake of L-BMAA by the central nervous system (CNS) was determined by injecting [^{14}C]-BMAA (2.0 µCi) into the right common carotid artery of adult male Sprague-Dawley rats (n=6) and measuring the radioactivity in the brain (hippocampus, cortex, striatum, sub-cortex, midbrain, cerebellum) and spinal cord tissue. L-BMAA was rapidly taken up by the CNS tissues (Kisby et al., 1991 abstr.).

The mechanism of L-BMAA uptake into brain measured in adult Sprague-Dawley rats using the *in situ* brain perfusion technique demonstrated that L-BMAA is transported into brain via the large neutral amino acid carrier (L-system) of the blood-brain barrier and that uptake is critically sensitive to competition from plasma neutral amino acids. Factors such as diet, disease, and age were considered when evaluating L-BMAA delivery to the CNS (Smith et al., 1992 [PMID:1548467]).

Pretreatment of mouse cortical explants (2-3 weeks *in vitro*) with the non-selective gamma amino butyric acid-uptake inhibitor, nipecotic acid (100 µM and 1.0 mM [0.012 and 0.1 mg/mL]), blocked L-BMAA uptake and L-BMAA-induced excitotoxicity, respectively. Metabolism of L-BMAA by purified brain microsomes and mitochondria was inhibited by P450 SKF 525A and piperonyl butoxide. Brain microsomal metabolism of L-BMAA (1.6 mM [189 µg/mL]) was inhibited by deprenyl (0.1-0.5 µM) (Kisby et al., 1991 abstr., 1992 abstr.).

Bioavailability of L-BMAA in cynomologous monkeys (n=3) was determined by simultaneous administration of two isotropic forms of BMAA using two different routes of exposure:

[^{15}N]-BMAA i.v. (~1 mg/kg [8 µmol/kg]), and unlabeled L-BMAA orally (~2 mg/kg [16 µmol/kg]). Approximately 1.4% of orally-administered BMAA and 1.8% of i.v.-administered [^{15}N]-BMAA was excreted unmetabolized in the 0-48 hour urine sample. The low recoveries of unmetabolized compound indicated that most of the BMAA was metabolized by a route other than conjugation, and/or that biliary and fecal excretion are significant pathways for BMAA elimination (Duncan et al., 1991 abstr., 1992a [PMID:1522948]).

9.1.3 Acute Exposure

A single dose of BMAA (10 µL of a 100 mM [11.8 mg/mL] solution) injected intracranially into the striatum of male 12-14 week old C57BL/6J mice induced damage in the CA1 region of the hippocampus. Evaluation of the injection site confirmed that BMAA was not administered directly into the hippocampus of any of the mice evaluated. Some animals showed intermittent pyknotic neurons in the pyramidal layer while others exhibited significant neuronal death and complete regression of the apical processes (Buenz and Howe, 2007 [PMID:17379313]).

Single doses of L-BMAA (not provided in abstract) induced seizures in neonatal mice, as well as postsynaptic neuronal edema and degeneration in explants of mouse spinal cord and frontal cortex (Spencer et al., 1987b [PMID:3107939]). Intraperitoneal (i.p.) injection produced convulsions in neonatal mice (1.68-3.34 mg/g [14.2-28.3 µmol/g] bw) and rats (0.84 or 1.68 mg/g [7.1 or 14.2 µmol/g] bw) (Polsky et al., 1972). [*Note: Authors use DL-BMAA; however, Nunn et al. (1987 [PMID:3109690]) and others cite the study as using the L-isomer.*] In male Swiss Webster mice, intracerebroventricular (i.c.v.) injection of L-BMAA (0.5-1.5 µmol [59-180 µg]) or its racemate DL-BMAA (1-10 µmol [0.1-1.2 mg]) induced ataxia, ptosis, rolling, unsteady gait, scratching, jumping, myoclonic jerks, forelimb clonus, hyperlocomotion, clonic muscle spasms, tonic seizure, and hypolocomotion; all animals fully recovered. The dose required to induce the seizure response for 50% of animals was 975 nmol for L-BMAA and 1620 nmol for DL-BMAA (Smith and Meldrum, 1990 [PMID:1980247]).

Intraperitoneal injections of L-BMAA (0.7-1.7 mg/g [6-14 µmol/g] bw) into young Wistar rats produced unsteadiness, ataxia, slow torsion spasm, and significant reductions in body weights; complete recovery was seen within two to three days with the lower doses. L-BMAA also produced acute signs of cerebellar dysfunction and degeneration of cerebellar stellate, basket, Purkinje, and Golgi cells; lesions occurred only in the cerebellum (Seawright et al., 1990 [PMID:2345599], 1992). In male Sprague-Dawley rats, i.c.v. injection of L-BMAA (500 µg [4.23 µmol]) induced clonic convulsions in 49% of test animals for ~26 seconds, splay in 66% for 30 seconds, and rigidity in 73% for 3 minutes. Other effects included irritability, forepaw and hindpaw grooming, wet-dog shaking, and deep breathing (Matsuoka et al., 1993 [PMID:8095728]). In another rat study, i.c.v. administration of L-BMAA (10 nmol [1.18 µg]) caused slow growth and a typical behavioral pattern which included loss of mobility (Chang et al., 1993 [PMID:7904554]).

Male albino Wistar rats (270-320 g) were perfused with BMAA (1, 10, or 50 mM [0.1, 1.2, or 5.9 mg/mL] for 1 hour; flow rate 3 µL/minute) 1 day after implantation of a microdialysis probe into both corpus striata and extracellular output of dopamine and its metabolites was measured. After an additional 24 hours, animals were perfused with 1-methyl-4-phenylpyridinium ion (MPP^+) (1 mM for 15 minutes) and measurements repeated. BMAA increased basal dopamine

levels in a dose-dependent manner. The 1 and 10 mM dose increased dopamine output 2-3 times and the 50 mM dose increased the output 23 times that of the basal level (14.6 ±1.7 fmol/min). The increase was transient and levels returned to baseline within ~75 minutes after BMAA was removed from the perfusions solution. A significant decrease in the levels of the dopamine metabolites 3,4-dihydropphenylacetic acid (DOPAC) and homovanillic acid (HVA) was only observed during perfusion with the 50 mM BMAA solution. Levels returned to baseline when BMAA was removed. Challenge with MPP^+ produced an inverse effect on dopamine levels relative to the BMAA dose used on day one (1 mM: 1885 ± 309.6 fmol dopamine/min, 10 mM: 1628.0 ± 190.34 fmol dopamine/min; 50 mM: 705.6 ± 79.7 fmol dopamine/min). Decreases in DOPAC and HVA levels also were observed (Santiago et al., 2006 [PMID:16979309]).

9.1.4 Short-term and Subchronic Exposure

In six-month-old male CD-1 mice, no effects were observed in five different neurobehavioral tests or a memory test in mice fed a diet containing L-BMAA (1 mg [8 µg/kg bw]) for 30 days, when compared to control animals. Analysis of brain and spinal cord tissues revealed no signs of neuronal damage (Cruz-Aguado et al., 2006 [PMID:16808967]).

Daily administration of BMAA (500 mg/kg [4.23 mmol/kg] daily for 18 days, then 500 mg/kg every other day for over 28 days, and 1000 mg/kg [8.465 mmol/kg] every other day for 30 days, totaling 15.5 g/kg [131 mmol/kg] of the *L*-isomer) via gavage to female CD1 mice for 11 weeks produced no neurotoxic effects. Animals appeared healthy; no changes in weight and no behavioral abnormalities were observed. Dopamine contents in the striatum and glutamate and aspartate contents in the cerebral cortex were normal (unlike those seen in sporadic human ALS, where the amino compound amounts are reduced). Additionally, there were no pathological changes in the brain (i.e., cerebral cortex, hippocampus, striatum, and substantia nigra) or spinal cord (Perry et al., 1989 [PMID:2614465]).

In male Sprague-Dawley rats, daily i.c.v. injection of L-BMAA (500 µg [4.23 µmol]) for ten days induced clonic convulsions, splay, and rigidity, which were reduced four days after initial treatment (Matsuoka et al., 1993 [PMID:8095728]). In a similar study, daily i.c.v. administration of L-BMAA (500 µg [4.23 µmol]) for up to 60 days produced similar behavioral changes (e.g., splay, jerking movements, and rigidity, hyperactivity, and facial tremor) that were seen for at least six days after treatment. At day 10, the behavior of the treated animals was comparable to that of controls. L-BMAA resulted in a marked decrease in acetylcholinesterase activity (53%) and increase in choline acetyltransferase activity (60%) at day 16. During days 30 to 60, acetylcholinesterase activity gradually returned to control levels, while choline acetyltransferase activity decreased by 23% (Rakonczay et al., 1991 [PMID:1653366]).

Repeated daily oral administration of L-BMAA (0.81 mmol/kg [96 mg/kg]) for up to 13 weeks to male cynomolgus monkeys produced signs of behavioral changes, pyramidal dysfunction, limb weakness, atrophy, upper extremity tremor, wrist drop, bradykinesia, conduction deficits in central and peripheral motor pathways, and neuropathological changes within eight weeks (Spencer et al., 1986 lett., 1987c).

9.1.5 Chronic Exposure

No data were available.

9.1.6 Synergistic/Antagonistic Effects

L-BMAA is a neurotoxic glutamate agonist; its neuroexcitatory and neurotoxic effects *in vivo* and *in vitro* depend on the presence of bicarbonate ions (Richter and Mena, 1989 [PMID:2568879]; Weiss et al., 1989a [PMID:2561969]). Details of the following studies are provided in **Table 1**.

In Vivo Assays

In rats, L-BMAA-induced behavioral changes were affected by several antagonists. Duration and severity of clonic convulsions were reduced by 6,7-dinitroquinoxaline-2,3-dione (DNQX), a non-*N*-methyl-*D*-aspartate (NMDA) glutamate receptor antagonist, while latency was significantly prolonged by DNQX, as well as by *DL*(±)2-amino-5-phosphonopentanoic acid (AP5), a NMDA receptor antagonist, and [(+)5-methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate (MK-801), a noncompetitive NMDA antagonist. Splay was slightly enhanced by MK-801, while rigidity was destroyed by MK-801 and partially inhibited by DNQX and AP5 (Matsuoka et al., 1993 [PMID:8095728]).

In Vitro Assays

In the presence of MK-801 and CNQX, L-BMAA dose-dependently increased inositol phosphate (InsP) formation in murine striatal neuronal cultures, which suggests that L-BMAA is a full agonist of the glutamate metabotropic receptor. The effects of L-BMAA were additive with carbachol-induced InsP formation but were completely blocked by phorbol ester phorbol-12,13-dibutyrate (Manzoni et al., 1991 [PMID:1684520]). In neonatal mouse cortex explants, L-BMAA-induced cellular changes were dose-dependently protected by 2-amino-7-phosphonoheptanoic acid (AP7) (Ross et al., 1987 [PMID:3123008]).

In fetal rat cell cultures, tetrandrine significantly reduced L-BMAA-induced neuronal injury, while in rat cerebellar granule cells, *D*-AP5, (*RS*)-α-methyl-4-carboxyphenylglycine, and (*RS*)-α-methylserine-*O*-phosphate reduced L-BMAA-induced cell death (Che et al., 1997; Staton and Bristow, 1997 [PMID:9326280], 1998 [PMID:9721754]). In hippocampal slices from adult rats, L-BMAA-induced increase in InsP hydrolysis was antagonized by *L*-2-amino-4-phosphonobutanoate (AP4) (Copani et al., 1990).

Table 1. Synergistic or Antagonistic Effects on L-BMAA-Induced Effects

Species, Strain, and Age, Number, and Sex of Animals or Test System	Dose of L-BMAA	Synergist or Antagonist	Results/Comments	Reference
In Vivo Assays				
Rat, Sprague-Dawley, age n.p., 6-12M	500 µg (4.23 µmol)	AP5, DNQX, and MK-801	DNQX significantly and dose-dependently decreased L-BMAA-induced rigidity and duration and severity of clonic convulsions; it delayed latency of clonic convulsion onset. DNQX (20 µg) inhibited forepaw and hindpaw grooming, and decreased wet-dog shaking and deep breathing. AP5 (50 µg) prolonged L-BMAA-induced latency of clonic convulsion onset, inhibited forepaw grooming and duration and severity of rigidity, decreased hindpaw grooming, and intensified facial tremor. MK-801 (5 µg) extended L-BMAA-induced latency of convulsion onset, and at 50 µg, it slightly increased duration of clonic convulsions. At 100 µg, it completely inhibited rigidity, increased duration and severity of splay and duration of convulsions, inhibited forepaw grooming, and decreased (or tended to decrease) hindpaw grooming, facial tremor, and chewing.	Matsuoka et al. (1993) [PMID:8092758]
In Vitro Assays				
Murine primary cortical cell cultures	0-100 µM (0-11.8 µg/mL)	NMDA, kainite, iron, BSO, amyloid-β, MPP+, C-2 ceramide, staurosporine	Alone, L-BMAA was not neurotoxic. However, L-BMAA potentiated the neurotoxic effects of NMDA, iron, BSO, amyloid-β, and MPP^+.	Lobner et al. (2007) [PMID:17098435]
Murine primary cultured striatal neurons	1 mM (118 µg/mL)	carbachol and phorbol ester phorbol-12,13-dibutyrate	In the presence of MK-801 and CNQX, L-BMAA dose-dependently increased InsP formation (EC_{50} = 0.97 mM; maximal stimulation = 246% of basal InsP formation). The effects of L-BMAA were additive with 1 mM carbachol-induced InsP formation but were completely blocked by 0.1 µM phorbol ester phorbol-12,13-dibutyrate.	Manzoni et al. (1991) [PMID:1684520]

Table 1. Synergistic or Antagonistic Effects on L-BMAA-Induced Effects (Continued)

Species, Strain, and Age, Number, and Sex of Animals or Test System	Dose of L-BMAA	Synergist or Antagonist	Results/Comments	Reference
Neonatal mouse cortex explants	0.5-1.6 mM (60-190 µg/mL) (EC_{100})	AP7, PDA, glutamate diethyl ester, and streptomycin	AP7 antagonized L-BMAA-induced cellular changes (postsynaptic vacuolation and neuronal degeneration) in a dose-dependent manner; 88% maximum protection was seen at 1.0 and 0.5 mM, respectively. No effects were observed with PDA, glutamate diethyl ester, or streptomycin.	Ross et al. (1987) [PMID:3123008]
Cultured fetal rat cerebral cortical cells	300 µg (2.54 µmol)	tetrandrine	Tetradrine partially reduced L-BMAA-induced neuronal injury (neuronal degeneration, neuronal loss, and LDH efflux).	Che et al. (1997)
Cultured rat cerebellar granule cells	1 or 3 mM (118 or 354 µg/mL)	*D*-AP5	*D*-AP5 reduced L-BMAA-induced cell death by 90% (1 mM) and 86% (3 mM).	Staton and Bristow (1997) [PMID:9326280]
Cultured rat cerebellar granule cells	n.p.	(*RS*)-α-methyl-4-carboxyphenylglycine; (*RS*)-α-methylserine-*O*-phosphate	(*RS*)-α-Methyl-4-carboxyphenylglycine reduced L-BMAA-induced cell death by 37%. (*RS*)-α-Methylserine-*O*-phosphate was also effective; no other data were provided.	Staton and Bristow (1998) [PMID:9721754]
Hippocampal slices from rats, Sprague-Dawley, 9-day-old or 3-mo-old, number n.p., F	50 µM, 200 µM, or 1 mM (5.9, 23.6, or 118 µg/mL)	AP4	AP4 antagonized L-BMAA-induced InsP hydrolysis in tissues of adult rats; at 1 mM, [^{3}H]inositol monophosphate formation decreased from 52 to 37 dpm/mg protein x 10^{-2}.	Copani et al. (1990)

Abbreviations: AP4 = *L*-2-amino-4-phosphonobutanoate; AP5 = *DL*(±)2-amino-5-phosphonopentanoic acid; AP7 = 2-amino-7-phosphonoheptanoic acid; BSO = buthionine sulfoxamine, CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione; DNQX = 6,7-dinitroquinoxaline-2,3-dione; EC_{50} = effective concentration for 50% of organisms tested; EC_{100} = effective concentration for 100% of organisms tested; InsP = inositol phosphate; LDH = lactate dehydrogenase; M = male(s); MK=801 = [(+)5-methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate; mo = month(s); MPP+ = 1-methyl-4-phenylpyridinium ion; NMDA = N-methyl-D-aspartic acid; n.p. = not provided; PDA = *cis*-2,3-piperidine dicarboxylic acid

9.1.7 Cytotoxicity

Details of the following studies are presented in **Table 2**.

In mouse cortical cells, L-BMAA (up to 10 M [1.2 g/mL]) induced dose-dependent neuronal degeneration; neurotoxicity was found to depend on the presence and concentration of bicarbonate (Weiss and Choi, 1988 [PMID:3136549]; Weiss et al., 1989b [PMID:2551452]). L-BMAA (doses not provided in abstract) did not affect NADH-dehydrogenase activity in mouse brain slices but caused lactate dehydrogenase (LDH) leakage into the medium (Pai and Ravindranath, 1993 [PMID:8242335]). In spinal cord cultures, vacuolar change, pathological change (primarily in synapses), and postsynaptic edematous swelling were observed (Nunn et al., 1987 [PMID:3109690]). In a murine neuroblastoma cell line, L-BMAA (up to 10 µM [1.2 µg/mL]) decreased neurite growth at low doses (≤0.1 µM [0.012 µg/mL]) but increased growth at high doses (≥1 µM) (Abdulla and Campbell, 1993).

L-BMAA induced effects in rat CNS monoamine neurons both *in vivo* and *in vitro*. Intracerebral and intracisternal injections of L-BMAA (10 or 400 µg [0.08 or 3.39 µmol]) produced a lesion in substantia nigra and caused loss of tyrosine hydroxylase (TH) immunoreactivity, the occurrence of TH-immunoreactive pyknotic neurons, loss of substance P-immunoreactive terminals, and a decrease in norepinephrine levels in the hypothalamus, while incubations with L-BMAA (1 mM [0.1 mg/mL]) resulted in a decrease in high-affinity norepinephrine uptake in cortical synaptosomes (Lindström et al., 1990 [PMID:1974606]). In newborn rat brain cells, L-BMAA (5 mM [0.6 mg/mL]) increased intracellular calcium levels in a dose-dependent manner in the presence of bicarbonate ion (Brownson et al., 2002 [PMID:12241991]). In rat cerebellar granule cells and ventral spinal cord cells, cell death was induced (La Bella et al., 1996 [PMID:8848166]; Staton and Bristow, 1996 abstr., 1997 [PMID:9326280]). Dose-dependent toxicity was also seen in rat neuronal cells and hippocampal neurons (Pean et al., 1995 [PMID:7595598]; Rapp et al., 1988 abstr.). At >100 µM [11.8 µg/mL], L-BMAA inhibited forskolin-stimulated cAMP formation in rat brain slices and cortical membranes (Genazzani et al., 1993 [PMID:8242352]).

In guinea pig synaptosomes, L-BMAA (100 µM or 1 mM [0.012 or 0.1 mg/mL]) had no effect on basal cytosolic or potassium chloride-stimulated vesicular release of glutamate or calcium-independent release of glutamate (Gannon and Terrian, 1989 [PMID:2575728]; McMahon et al., 1989 [PMID:2566656]).

In cortex and cord cultures of macaques, L-BMAA (125-500 µg/mL [1.06-4.23 mM]) produced synaptic edematous vacuolation and degeneration of selected neurons (Spencer et al., 1986 abstr.).

L-BMAA (0.1-3 mM [0.01-0.4 mg/mL]) significantly reduced input membrane resistance in leech Retzius nerve cells, indicating that an increase of membrane permeability led to cell depolarization by L-BMAA. The effect on membrane potential was enhanced in the presence of bicarbonate compared to standard Ringer solution (Nedeljkov et al, 2005 [PMID:15164949]).

Note: In a study using cultured rat cerebellar granule cells and ventral mesencephalic neurons, a correlation between toxicity and L-BMAA concentratioon was not observed; L-BMAA concentrations in the Guamanian flour extracts were too low to be neurotoxic (<5 µM). Instead, neurotoxicity of the extracts correlated with their zinc levels (Duncan et al., 1992b).

Table 2. Studies of L-BMAA in Neural Cell and Tissues

Test System	Dose	Exposure Period	Results/Comments	Reference
Cultured mouse cortical neurons	3 mM - 10 M (0.4-1.2 mg/mL)	24 hr	L-BMAA induced dose-dependent neuronal degeneration without glial damage (neurotoxic EC_{50} ~1 mM).	Weiss et al. (1989b) [PMID:2551452]
Murine cortical cell cultures	1-3 mM (0.1-0.4 mg/mL)	1 hr	In Hepes-buffered salt solution (HSS) or tris-buffered exposure solution, L-BMAA was not neurotoxic. In Eagle's minimal essential medium (MEM), which contains bicarbonate, L-BMAA (1-3 mM) produced acute neuronal swelling and extensive late neuronal degeneration. With the addition of 10 mM $NaHCO_3$ in HSS, 3 mM L-BMAA produced significant neuronal degeneration by the next day. Neurotoxicity of L-BMAA depended on the concentration of HCO_3^- between 6 and 24 mM: at <6 mM HCO_3^-, little toxicity occurred; at 24 mM, L-BMAA destroyed 50-80% of the cell population.	Weiss and Choi (1988) [PMID:3136549]
Mouse brain slices	n.p.	n.p.	L-BMAA did not affect NADH-dehydrogenase activity but cased LDH leakage into the medium.	Pai and Ravindranath (1993) [PMID:8242335] [abstract used]
Mouse spinal cord cultures	0.19, 0.38, 0.81, 1.62, or 3.2 mM (22, 45, 96, 191, or 380 µg/mL)	1 hr	At ≥0.81 mM, L-BMAA induced vacuolar change within minutes of exposure. After 10 min, pathological change mainly in synapses was observed; adjacent postjunctional elements were swollen by edematous fluid. After 3 hr, postsynaptic edematous swelling with neuronal perikarya was seen.	Nunn et al. (1987) [PMID:3109690]
Mouse neuroblastoma cell line (NB41A3)	0.001-10 µM (0.118 ng/mL - 1.2 µg/mL)	n.p.	At low doses (0.001-0.1 µM), L-BMAA decreased neurite outgrowth (seen as a decrease of levels of the neurofilament proteins 68 and 160 kDa). At higher doses (≥1 µM), growth was significantly increased.	Abdullah and Campbell (1993)
Mouse spinal motor neuron-like cell line (NSC-34)[a]	50-1000 µM (5.9-118.1 µg/mL)	18 hr	L-BMAA induced cell death in a dose-dependent manner, with 100 µM as the minimum effective concentration.	Buenz and Howe (2007) [PMID:17379313].

Table 2. Studies of L-BMAA in Neural Cell and Tissues (Continued)

Test System	Dose	Exposure Period	Results/Comments	Reference
Dissociated mouse (embryonic day 13) spinal cord cultures	30-1000 µM (3.5-118.1 µg/mL)	24 hr	L-BMAA induced dose-dependent cell death in motor neurons. While the highest dose (1000 µM) induced widespread death, selective damage was observed at the lower doses. No injury was reported to the astrocytes. L-BMAA also induced intracellular Ca^{+2} release and reactive oxygen species generation in motor neurons. Extracts of cycad seeds (containing 107 µg/ml L-BMAA) caused selective dose-dependent motor neuron injury, with spinal neurons showing less damage.	Rao et al. (2006) [PMID:16764863]
Dissociated newborn Sprague-Dawley rat (<24 hr) brain cells	5 mM (0.6 mg/mL)	51 seconds	L-BMAA increased intracellular calcium levels in a dose-dependently in the presence of bicarbonate ion; peak levels were observed at 20 seconds. The effect of L-BMAA was also dependent on extracellular calcium levels.	Brownson et al. (2002) [PMID:12241991]
Rat CNS monoamine neurons	10 or 400 µg (0.08 or 3.39 µmol) [injection]; 0.001-10 µM (0.118 ng/mL - 1.2 µg/mL) [incubation]	20 min (incubation)	Given via intracerebral injection, L-BMAA produced a lesion in substantia nigra and caused loss of TH immuno-reactivity in the intranigral injection sites, the presence of TH-immunoreactive pyknotic neurons near the borders of the injection sites, and loss of substance P-immunoreactive terminals in substantia nigra pars reticulate within lesioned areas. Intracisternal administration of L-BMAA (400 µg) decreased norepinephrine levels in the hypothalamus to 86% of controls but had a tendency to increase in pons-medulla; it had no effects on [^{3}H]norepinephine uptake in cortical synaptosomes. Both injection routes did not affect dopamine levels. Incubations with L-BMAA (10 µM) resulted in a 41% decrease in high-affinity norepinephrine uptake in cortical synaptosomes compared to controls; at $\leq 10^{-7}$, no reductions were seen. At ≤10 µM L-BMAA, striatal dopamine uptake and cortical serotonin uptake were not affected.	Lindström et al. (1990) [PMID:1974606]
Rat cerebellar granule cells	n.p.	30 min, 24 or 48 hr	L-BMAA dose-dependently induced cell death. At 30 minutes, a maximum of 16.4% (mean) of cells was killed; at 24 hours, 39.4%; and at 48 hours, 43.4%. The results also suggested the induction of apoptosis.	Staton and Bristow (1996 abstr.)

Table 2. Studies of L-BMAA in Neural Cell and Tissues (Continued)

Test System	Dose	Exposure Period	Results/Comments	Reference
Rat cerebellar granule cells	up to >1000 µM (118.1 µg/mL)	30 min, 24 or 28 hr	After exposure for 30 min, L-BMAA induced a maximal cell death of 7% above control in cells aged 11-14 DIV. In addition, maximal cell death of 39% above control was seen over 24 hr and 38% over 48 hr. In cells aged 9-10 DIV, maximal cell death was 21% above control over 24 hr. In cells 11-14 DIV, L-BMAA (3 mM) for 24 hr produced DNA fragmentation. L-BMAA (3 mM) also caused maximal necrotic-like cell death of 48% above control at 72 hr and maximal apoptotic-like cell death of 35% above control at 18 hr; necrotic-like cell death was significantly greater at 12 and 72 hr after exposure.	Staton and Bristow (1997) PMID:9326280]
Rat motoneuron hybrid ventral spinal cord 4.1 cells	1 or 10 mM (0.1 or 1.2 mg/mL)	4 days	At the low dose, L-BMAA caused about 10% cell death. At the high dose, cell viability was decreased to 70% of controls. Additionally, LDH release was increased in a time-dependent manner in the growth media, with maximal release at day 4.	La Bella et al. (1996) [PMID:8848166]
Rat neuronal cell line B50	0.1-1.0 mM (0.01-0.1 mg/mL)	>1 hr	At 1 hr, dose-dependent toxicity was observed (measured by release of LDH from cells). Increasing incubation time produced no effect, suggesting the cells had "substantial repair capacity."	Pean et al. (1995) [PMID:7595598]
Rat brain slices (hippocampal slices) and cortical membranes	(-log5) – (-log3) M	15 min	At >100 µM [11.8 µg/mL], L-BMAA slightly inhibited forskolin-stimulated cAMP formation. At 1 mM, it did not increase basal cAMP formation.	Genazzani et al. (1993) [PMID:8242352]
Rat hippocampal neurons	1 µM - 1 mM (0.1 µg/mL - 0.1 mg/mL)	up to 24 hr	L-BMAA caused time- and dose-dependent neuronal cytotoxicity.	Rapp et al. (1988 abstr.)
Guinea pig hippocampal mossy fiber synaptosomes	1 mM (0.1 mg/mL)	n.p.	L-BMAA had no effect on basal cytosolic or potassium chloride-stimulated vesicular release of *L*-glutamate or on dynorphin A (1-8)-like immunoreactivity.	Gannon and Terrian (1989) [PMID:2575728]
Guinea pig cerebral cortical synaptosomes	100 µM (11.8 µg/mL)	n.p.	L-BMAA had no effect on the calcium-independent release of glutamate.	McMahon et al. (1989) [PMID:2566656]
Primate (*Macaca fascicularis*) cortex and cord cultures	125-500 µg/mL (1.06-2.67 mM)	2 mo	L-BMAA produced post-synaptic edematous vacuolation and degeneration of selected neurons.	Spencer et al. (1986 abstr.)

Table 2. Studies of L-BMAA in Neural Cell and Tissues (Continued)

Test System	Dose	Exposure Period	Results/Comments	Reference
Leech (*Haemopis sanguisuga*) Retzius nerve cells	0.1-3 mM (0.01-0.4 mg/mL)	3 min	L-BMAA significantly reduced input membrane resistance in the cells, indicating that an increase of membrane permeability led to cell depolarization by L-BMAA. The effect on membrane potential was enhanced in the presence of bicarbonate (20 mM) compared to standard Ringer solution.	Nedeljkov et al. (2005) [PMID:16154949]

Abbreviations: AMPA = α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS = central nervous system; DIV = day(s) *in vitro*; hr = hour(s); InsP = inositol phosphate; LDH = lactic dehydrogenase; min = minute(s); MK-801 = [(+)5-methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate; mo = month(s); NMDA = *N*-methyl-*D*-aspartate; n.p. = not provided; TH = tyrosine hydroxylase

[a]NSC-34 is a hybrid cell line; fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma (Durham et al., 1993 [PMID:7909362]).

9.2 Reproductive and Developmental Effects

In Sprague-Dawley rat pups, s.c. injection of L-BMAA (500 mg/kg [4.23 mmol/kg] on postnatal day 5 only [group A], 500 mg/kg [4.23 mmol/kg] on postnatal days 2 and 5 [B], or 100 mg/kg [0.847 mmol/kg] on postnatal days 2 and 5 [C]) produced an increase in open field behavior in males in groups A and B and in hindlimb splay in females in group A. Deaths prior to postnatal day 7 occurred in 1/12 rats in group A, 7/24 in group B (four due to infanticide by a dam), and none in group C. Significant increases were seen in anterior pituitary weight for group B males (13 mg) and in ovarian weight for group A and B females (115 and 105 mg, respectively) compared to controls (10 mg [pituitary] and 88 mg [ovary]). Significant decreases were seen in spinal cord weight for group A males (202 mg) and in cerebellar weight for Group A males (134 mg) and group B females (128 mg) compared to controls (247, 163, and 145 mg, respectively). Additionally, changes in monoamines were seen in the spinal cord, cerebellum, mediobasal hypothalamus, and hippocampus and increases in α_2-adrenergic binding sites were observed in the cerebellum (Dawson et al., 1998 [PMID:9536463]).

9.3 Carcinogenicity

No data were available.

9.4 Initiation/Promotion Studies

No data were available.

9.5 Anticarcinogenicity

No data were available.

9.6 Genotoxicity

No data were available.

9.7 Cogenotoxicity

No data were available.

9.8 Antigenotoxicity

No data were available.

9.9 Immunotoxicity

No data were available.

9.10 Other Data

Endocrine Activity

In cultured fetal rat brain cells, L-BMAA (doses not provided in abstract) increased thyrotrophin-releasing hormone levels twofold but had no effect on somatostatin (Lewis et al., 1990 [PMID:1982241]). In rat pups [see Section 9.2 for study details], L-BMAA did not affect the day of vaginal opening. Pituitary luteinizing hormone content was decreased in group A females, while significant increases in growth hormone and free thyroxine levels were seen in group B females compared to controls. Group A males had significant increases in serum thyroid-stimulating hormone levels, while group B males had significantly increased insulin growth factor 1 levels (Dawson et al., 1998 [PMID:9536463]).

In rat prostate tissue or a human prostate adenocarcinomas cell line (LNCaP), L-BMAA (1-100 µM [0.1-11.8 µg/mL]) for 0.5 to 18 hours dose-dependently delayed androgen-dependent cell function. At the lowest dose, it reduced androgen levels in the nuclear fraction of rat prostate tissue by 23% within 30 minutes. In LNCaP cells, L-BMAA (3 mM [0.4 mg/mL]) reduced the amount by >20% in the nuclear and cytosol fractions within 18 hours (Yu et al., 1990 abstr.).

Mechanisms of Action

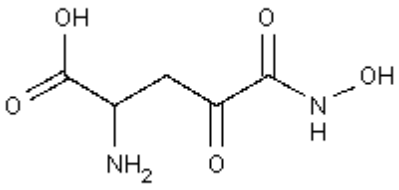
In neonatal mice, L-BMAA was stated to likely exert most of its acute neurotoxic action via the A1 glutamate receptor (Ross and Spencer, 1987 [PMID:3145580]). L-BMAA was a potent agonist of "metabolotropic" glutamate receptors and a proposed mixed agonist of "metabotropic" NMDA receptors in rats (Copani et al., 1990, 1991 [PMID:1657313]). It also modulated glutamate-induced neurotoxicity in the animals (Lysko and Henneberry, 1988 abstr.). Stimulation of both NMDA and non-NMDA glutamate receptors by L-BMAA has been suggested as the mechanisms mediating the neurotoxic effects (Rakonczay et al., 1991 [PMID:1653366]). Based on neurochemical and binding studies, the latter has been strongly suggested (Copani et al., 1991 [PMID:1657313]). In dissociated rat brain cells, L-BMAA dose-dependently increased intracellular calcium levels only in the presence of bicarbonate ion, suggesting its neurotoxicity was due to an excitotoxic mechanism (Brownson et al., 2002 [PMID:12241991]). In primary cortical cells, L-BMAA was reported to exert its neurotoxic effects through three different pathways: activation of NMDA and mGluR5 receptors and induction of oxidative stress (Lobner et al., 2007 [PMID:17098435]).

Effects on Blood

L-BMAA (0.30-5.00 mM [35-591 µg/mL]) produced a significant and dose-dependent change in the physical state of the cytoskeletal proteins of human erythrocyte membranes *in vitro*; the half-maximal effect on protein-protein interactions was ~0.8 mM (Butterfield et al., 1993 [PMID:8397004]).

10.0 Structure-Activity Relationships

β-Oxalylamino-*L*-alanine (L-BOAA) [5302-45-4; 118267-16-6]



L-BOAA is an amino acid structurally related to L-BMAA and is present in the seeds of *Lathyrus sativus* at levels of 1-2%. It is associated with lathyrism, and like L-BMAA has caused abnormal neurological signs (e.g., tremor, mild spasms, and bilateral extensor plantar reflexes) in macaques. In well-nourished humans, oral doses of L-BOAA (hundreds of mg/kg bw) has caused neurological signs after several weeks, while intake of 100 mg/kg bw produced beginning signs of lathyrism in minimally nourished humans after two to three months (Spencer et al., 1987b [PMID:3107939], 1987c [PMID:3603037]).

Studies in mouse CNS cultures have allowed for the detection and characterization of L-BOAA (Spencer et al., 1987a). Single doses of L-BOAA given via systemic administration or intraventricular injection have caused convulsions in newborn mice (Spencer et al., 1987b [PMID:3107939]. An i.p. LD_{50} of 301 mg/kg was reported in the mouse and an LD_{50} value of 1043 mg/kg in mammals [route and species not specified] (ChemIDplus, undated). Like L-BMAA, seizures and prominent excitotoxic pathology were seen in rodents administered L-BOAA (Spencer et al., 1987c [PMID:3603037], 1994).

Comparative toxicity studies of L-BMAA and L-BOAA have been conducted that show L-BOAA is more neurotoxic by a factor of 10^9 (e.g., Pai et al., 1993 [PMID:7901822]). L-BOAA caused dose-dependent inhibition of mitochondrial NADH-dehydrogenase in mouse brain slices as well as in isolated mouse brain mitochondria and induced LDH leakage from the slice into the medium. No effects on other mitochondrial enzymes (e.g., isocitrate dehydrogenase and cytochrome c oxidase) were seen (Pai and Ravindranath, 1993 [PMID:8242335]). In rat striatal homogenates, L-BOAA reduced glutamine synthetase activity and high affinity transport of D-^{3}H-asparate (Doorty and McBean, 1994). However, L-BOAA and glutamate together had a synergistic stimulation of cGMP synthesis in cultured rat cerebellar granule cells (Lysko and Henneberry, 1988 abstr.).

Other Analogues

There are two neuroactive carbamate compounds of L-BMAA, the α-*N*-carboxy adduct and the β-*N*-carboxy adduct. The latter, similar in structure ("practically isosteric") to glutamate, was determined to be the compound probably responsible for the bicarbonate-dependent cytotoxicity of L-BMAA (Myers and Nelson, 1990).

Similar to the effects of L-BMAA, the neurotoxic excitatory amino acid analog kainite [2-carboxy-4-isopropenyl-3-pyrrolidineacetic acid; CASRN 487-79-6] decreased neurite outgrowth of mouse neuroblastoma cells at low doses (10^{-9} to 10^{-7} M) but increased growth at higher doses (10^{-6} to 10^{-5} M) (Abdulla and Campbell, 1993). In guinea pig cerebellar slices, *N*-methylamino acids (*N*-methyl-γ-aminobutyrate, *N*-methylglycine, *N*-methyltaurine, and *N*-methyl-β-alanine) diminished the frequency of spontaneous spike discharges (Okamoto and Quastel, 1977 [PMID:870120]). Like L-BMAA, the neurotoxic and neuroexcitatory effects of the structural analogs *DL*-2,4-diaminobutyrate and *DL*-2,3-diaminopropionate in isolated membrane patches were potentiated by bicarbonate (Weiss et al., 1989a [PMID:2561969]).

11.0 Online Databases and Secondary References

11.1 Online Databases

National Library of Medicine Databases (TOXNET)

ChemIDplus	Household Products
CCRIS	HSDB
DART	IRIS
EMIC and EMICBACK	TOXLINE
GENETOX	TRI

STN International Files

AGRICOLA	IPA
BIOSIS	MEDLINE
BIOTECHNO	NIOSHTIC
CABA	NTIS
CANCERLIT	Registry
EMBASE	RTECS
ESBIOBASE	TOXCENTER

TOXCENTER includes toxicology data from the following files:

Aneuploidy	ANEUPL*
BIOSIS Previews® (1969-present)	BIOSIS*
CAplus (1907-present)	CAplus
International Labour Office	CIS*
Toxicology Research Projects	CRISP*
Development and Reproductive Toxicology	DART®*
Environmental Mutagen Information Center File	EMIC*
Epidemiology Information System	EPIDEM*
Environmental Teratology Information Center File	ETIC*
Federal Research in Progress	FEDRIP*
Health Aspects of Pesticides Abstract Bulletin	HAPAB
Hazardous Materials Technical Center	HMTC*
International Pharmaceutical Abstracts (1970-present)	IPA*
MEDLINE (1951-present)	MEDLINE
Pesticides Abstracts	PESTAB*
Poisonous Plants Bibliography	PPBIB*
Swedish National Chemicals Inspectorate	RISKLINE
Toxic Substances Control Act Test Submissions	TSCATS*

*These are also in TOXLINE. Missing are TOXBIB, NIOSHTIC®, NTIS.

National Archives and Records Administration
Code of Federal Regulations (CFR)

In-House Databases
Current Contents on Diskette®
The Merck Index, 2006, on CD-ROM

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No secondary sources were cited.

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Appendix A: Units and Abbreviations

ºC = degrees Celsius
µg/L = microgram(s) per liter
µg/m^3 = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
ALS = amyotrophic lateral sclerosis
AMPA = α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid
AP4 = *L*-2-amino-4-phosphonobutanoate
AP5 = *DL*(±)2-amino-5-phosphonopentanoic acid
AP7 = 2-amino-7-phosphono-heptanoic acid
bw = body weight
CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione
CNS = central nervous system
DNQX = 6,7-dinitroquinoxaline-2,3-dione
DIV = day(s) *in vitro*
EC_{50} = effective concentration for 50% of organisms tested
EC_{100} = effective concentration for 100% of organisms tested
F = female(s)
g = gram(s)
g/mL = gram(s) per milliliter
hr = hour(s)
HABs = harmful algal blooms
HPLC = high performance liquid chromatography
i.c.v. = intracerebroventricular
InsP = inositol phosphate
i.p. = intraperitoneal(ly)
i.v. = intravenous(ly)
kg = kilogram(s)
L = liter(s)
lb = pound(s)
LC = liquid chromatography
LC_{50} = lethal concentration for 50% of test animals
LD_{50} = lethal dose for 50% of test animals
LDH = lactic dehydrogenase
M = male(s)
mg/kg = milligram(s) per kilogram
mg/m^3 = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
min = minute(s)
MK-801 = [(+)5-methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate
mL/kg = milliliter(s) per kilogram
mm = millimeter(s)
mM = millimolar
mmol = millimole(s)
mmol/kg = millimoles per kilogram

mo = month(s)
mol = mole(s)
mol. wt. = molecular weight
NMDA = *N*-methyl-*D*-aspartate
n.p. = not provided
NTP = National Toxicology Program
PD = Parkinson's disease/dementia
PDA = *cis*-2,3-piperidine dicarboxylic acid
PDC = Parkinson dementia complex
TH = tyrosine hydroxylase

Appendix B: Description of Search Strategy

On June 2-3, 2005, a search for L-BMAA in the files MEDLINE, CANCERLIT, NIOSHTIC, AGRICOLA, CABA, BIOTECHNO, EMBASE, ESBIOBASE, BIOSIS, IPA, TOXCENTER, and NTIS used the following strategy:

```
L1          190 S 15920-93-1
L2            0 S 16674-91-8
L3          245 S 16676-91-8
L4            1 S 16012-55-8
L5            2 S 20790-76-5
L6          431 S L1 OR L2 OR L3 OR L4 OR L5
L7           66 S (BETA OR β OR 3)(W)METHYLAMINO(2A)ALANINE
L8           61 S β(2A)METHYLAMINOALANINE
L9           61 S BETA(2A)METHYLAMINOALANINE
L10          12 S BETA(W)METHYL(W)AMINO(2A)ALANINE
L11         407 S BETA(2A)METHYLAMINO(2A)ALANINE
L12           2 S 2(W)AMINO(W)3(W)METHYLAMINO(W)L(W)(PROPION? OR PROPANO?)
L13           0 S BETA(W)METHYL(W)α(W)β(W)(DIAMINOPROPRION? OR DIAMINOPROPANO?)
L14          96 S L(W)BMAA
L15         410 S BMAA NOT (BOVINE OR MELANIN)
L16         262 S L11 AND L15
L17         262 S L11 AND (L15 OR L14)
L18         617 S L6 OR L7 OR L8 OR L10 OR L11 OR L12 OR L14 OR L16
L19          49 S L18 AND (REVIEW? OR REVIEW/DT)
                SET DUPORDER FILE
L20          29 DUP REM L19 (20 DUPLICATES REMOVED)
L21          29 SORT L20 1-29 TI
                SAVE L21 X240REVU/A
L22         568 S L18 NOT L19
L23         202 DUP REM L22 (366 DUPLICATES REMOVED)
L24         202 SORT L23 1-202 TI
L25          35 S L22 AND (CHRONIC OR SUBCHRONIC OR WEEK? OR MONTH? OR 90(W)DAY
L26         238 S L22 AND (METAB? OR HYDROLY? OR URIN? OR BLOOD? OR SERUM? OR PLASMA?)
L27           8 S L22 AND (CYTOTOX? OR PROLIFERAT? OR PEROXISOM?)
L28         528 S L22 AND (NEURO? OR BRAIN OR CEREBR? OR CEREBELL? OR NERV? OR
                DEMENTIA? OR PARKINSON?)
L29          80 S L22 AND (ALZHEIMER? OR EPIDEMIOL? OR ENDEMIC?)
L30          23 S L22 AND (REPRODUCTI? OR DEVELOPMENTAL? OR TERAT?)
L31          74 S L22 AND (ANDROGEN? OR ESTROGEN? OR TESTES OR TESTIS OR SPERM? OR
                EPIDIDYM?)
L32          46 S L22 AND (GENE? OR MUTA?)
L33          17 S L22 AND (CARCINO? OR CANCER? OR TUMOR? OR TUMOUR? OR NEOPLAS
L34          47 S L22 AND ENDOCRIN?
L35         150 S L22 AND (RATS OR MICE OR PIG OR PIGS OR RABBIT? OR HAMSTER?
L36         213 S L22 AND (HUMAN? OR MONKEY? OR PRIMATE?)
L37         558 S L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33 OR L34
                OR L35 OR L36 OR L37
L38          10 S L22 NOT L37
L39           7 DUP REM L38 (3 DUPLICATES REMOVED)
L40           7 SORT L39 1-7 TI
=> DUP REM L37
PROCESSING COMPLETED FOR L37
L41         198 DUP REM L37 (360 DUPLICATES REMOVED)
                ANSWERS '1-85' FROM FILE MEDLINE
                ANSWERS '86-87' FROM FILE AGRICOLA
                ANSWERS '88-90' FROM FILE CABA
                ANSWER '91' FROM FILE BIOTECHNO
                ANSWERS '92-119' FROM FILE EMBASE
                ANSWER '120' FROM FILE ESBIOBASE
```

```
                ANSWERS '121-176' FROM FILE BIOSIS
                ANSWERS '177-197' FROM FILE TOXCENTER
                ANSWER '198' FROM FILE NTIS
```

PubMed searches were performed in June 2005 with the CASRN, l bmaa, lbmaa, bmaa, alpha amino beta methylaminoproprionate, and other synonyms (e.g., methylamino propanoic) alone and with the following terms: AND (brain OR cerebr* OR cerebella* OR nervous); AND (dement* OR neurology* OR neurotoxic*): NOT bovine; NOT melanin. Additionally, a search was performed on all cycad toxins publications and on the epidemiology of ALS-PDC in Guam using the following terms: guam AND (pd OR Parkinson*) AND als (115 hits); guam AND als (131 hits); (neurotox* AND cyca) OR (neurotox* [mh] AND cyca) (114 hits); excitotoxin* AND NMDA (319 hits); excitotoxin AND cyca* (1 hit); and excitotoxin* (1058 hits).

Brief Internet searches with the Google search engine were also done. Using the term "methylamino l alanine" produced ~444 results; the term "methylaminoalanine" produced ~56 hits. Attachment A includes URLs from the searches.

Updated Search Strategy (2006)

On January 30, 2006, a search for L-BMAA in the files MEDLINE, NIOSHTIC, AGRICOLA, CABA, BIOTECHNO, EMBASE, ESBIOBASE, BIOSIS, IPA, TOXCENTER, and NTIS used the following strategy for any new information since submission of the draft report in July 2005:

```
L1          198 S 15920-93-1 OR 16674-91-8 OR 16012-55-8 OR 20790-76-5
L2          249 S 16676-91-8
L3          441 S L1 OR L2
L4           76 S (BETA OR 3)(W)METHYLAMINO(2A)ALANINE
L5           67 S BETA(2A)METHYLAMINOALANINE
L6           18 S BETA(W)METHYL(W)AMINO(2A)ALANINE
L7          423 S BETA(2A)METHYLAMINO(2A)ALANINE
L8            2 S 2(W)AMINO(W)3(W)METHYLAMINO(W)L(W)(PROPION? OR PROPANO?)
L9           97 S L(W)BMAA
L10         423 S BMAA NOT (BOVINE OR MELANIN)
L11         518 S L4 OR L5 OR L6 OR L7 OR L8 OR L9
L12         648 S L3 OR L11
L13          74 S L10 NOT L12
L14         722 S L12 OR L13
L15          44 S L14 AND (2005-2006)/PY
                SET DUPORDER FILE
L16          15 DUP REM L15 (29 DUPLICATES REMOVED)
                ANSWERS '1-6' FROM FILE MEDLINE
                ANSWER '7' FROM FILE AGRICOLA
                ANSWER '8' FROM FILE CABA
                ANSWERS '9-11' FROM FILE EMBASE
                ANSWER '12' FROM FILE BIOSIS
                ANSWERS '13-15' FROM FILE TOXCENTER
L17          15 SORT L16 1-15 TI
```

Updated Search Strategy (2008)

On July 16, 2008, STN International files MEDLINE, CABA, AGRICOLA, EMBASE, ESBIOBASE, BIOTECHNO, IPA, BIOSIS, TOXCENTER, FSTA, FROSTI, and PASCAL were searched simultaneously. This second update search strategy (shown below) added additional synonyms (primarily query L10 below) so that a second search was done to see if use

of the additional synonyms in older searches would have missed useful information. The history of the main online session for update 2 is reproduced below.

```
L1          226 S 15920-93-1 OR 16012-55-8 OR 20790-76-5
L2          304 S 16676-91-8
L3          574 S (BETA OR 3)(2A)METHYLAMINO(2A)ALANINE
L4           78 S (BETA OR 3)(2A)METHYLAMINOALANINE
L5           60 S (ALPHA OR 2)(W)AMINO(W)(3 OR BETA)(W)
                METHYLAMINO(2A)(PROPION? OR PROPANO?)
L6          111 S L(W)BMAA
L7          574 S BMAA NOT (BOVINE OR MELANIN)
L8          941 S L1-L7
L9            0 S MONOMETHYL(W)(BETA OR 3)(W)AMINOALANINE
L10         176 S (ALPHA OR 2)(2A)AMINO(W)(3 OR BETA)
                (2A)(METHYLAMINOPROPIONIC OR METHYLAMINOPROPANOIC)(W)ACID)
L11         963 S L8 OR L10
                SET DUPORDER FILE
L12         332 DUP REM L11 (631 DUPLICATES REMOVED)
                ANSWERS '1-140' FROM FILE MEDLINE
                ANSWERS '141-158' FROM FILE CABA
                ANSWERS '159-161' FROM FILE AGRICOLA
                ANSWERS '162-211' FROM FILE EMBASE
                ANSWER '212' FROM FILE ESBIOBASE
                ANSWERS '213-283' FROM FILE BIOSIS
                ANSWERS '284-324' FROM FILE TOXCENTER
                ANSWERS '325-326' FROM FILE FSTA
                ANSWER '327' FROM FILE FROSTI
                ANSWERS '328-332' FROM FILE PASCAL
L13          53 S L12 AND (2006-2008)/PY
L14          53 DUP REM L13 (0 DUPLICATES REMOVED)
                ANSWERS '1-25' FROM FILE MEDLINE
                ANSWERS '26-27' FROM FILE CABA
                ANSWERS '28-29' FROM FILE AGRICOLA
                ANSWERS '30-42' FROM FILE EMBASE
                ANSWERS '43-51' FROM FILE BIOSIS
                ANSWERS '52-53' FROM FILE TOXCENTER
L15          53 SORT L14 1-53 TI
                SAVE L11 LBMAANAMES/Q
```

Of the 53 presumably unique results, 39 were selected for printing. Of those not selected, two were duplicates from update search 1 and the rest were seemingly general reviews or on syntheses or analytical methods. Two pairs of answers were duplicates with somewhat different titles and three records appeared to offer nothing useful for the update after examination of their abstracts. Of the 62 titles examined from the possibly overlooked answer set, only 9 were selected for printing. They had not been retrieved before and might provide additional information. No ready explanation occurred to us to explain why the PASCAL result in column 4 was higher than the result in column 2.

Database Tallies

Database	1907-2008 (after full-set dupl. removal)	Update 2 (2006-2008)	Possibly Overlooked (1911-2005)	Selected Update 2 (Printed full records)	Selected Overlooked (Printed full records)	Final Selections Minus 2 Duplicates & 3 Unwanted
MEDLINE	140	25	13	22	1	22
CABA	18	2	4	2	1	3
AGRICOLA	3	2	2	1	0	1
EMBASE	50	13	3	7	1	7
ESBIOBASE	1	0	2	0	0	0
BIOSIS	71	9	12	6	0	4
TOXCENTER	41	2	12	1	3	3
FSTA	2	0	1	0	0	0
FROSTI	1	0	0	0	0	0
PASCAL	5	0	13	0	3	3
Totals	*332*	*53*	*62*	*39*	*9*	*43*