# Supplemental material

Table S1. Tissue Distribution of Radioactivity 24 h Following a Single Gavage or Intravenous Administration of [14C]L-BMAA to Harlan Sprague Dawley Rats

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Concentration (nmol-Equivalents/g Tissue)a | | | | | | | | | | | | | | | | |
| Tissue | Male, Gavage  1 mg/kg | | | Male, Gavage  10 mg/kg | | | Male, Gavage  100 mg/kg | | | Male, IV  1 mg/kg | | | | Female, Gavage  10 mg/kg | | |  |
| Bloodb | 1.38 | ± | 0.18 | 12.0 | ± | 1.8 | 111 | ± | 8 | 2.14 | ± | 0.20 | | 10.6 | ± | 1.8 |
| Adipose | 0.80 | ± | 0.28 | 8.35 | ± | 2.42 | 51.3 | ± | 12.1 | 1.25 | ± | 0.33 | | 6.72 | ± | 1.01 |
| Muscle | 0.85 | ± | 0.12 | 6.73 | ± | 1.02 | 98.9 | ± | 20.7 | 2.07 | ± | 0.52 | | 9.46 | ± | 1.75 |
| Skin | 1.58 | ± | 0.19 | 12.0 | ± | 1.2 | 133 | ± | 14 | 3.48 | ± | 0.91 | | 10.7 | ± | 1.0 |
| Brain | 0.66 | ± | 0.18 | 6.67 | ± | 3.52 | 106 | ± | 24 | 1.84 | ± | 0.78 | | 8.45 | ± | 2.67 |
| Heart | 1.27 | ± | 0.42 | 9.09 | ± | 1.52 | 104 | ± | 14 | 2.48 | ± | 2.41 | | 8.50 | ± | 0.84 |
| Kidneys | 3.10 | ± | 0.94 | 23.9 | ± | 4.1 | 344 | ± | 75 | 5.20 | ± | 0.90 | | 21.0 | ± | 1.6 |
| Liver | 8.26 | ± | 2.63 | 73.4 | ± | 24.1 | 738 | ± | 460 | 8.77 | ± | 1.30 | | 108 | ± | 63 |
| Lung | 1.86 | ± | 0.42 | 15.3 | ± | 2.0 | 149 | ± | 19 | 2.71 | ± | 0.61 | | 13.5 | ± | 0.9 |
| Spleen | 2.29 | ± | 0.80 | 16.1 | ± | 2.5 | 171 | ± | 19 | 2.22 | ± | 1.53 | | 14.3 | ± | 0.9 |
| Adrenals | 3.32 | ± | 1.21 | 24.2 | ± | 4.2 | 268 | ± | 75 | 4.58 | ± | 0.69 | | 27.2 | ± | 3.3 |
| Thymus | 2.40 | ± | 1.78 | 19.7 | ± | 4.3 | 214 | ± | 40 | 2.12 | ± | 1.18 | | 20.2 | ± | 1.8 |
| Thyroid | 3.75 | ± | 1.75 | 21.9 | ± | 6.2 | 431 | ± | 109 | 4.04 | ± | 0.92 | | 37.9 | ± | 20.5 |
| Urinary Bladder | 1.90 | ± | 1.64 | 15.7 | ± | 6.5 | 415 | ± | 299 | 3.61 | ± | 2.05 | | 10.7 | ± | 1.8 |
| Pancreas | 1.32 | ± | 0.44 | 16.8 | ± | 2.0 | 246 | ± | 50 | 2.74 | ± | 1.57 | | 15.3 | ± | 1.9 |
| Testes | 0.68 | ± | 0.19 | 6.11 | ± | 2.02 | 72.1 | ± | 14.8 | 0.69 | ± | 0.266 | | 9.44 | ± | 0.75 |
| Cecum | 1.75 | ± | 0.50 | 11.2 | ± | 2.9 | 134 | ± | 25 | 2.39 | ± | 0.41 | | 9.40 | ± | 1.55 |
| Large Intestine | 1.63 | ± | 0.37 | 12.0 | ± | 2.5 | 143 | ± | 50 | 2.07 | ± | 0.66 | | 11.7 | ± | 2.4 |
| Small Intestine | 2.61 | ± | 0.47 | 21.5 | ± | 5.2 | 206 | ± | 22.3 | 4.42 | ± | 0.45 | | 17.3 | ± | 1.5 |
| Stomach | 2.40 | ± | 1.75 | 12.3 | ± | 1.5 | 129 | ± | 6 | 3.38 | | ± | 0.92 | 12.2 | ± | 2.1 |

aMean ± SD (N = 5).   
bTissue weights for the dispersed tissues were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (International Life Sciences Institute, 1994).

Table S2. Tissue Distribution of Radioactivity 24 h Following 1,5 or 10 daily gavage doses of 1 mg/kg [14C]L-BMAA in male Harlan Sprague Dawley rats

|  |  |  |  |
| --- | --- | --- | --- |
| Tissue | Concentration | (nmol-Equivalents/g Tissue)a |  |
|  |  | 1 mg/kg |  |
|  | 1 (1 d)b | 5 (1 d) | 10 (1 d) |
| Bloodc | 1.38 ± 0.18 | 3.30 ± 0.29 | 4.32 ± 0.70 |
| Adipose | 0.80 ± 0.28 | 2.86 ± 0.67 | 18.9 ± 10.6 |
| Muscle | 0.85 ± 0.12 | 2.73 ± 0.47 | 4.85 ± 0.68 |
| Skin | 1.58 ± 0.19 | 5.25 ± 0.79 | 8.90 ± 0.76 |
| Brain | 0.66 ± 0.18 | 1.77 ± 0.26 | 2.67 ± 0.32 |
| Heart | 1.27 ± 0.42 | 3.63 ± 1.06 | 5.09 ± 0.57 |
| Kidney | 3.10 ± 0.94 | 7.93 ± 0.46 | 10.3 ± 1.04 |
| Liver | 8.26 ± 2.63 | 15.0 ± 1.82 | 22.5 ± 2.47 |
| Lung | 1.86 ± 0.42 | 4.35 ± 0.34 | 5.72 ± 0.74 |
| Spleen | 2.29 ± 0.80 | 4.34 ± 0.53 | 6.37 ± 0.61 |
| Adrenal | 3.32 ± 1.21 | 9.96 ± 2.09 | 15.5 ± 0.65 |
| Thymus | 2.40 ± 0.63 | 5.54 ± 0.92 | 7.95± 1.02 |
| Thyroid | 3.75 ± 1.75 | 10.5 ± 6.86 | 7.84 ± 1.17 |
| Bladder | 1.90 ± 1.64 | 5.61 ± 1.27 | 7.22 ± 1.04 |
| Pancreas | 2.32 ± 0.44 | 4.99 ± 1.34 | 8.98 ± 1.51 |
| Testes | 0.68 ± 0.19 | 2.10 ± 0.32 | 3.05 ± 0.43 |

aMean ± SD (N = 5).

bNumber of doses (sacrifice time in days) are shown.  
cTissue weights for the dispersed tissues were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (International Life Sciences Institute, 1994).

Table S3. Tissue Distribution of Radioactivity Following Single Gavage Administration of 10 mg/kg [14C]L-BMAA in Male Harlan Sprague Dawley Rats and Sacrificed 1, 3, 5 or 7 days Following Administration

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tissue | Concentration | (nmol-Equivalents/g Tissue)a |  |  |
|  | 1 (1d)b | 1 (3 d) | 1 (5d) | 1 (7d) |
| Bloodc | 12.0 ±1.81 | 10.3 ± 2.80 | 5.40 ± 0.61 | 4.45 ± 0.68 |
| Adipose | 8.35 ± 2.42 | 7.31 ± 0.87 | 4.17 ± 0.82 | 4.29 ± 1.13 |
| Muscle | 6.73 ± 1.02 | 7.47 ± 2.12 | 6.05 ± 1.20 | 5.25 ± 0.56 |
| Skin | 12.0 ± 1.20 | 10.3 ± 1.10 | 12.9 ± 1.68 | 9.50 ± 1.65 |
| Brain | 6.67 ± 3.52 | 7.94 ± 2.25 | 3.60 ± 1.03 | 3.16 ± 1.27 |
| Heart | 9.09 ± 1.52 | 8.36 ± 1.59 | 5.84 ± 0.62 | 4.77 ± 0.54 |
| Kidney | 23.9 ± 4.10 | 16.4 ± 2.90 | 9.93 ± 0.73 | 7.11 ± 0.94 |
| Liver | 73.4 ± 24.1 | 67.8 ± 18.4 | 14.8 ± 2.60 | 11.9 ± 4.80 |
| Lung | 15.3 ± 2.01 | 11.0 ± 1.00 | 6.63 ± 0.52 | 5.13 ± 0.62 |
| Spleen | 16.1 ± 2.50 | 10.7 ± 1.90 | 7.30 ± 0.71 | 5.47 ± 0.85 |
| Adrenal | 24.2 ± 4.20 | 20.7± 3.91 | 11.9 ± 1.72 | 11.2 ± 2.60 |
| Thymus | 19.7 ± 4.30 | 16.9 ± 4.90 | 8.40 ± 1.20 | 5.20 ± 0.95 |
| Thyroid | 21.9 ± 6.21 | 20.2 ± 7.40 | 19.9 ± 6.84 | 15.2 ± 2.20 |
| Bladder | 15.7 ± 6.50 | 13.0 ± 5.10 | 11.6 ± 3.48 | 8.64 ± 1.85 |
| Pancreas | 16.8 ± 2.01 | 12.0 ± 1.81 | 6.04 ± 0.72 | 5.04 ± 0.78 |
| Testes | 6.11 ± 2.02 | 5.81 ± 1.42 | 4.03 ± 1.08 | 3.21 ± 0.60 |

aMean ± SD (N = 5).

bNumber of doses (sacrifice time in days) are shown.  
cTissue weights for the dispersed tissues were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (International Life Sciences Institute, 1994).

Table S4. Tissue Distribution of Radioactivity 24 h Following a Single Gavage or Intravenous Administration of [14C]L-BMAA to B6C3F1/N Mice

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Disposition in Tissues (nmol-Equivalents/per g Tissue)a | | | | | | | | | | | | | | | |
| Tissue | Male, Gavage  1 mg/kg | | | Male, Gavage  10 mg/kg | | | Male, Gavage  100 mg/kg | | | Male, IV  1 mg/kg | | | Female, Gavage  10 mg/kg | | |
| Bloodb | 0.44 | ± | 0.11 | 6.64 | ± | 1.71 | 54.2 | ± | 8.1 | 0.47 | ± | 0.12 | 4.57 | ± | 0.68 |
| Adipose | 0.39 | ± | 0.17 | 4.06 | ± | 0.98 | 64.0 | ± | 23.7 | 0.46 | ± | 0.09 | 9.41 | ± | 5.67 |
| Muscle | 0.72 | ± | 0.15 | 5.89 | ± | 1.88 | 62.4 | ± | 21.4 | 0.96 | ± | 0.25 | 12.7 | ± | 1.4 |
| Skin | 0.67 | ± | 0.15 | 7.83 | ± | 1.75 | 52.6 | ± | 10.7 | 0.93 | ± | 0.19 | 8.82 | ± | 0.84 |
| Brain | 0.31 | ± | 0.09 | 3.48 | ± | 0.81 | 36.7 | ± | 7.2 | 0.49 | ± | 0.04 | 3.79 | ± | 0.92 |
| Heart | 0.50 | ± | 0.04 | 6.11 | ± | 0.42 | 69.1 | ± | 28.2 | 0.73 | ± | 0.16 | 7.60 | ± | 2.79 |
| Kidneys | 1.45 | ± | 0.12 | 16.6 | ± | 3.1 | 203 | ± | 82 | 1.71 | ± | 0.16 | 16.6 | ± | 1.6 |
| Liver | 4.61 | ± | 2.12 | 36.7 | ± | 2.7 | 691 | ± | 223 | 4.14 | ± | 1.44 | 37.5 | ± | 7.7 |
| Lung | 0.65 | ± | 0.10 | 9.59 | ± | 1.95 | 110 | ± | 39 | 0.92 | ± | 0.11 | 10.1 | ± | 1.1 |
| Spleen | 0.66 | ± | 0.05 | 10.3 | ± | 6.2 | 131 | ± | 52 | 0.98 | ± | 0.26 | 13.1 | ± | 3.5 |
| Adrenals | 1.19 | ± | 0.78 | 30.9 | ± | 20.6 | 242 | ± | 53 | 2.38 | ± | 0.90 | 36.7 | ± | 7.7 |
| Thymus | 1.22 | ± | 0.12 | 12.7 | ± | 1.7 | 223 | ± | 133 | 1.37 | ± | 0.38 | 16.2 | ± | 1.9 |
| Thyroid | 1.59 | ± | 0.96 | 68.6 | ± | 46.8 | 340 | ± | 83 | 2.29 | ± | 1.07 | 34.0 | ± | 2.9 |
| Urinary Bladder | 0.98 | ± | 0.33 | 9.92 | ± | 1.83 | 150 | ± | 77 | 1.48 | ± | 0.49 | 23.8 | ± | 5.3 |
| Pancreas | 0.77 | ± | 0.06 | 9.83 | ± | 1.20 | 127 | ± | 70 | 0.88 | ± | 0.22 | 10.2 | ± | 1.4 |
| Testes/Ovaries | 0.36 | ± | 0.05 | 4.31 | ± | 1.17 | 44.1 | ± | 17.4 | 0.42 | ± | 0.07 | 13.2 | ± | 4.4 |
| Cecum | 0.66 | ± | 0.16 | 9.04 | ± | 3.60 | 110 | ± | 43 | 0.87 | ± | 0.11 | 10.8 | ± | 2.5 |
| Large Intestine | 0.79 | ± | 0.13 | 13.1 | ± | 1.4 | 92.8 | ± | 24.3 | 0.88 | ± | 0.33 | 11.5 | ± | 1.8 |
| Small Intestine | 1.08 | ± | 0.21 | 14.8 | ± | 1.1 | 123 | ± | 23.8 | 1.22 | ± | 0.17 | 11.8 | ± | 1.8 |
| Stomach | 0.85 | ± | 0.06 | 10.7 | ± | 1.3 | 106 | ± | 34 | 1.06 | ± | 0.08 | 11.6 | ± | 0.9 |

aMean ± SD (N = 5).   
bTissue weights for the dispersed tissues were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (International Life Sciences Institute, 1994).

Table S5. Expected peptide mass shifts associated with substitution of amino acids with L-BMAA or its metabolites

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Table S6. Potential adduct forming electrophiles arising from L-BMAA and expected masses

|  |  |  |
| --- | --- | --- |
| **Adduct** | **Molecular Weight** | **Expected Mass Shift** |
| L-BMAA | 118 | 116 |
| 2-amino-3-oxopropionic acid | 103 | 101 |
| Formaldehyde | 30 | 28 |
| Formaldehyde –H2O | NA | 12 |

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Table S7. Summary of counts for mass shifts observed in brains of control and L-BMAA-exposed rats based on Z-score and Chi Squared tests

Table S7. Summary of counts for mass shifts observed in brains of control and L-BMAA-exposed rats based on Z-score and Chi Squared tests

Table S8. Summary of counts for mass shifts that had at least 20 counts and were at least 2-fold different between brains of control and L-BMAA-exposed rats

Table S8. Summary of counts for mass shifts that had at least 20 counts and were at least 2-fold different between brains of control and L-BMAA-exposed rats 

Figure S1. HPLC radiochromatogram of rat urine collected following a single gavage administration of 100 mg/kg [14C]L-BMAA

**Figure S1. HPLC radiochromatogram of rat urine collected following a single gavage administration of 100 mg/kg [14C]L-BMAA**

Figure S2. Radiochemical regions observed in urine of L-BMAA-exposed rats during LC-MS/MS metabolite identification couple to beta ram detector

**Figure S2. Radiochemical regions observed in urine of L-BMAA-exposed rats during LC-MS/MS metabolite identification couple to beta ram detector **

**Method S1**

***Plasma sample preparation.*** One hundred microgram of plasma was dried under vacuum and in dissolved in 50 μL of 8M deionized urea, containing 1.0 M tris (pH 8.5), 8 mM CaCl2 and 0.2M methylamine. Following reduction of disulfides with 4μL of 0.2 M dithioerythritol (DTT) at 50°C for 15 min, cysteines were alkylated using 4 μL of 0.5 M iodoacetamide at room temperature for 30 min. Excess iodoacetamide was scavenged by reacting with 8 μL of 0.2 M DTT at room temperature for 15 min. After adding 94 μL of water samples was digested overnight at 37°C with 40 μL (0.1 μg/μL) trypsin (Sigma, Proteomics Grade) in 1 mM HCl. Reaction was stopped with 10 μL of formic acid. The peptide digests were subjected to solid phase extraction (Waters Sep Pak Light cartridges), followed by fractionation by cation exchange. The recovered peptides were dissolved in 1.0 ml of 75% acetonitrile, 0.1 % formic acid were applied to a 20 μL bed volume Reliasil SCX cartridge (Optimize Technologies) using a glass tight syringe and syringe pump flowing at 100 μL per min. The eluate from the Sep Pak cartridges was passed through twice to assure binding of all peptides and the flow through retained. Bound peptides were then eluted by sequential 300 μL injections of 17, 21, 25, 37.5, 56.5, 75, 150, and 375 mM ammonium formate solution containing 25% ACN, 0.4% formic acid. These 8 fractions and the flow through were then dried by vacuum centrifugation and each dissolved in 40 μL of 5% formic acid in preparation for mass spectrometry as described below.

***Brain sample preparation***. Brains were thawed and homogenized in 1.0 mL of 100 mM ammonium bicarbonate buffer using a Wheaton ground glass homogenizer and homogenate was centrifuged at 14,000 g for 15 min at 4°C. The pellet was suspended in 1.0 mL of 100 mM ammonium bicarbonate buffer by brief probe sonication (Fisher Scientific, Sonic Dismembrator 60) to produce a uniform suspension. A BCA protein assay (Pierce) was then performed on both the supernatant (concentration ~ 6 mg/mL) and suspended pellet (concentration ~ 18-20 mg/mL) using bovine serum albumin as a standard. One hundred microliter aliquots of each of these fractions were then vacuum dried, trypsinized, and separated by cation exchange as described above.

***2D- LC-MS/MS.*** LC-MS was performed using an Agilent 1100 series capillary LC system (Agilent Technologies) and an LTQ Velos Pro linear ion trap mass spectrometer (Thermo Scientific). Electrospray ionization was performed with an ion max source fitted with a 34 gauge metal needle (Thermo Scientific, cat. no. 97144-20040) and 2.7 kV source voltage. Samples were applied at 20 μL/min to a trap cartridge (Michrom BioResources, Inc.), and then switched onto a 0.5 x 250 mm Zorbax SB-C18 column with 5 μM particles (Agilent Technologies) using a mobile phase containing 0.1% formic acid, 7-30% acetonitrile gradient over 110 min, and 10 μL/min flow rate. Data-dependent collection of MS/MS spectra used the dynamic exclusion feature of the instrument‟s control software (repeat count equal to 1, exclusion list size of 100, exclusion duration of 30 sec, and exclusion mass width of -1 to +4) to obtain MS/MS spectra of the five most abundant parent ions (minimum signal of 10,000) following each survey scan from m/z 350-2000. The tune file was configured with no averaging of microscans, a maximum MS1 inject time of 200 msec, a maximum MS2 inject time of 100 msec, and automatic gain control targets of 3 x 104 in MS1 mode and 1 x 104 in MS2 mode.

***Mass Spectrometry Data analysis.***

***Database***: Rattus norvegicus FASTA protein database was downloaded from the Uniprot website (www.uniprot.org), and appended with 179 common contaminant sequences. A sequence-reversed database was used to estimate error thresholds (Elias and Gygi, 2007) making a final database size of 16,046 entries. The database processing was performed

with python scripts available at <http://www.ProteomicAnalysisWorkbench.com>.

***Sequest searching:*** RAW data from the mass spectrometer were converted to DTA files representing individual MS2 spectra using extract\_msn.exe (version 5.0; Thermo Scientific); duplicate 2+/3+ spectra were created for non-singly-charged ions. The group scan minimum count was 1, a minimum of 25 ions were required, the mass tolerance for combining DTAs was set to a small value to prevent combining, an absolute intensity of greater than 500 was required, and MH+ values had to be in the range of 550 to 4000 Da. SEQUEST (version 28, revision 12, Thermo Scientific) searches for all samples were performed with trypsin specificity. Average parent ion mass tolerance was 2.5 Da. Average fragment ion mass tolerance was 1.0 Da. The ion series used in scoring were b and y. A static modification of +57.02 Da was added to all cysteine residues. A variable modification of +16 Da on methionine residues was also allowed with a maximum of 3 modifications per peptide.

***Statistical Validation:*** A linear discriminant transformation was used to improve the identification sensitivity from the SEQUEST analysis (Keller et al., 2002; Wilmarth et al., 2009). SEQUEST DTA and OUT files were compressed using in-house Python scripts (PAW\_MudPIT\_Zipper.py). The zipped results files were converted to SQT and MS2 files, SEQUEST scores combined into linear discriminant function scores, and discriminant score histograms created separately for each peptide charge state (1+, 2+, and 3+). Separate histograms were created for matches to forward sequences and for matches to reversed sequences (PAW\_convert.py) for all peptides of 7 amino acids or longer. The score histograms for reversed matches were used to estimate peptide false discovery rates (FDR) and set score thresholds for each peptide class. Any identifications not passing the score thresholds were removed from the SQT and MS2 files by another script (PAW\_filter.py). The entire set of confidently identified peptides was collectively mapped to the protein database. Any proteins identified by identical sets of peptides were grouped together as redundant proteins. Any proteins identified by a peptide set that was a formal subset of another protein‟s peptide set were removed (parsimony principle). Any proteins that were not identified by at least two distinct peptides having two tryptic termini per sample were removed from the final list of identified proteins. Peptide to protein mapping and protein filtering was performed using PAW\_results\_6.py (version 6.0). As a result of the above filtering, the 2,243,757 MS2 spectra collected during the analysis of the 4 brain 2D-LC MS/MS datasets were reduced to a subset of 332,724 confidently matched spectra, with 899 additional spectra matched to reversed peptide sequences, giving a false discovery rate (FDR) of 0.27%. After mapping peptide sequences to protein entries and parsimony filtering we were left with 2,568 protein identifications, with an additional 21 matches to reversed sequences, giving a FDR of 0.82%. The numbers of MS2 spectra collected during the analysis of the plasma digests were 725,176 and after filtering this was reduced to 65,732 spectra assigned to forward sequences and 457 assigned to reversed sequences, giving a peptide FDR of 0.70%. After mapping peptide sequences to protein entries and parsimony filtering this resulted in 223 serum protein identifications, with a FDR of 0.90%. These lists of confidently identified proteins were next used to create a database of proteins to perform wildcard searches of amino acid mass shifts using Byonic software.

***Byonic wildcard post-translational modification searches:*** A program designed to perform a wildcard search, where all possible mass shifts within a specified range are applied to each amino acid within candidate peptides, using the program Byonic (Bern, Kil, and Becker, 2012). In preparation for the Byonic searches, confidently matched proteins from the brain were put through a strict filtering requiring at least 8 unique peptides per protein. There were 1,204 proteins that passed this filtering in the brain samples. This resulted in the creation of a subset database enriched for the more abundant proteins, at a size that could be analyzed in approximately 36 hours using an 8-processor computer. The initial Sequest results from the plasma analysis did not require this filtering, since only 223 proteins were identified. The subset database creation was handled by an in-house python script (PAW\_database\_creation\_1.py) and .mgf files were created from the .dta files using merge.pl, a script available from Matrix Science. Each of the 6 2D-LC MS/MS datasets (2 plasma and 4 brain) were searched independently with Byonic. For the searches, precursor tolerance was set to 2.0 Da and fragment tolerance was set to 1.0 Da. The wildcard search was enabled with a mass range of -40 Da to +200 Da on every amino acid, with „Off by x isotopes‟ set to „Off by one or two‟. Carbamidomethyl at cysteines was set as a fixed modification and oxidation was set as a common1 variable modification, with a maximum of 2 common modifications per peptide set as a maximum value. Searches were performed using version PMI-Byonic-Com:v1.3beta-84.

After analysis, the results from the supernatant and pellet digests for the brain samples were combined for both the control and L-BMAA groups. The false discovery rate at the peptide level was estimated by Byonic to be about 10% in each of the analysis. Low quality spectra, below scores of 600 and 400 were then removed from the analysis of the brain and plasma samples, respectively. At this point we were left with 485,050 and 524,886 spectra successfully matched to sequences in the control and L-BMAA-exposed groups in brain, respectively, with 59,161 and 70,790 of these, respectively, containing detected mass shifts. Based on the number of modifications assigned to sequence reversed peptides after this filtering, the false discovery rate for modified peptides in the brain samples was 4.1% in the control group and 6.8% in the L-BMAA treated group.

During the analysis of plasma samples Byonic matched 45,719 and 24,141 spectra in the control and L-BMAA groups, respectively, with 22,584 and 9,740 of these, respectively, containing detected mass shifts. Based on the number of modifications assigned to sequence reversed peptides after this filtering, the false discovery rate for modified peptides in the serum samples was 0.3% in the control group and 0.08% in the L-BMAA-exposed group. To detect abundance differences in the number of assigned modifications between control and L-BMAA-exposed samples, heat maps displaying the number of specific amino acids containing each mass shift were generated for both groups using an in-house python script (byonic\_ptm\_filter\_clipboard\_PW).

***Quantitative analysis:*** Results from plasma samples were filtered using a Z-transformation of the data to control for dispersion in the lower count data, followed by a combination of Chi squared and Zscore thresholds to identify modifications that were differentially abundant in the two sample groups. In this method, a 61-modification-wide sliding window from highest to lowest counts was used for the Z-transformation to control for greater dispersion for the lower count modifications. Cutoffs were then set by fitting a normal distribution to a histogram of Z-transformed ratios of modifications between the two groups. Modifications with a p-value of more than 0.1 were considered non-candidates, 0.1-0.05 were considered low probability candidates, 0.05-0.01 were ranked medium, and 0.01 and less were ranked high. Z-score and Chi squared tests were not performed for modifications with a „0‟ value in one of the groups due to divide by zero errors. However, candidates with 12-13 counts in one sample and 0 counts in the other were also included in the “medium” group, and candidates with 11 counts in one sample and 0 counts in the other were included in the “low” group (“z-score candidates” tab). Due to the differences in the overall number of assigned MS/MS spectra in the control and L-BMAA-exposed groups, differential candidates were not identified using simple fold difference cut offs.

Results from brain were similarly filtered using a combination of z-scores and Chi squared tests as described above. Z-score and Chi squared tests were not performed for modifications with a

„0‟ value in one of the groups due to divide by zero errors. Candidates with 10 and 0 counts, was well as candidates with 9 and 0 counts were included in the “medium” group above, since the filtered data with 11 and 2 counts and 9 and 2 counts had p-values placing them in the “medium” group. The brain data was also filtered by using a fold difference cut off of at least 2, and requiring at least 20 assigned spectra to a specific modification.

Mass shift at specific amino acids where compared to a list of known modifications found at http://www.unimod.org/. It should be noted that Byonic restricts the results to only a single modification found through its wildcard PTM search. It is possible that some of the mass shifts seen could actually be the sum of two different modifications, however given the number of possible combinations of different modifications, and the lack of software to do this in an automated fashion, this was not pursued.