Northern Gulf of Mexico Bottlenose Dolphin UME
Algal Toxins Report

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Documented by: Spencer Fire
NOAA National Ocean Service, Marine Biotoxins Program
219 Fort Johnson Road, Charleston, SC 29412
(843) 762-8574 <spencer.fire@noaa.gov>

A. Suspected Event and Sample Details

Samples from 10 bottlenose dolphins (*Tursiops truncatus*) stranding in the northern Gulf of Mexico between February 2010 and June 2011 were sent to NOS Charleston by Jenny Litz of NOAA Fisheries. Sample types analyzed included gastric contents, feces and liver. Samples were assigned NOS ID numbers (NG04-24) upon receipt in preparation for toxin extraction and analysis. A complete sample list is given in the data summary below (section G).

B. Findings

No biotoxins were detected in any of the samples.

C. Saxitoxin (STX) Analyses

1. Preparation of Gastric, Feces and Liver Samples: Samples were extracted by combining and homogenizing a given weight of sample (typically 1-2 grams) with three volumes of 0.1N hydrochloric acid. The pH of the mixture was measured and adjusted if necessary to fall between 3.0 and 4.0. Homogenized samples were boiled for five minutes then allowed to cool to room temperature. These extracts were centrifuged at 3400 x *g*, then the supernatant was collected and syringe filtered (0.45 µm). Samples were prepared for analysis by Bennie Haynes.

2. RBA Methods: The acidic aqueous extracts were analyzed in a STX receptor binding assay (RBA). The receptor binding assay measures competition between radiolabeled STX and sample or FDA standard (S. Hall, USFDA/CFSAN, Washington, DC) for binding to the voltage-gated sodium channel, the pharmacological target of STX, to determine the total saxitoxin-like activity of the sample. Receptor binding analysis was performed by Trey Knott.

3. RBA Results: Saxitoxin-like activity was not identified in any of the samples analyzed. The detection limit of this method was 72 ng STX equivalents per gram of extracted sample or 72 ng STX equivalents per milliliter of urine.

D. Domoic Acid (DA) Analyses

1. Preparation of Gastric, Feces and Liver Samples: Samples were extracted by adding four volumes of 50% aqueous methanol to the sample volume, homogenizing, and then probe sonicating on ice for 2 min. These extracts were then centrifuged at 3400 x *g*, and the supernatant collected and syringe filtered (0.45µm) prior to analysis. Sample clean-up was performed by solid-phase extraction (SPE) using Varian 200 mg C18 columns. Samples were prepared for analysis by Bennie Haynes.

2. LC-MS Methods: The methanolic extracts were analyzed for the presence of domoic acid using tandem mass spectrometry coupled with liquid chromatographic separation (LC-MS/MS) by Zhihong Wang. This method utilized reversed phase chromatography, using an Agilent 1100 HPLC coupled to an ABI-SCIEX API-4000 triple quadrupole mass spectrometer in ESI+ mode. Chromatographic separation was performed on a Phenomenex Luna C18(2), 5µ, 150mm x 2 mm column. Mobile phase consisted of water and acetonitrile in a binary system, with 0.1% formic acid as an additive. Retention time of DA in samples was determined based on the retention time observed with a certified DA reference standard from the Institute for Marine Biosciences, NRC Canada (Halifax, NS). The detection of domoic acid by MS was achieved by Multiple Reaction Monitoring (MRM) method with Turboionspray interface. Four MRM transitions from protonated domoic acid were monitored: *m/z* 312 → 266, *m/z* 312 → 248, *m/z* 312 → 193, and *m/z* 312 → 161.

3. LC-MS Results: DA was not identified in any of the samples analyzed. The limit of quantification (LOQ) of this method was 2.5 ng domoic acid per mL urine or 2.5 ng per gram sample, with a signal to noise ratio above ten for standards.

E. Brevetoxin (PbTx) Analyses

1. Preparation of Gastric, Feces and Liver Samples: Samples were homogenized and extracted twice in 3 volumes of acetone, filtered via a 0.45 µm Acrodisc syringe filter, evaporated, resuspended in 80% aqueous methanol (6 or 30 mL), twice solvent partitioned with 30 mL hexane, and the methanolic fraction collected, evaporated and resuspended in 5 mL of 100% methanol. Extracts were stored at -20°C until analysis. Extractions were performed by Bennie Haynes.

2. ELISA Methods: Sample extracts were analyzed using a direct competitive enzyme-linked immunosorbent assay (ELISA) for PbTx. The ELISA utilizes cross-reactivity of PbTx to anti-PbTx antibodies to determine PbTx-like activity in a sample. Quantitation is determined via competition between PbTx in the sample and PbTx conjugated to a signal enzyme for binding to anti-PbTx antibodies. Analyses were conducted by Spencer Fire.

3. ELISA Results: Brevetoxin-like activity was not detected in any of the samples analyzed. The limit of detection for this assay was 1.2 ng PbTx per gram of sample and 1.2 ng PbTx per mL of urine.

F. Okadaic Acid (OA) Analyses

1. Preparation of Gastric, Feces and Liver Samples: Samples were homogenized and extracted in 3 volumes of 100% methanol, followed by centrifugation at 3000 x *g* for 5 min. The methanolic supernatants were filtered with a 0.2 µm syringe filter in preparation for analysis. Samples were prepared by Bennie Haynes.

2. LC-MS Methods: Samples were analyzed for the presence of okadaic acid (OA) and its two congeners (DTX1 and 2) , and pectenotoxin-2 using liquid chromatography (Agilent 110 series HPLC, Palo Alto, CA) coupled with tandem mass spectrometry (Applied Biosystems/MDS Sciex, Foster City, CA). For quantitation of okadaic congeners, LC separation was performed on X-Bridge™ C18 (150 × 3 mm, 5 µm)column, (Waters, Milford, MA) using a mobile phase of water (A) and acetonitrile/water (90:10, V/V) (B), both containing 6.7 mM ammonium hydroxide under gradient elution at a flow rate of 0.4 ml/min (linear gradient from 1min of 10% B to 90% B at 12 min, hold for 3 min, then return to 10% B at 17 min and hold for 4 min), MS detection was in multiple reaction monitoring (MRM) mode using an 4000 QTRAP mass spectrometer (for OA and DTX-2 with MRM transitions of *m/z* 803.5 →113.1and 255.1, for DTX-1 with MRM transitions of *m/z* 817.5 → 113.1and 255.1, for PTX-2 with MRM transitions of *m/z* 876.5 →213.1and 823.5) with LOD for OA about 0.1 ng/ml, for DTX2 about 0.08 ng/ml, for DTX1 about 0.13 ng/ml, for PTX-2 about 0.05 ng/ml with a 5 µl injection.. LC/MS analyses were performed by Zhihong Wang.

3. LC-MS Results: Okadaic acid congeners were not detected in any of the samples analyzed. The limit of quantification (LOQ) of this method was approximately 1 ng toxin per gram sample, with a signal to noise ratio slightly above ten for standards.

G. Data Summary

Table 1. Toxin concentrations detected by LCMS, ELISA or RBA. <dl = below detection limit.

NOS ID	Animal ID	Sample Type	Stranding Location	Species Name	Common Name	Date	OA	PbTx	STX	DA
NG06	04IMMS020710	Feces	AL - Baldwin Co.	*Tursiops truncatus*	Bottlenose dolphin	02/07/10	<dl	<dl	<dl	<dl
NG15	04IMMS020710	Liver	AL - Baldwin Co.	*Tursiops truncatus*	Bottlenose dolphin	02/07/10	<dl	<dl	<dl	<dl
NG11	27IMMS032510	Liver	MS - Harrison Co.	*Tursiops truncatus*	Bottlenose dolphin	03/25/10	<dl	<dl	<dl	<dl
NG18	70IMMS070810	Liver	AL - Baldwin Co.	*Tursiops truncatus*	Bottlenose dolphin	07/08/10	<dl	<dl	<dl	<dl
NG19	71IMMS071110	Liver	MS - Harrison Co.	*Tursiops truncatus*	Bottlenose dolphin	07/11/10	<dl	<dl	<dl	<dl
NG12	SDD-20110219-LA001 (LA454)	Liver	LA - Jefferson Co.	*Tursiops truncatus*	Bottlenose dolphin	02/19/11	<dl	<dl	<dl	<dl
NG04	SDD-20110221-LA001 (LA472)	Gastric	LA - Fourchon Beach	*Tursiops truncatus*	Bottlenose dolphin	02/21/11	<dl	<dl	<dl	<dl
NG08	SDD-20110221-LA001 (LA472)	Feces	LA - Fourchon Beach	*Tursiops truncatus*	Bottlenose dolphin	02/21/11	<dl	<dl	<dl	<dl
NG21	34IMMS022511	Liver	MS - Harrison Co.	*Tursiops truncatus*	Bottlenose dolphin	02/25/11	<dl	<dl	<dl	<dl
NG24	MRB-20110315-LA001 (LA470)	Feces	LA - Chaland Beach	*Tursiops truncatus*	Bottlenose dolphin	03/14/11	<dl	<dl	<dl	<dl
NG13	63IMMS031711	Liver	MS - EEZ	*Tursiops truncatus*	Bottlenose dolphin	03/17/11	<dl	<dl	<dl	<dl
NG17	63IMMS031711	Feces	MS - EEZ	*Tursiops truncatus*	Bottlenose dolphin	03/17/11	<dl	<dl	<dl	<dl
NG14	MCT-20110608-LA001 (LA558)	Liver	LA - Grand Terre Beach Co.	*Tursiops truncatus*	Bottlenose dolphin	06/08/11	<dl	<dl	<dl	<dl

Figure 1. Stranding Locations.