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Brevetoxin Concentrations in Marine Aerosol: Human Exposure Levels During a *Karenia brevis* Harmful Algal Bloom

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The dinoflagellate responsible for the Florida red tide, *Karenia brevis*, (formerly, *Gymnodinium breve*) (Duagbjerg et al. 2001) produces a suite of polyether neurotoxins called brevetoxins (Poli et al.1986; Shimizu et al.1990; Baden et al. 1995). A unique characteristic of these harmful algal blooms is the associated airborne (aerosolized) toxin component (Pierce et al. 1889; 1990). Toxins are released into the water by excretion and as cells rupture increasing the amount of extra-cellular toxins as a bloom progresses (Pierce et al. 2001). These extra-cellular toxins undergo bubble-mediated transport to the sea surface where they are ejected into the air as jet drops from the bursting bubbles. With on-shore winds and breaking surf, the toxins become incorporated into marine aerosol causing severe respiratory irritation to humans and other mammals along the shore (Pierce 1986; Pierce et al. 1990).

This study was undertaken in cooperation with a human effects study to assess the amount of aerosolized brevetoxin to which beach goers were exposed during a *Karenia brevis* (*K. brevis*) harmful algal bloom that occurred at Jacksonville Beach on the Atlantic coast of Florida during October, 1999 (Backer et al. 2002).

MATERIALS AND METHODS

To obtain a sample grid for brevetoxin aerosol distribution over the beach area, six high volume air samplers (TE-5000; Tisch Environmental, Inc., Village of Cleaves, OH) were placed 65 m apart along two transects (North and South), two samplers on the beach near the surf (Air-Surf), two just past the dunes (Air-Dune), and two behind the Jacksonville Beach lifeguard station in the parking lot (Air-Park). The air samplers were fitted with a 8"x10" glass fiber filter (Whatman EPM 2000, Maidstone, England.) and allowed to run for approximately 5 hours on October 9 and 3.5 hours on October 10, 1999. Two of the samplers were placed near the surf during the night of October 9 at a location 1 mile north of the lifeguard station (Air-Night). The volume of air passing through the filter was determined using a Tisch TE-5009 continuous flow recorder. All samplers were calibrated prior to deployment.

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Air filters were removed from the samplers, placed in glass jars and covered with dichloromethane (DCM) (HPLC grade, Burdick & Jackson, Muskegon, WI) for transport to the laboratory for processing. The filters were extracted in DCM overnight in a Soxhlet apparatus. The DCM was evaporated and replaced with methanol for analysis by high-performance liquid chromatography (HPLC) as described below (Pierce et al. 1990; 1992). The two most abundant brevetoxins present in these environmental samples were PbTx-2 and PbTx-3, reported according to the nomenclature presented by Poli et al. (1986).

Water samples were collected from near-shore (surf) in 1-liter glass bottles and brevetoxins were extracted on site by vacuum filtration through a C-18 solid-phase extraction disk (Ansys Technologies, Inc.; Lake Forest, CA). The C-18 disks were then rinsed with reverse osmosis water to remove remaining salts and then eluted with methanol, according to the method of Pierce et al. (1992). Method verification for extraction and analysis of brevetoxins was obtained by adding known amounts of brevetoxins to water and filter samples with subsequent extraction and HPLC analysis to determine recovery efficiency. Standard brevetoxins were obtained from Dr. Dan Baden, University of Miami, RSMAS. *Karenia brevis* cell concentrations also were enumerated in the near-shore (surf) water by preserving a 20-ml sample with 2 drops of Utermohls solution and enumeration in a 1-ml well plate using an inverted microscope at 100 to 200 magnification.

Sea foam samples also were collected from the beach surf zone. Foam was vacuum-collected into a 4-liter glass bottle. DCM was added to break the emulsion and to initiate extraction of the brevetoxins. The DCM extract was treated as above for the filter samples in preparation for brevetoxin analysis by HPLC. The volume of water represented by the foam was measured post extraction.

Qualitative and quantitative analyses were obtained by injection of concentrated extracts in methanol onto a Shimadzu SPDM6-A diode array HPLC (Shimadzu, Colombia, MD). The mobile phase was isocratic 85:15 methanol:water using a 250x4.6 mm 5 μm C-18 column and a flow rate of 1 ml per minute. The detector provided a scan from 200–300 nm with quantitation at 215 nm.

A portable, self-contained weather station (Complete Weather Station, Davis Instruments, Hayward, CA) was used near the ambient air monitoring stations to monitor the temperature, relative humidity, wind speed and wind direction.

RESULTS AND DISCUSSION

Results of the brevetoxin concentrations in water, sea foam and air samples are given in Table 1 for the two most abundant brevetoxins, PbTx-2 and PbTx-3. The efficiency for recovering standard brevetoxins from water and glass-fiber

filters was found to be approximately 100% from water and 81% to 90% from glass-fiber filters. Data reported for field samples were not adjusted for percent recovery. The *K. brevis* cell counts observed in water from the surf zone were $7x10^5$ cells/L on October 9, diminishing to $7x10^4$ cells/L on October 10.

The average values and standard deviation of the environmental conditions at the Jacksonville beach during the October 9 and 10 study are summarized in Table 2. The temperature and relative humidity remained relatively stable. The on shore wind was strong in the morning, decreasing somewhat in the afternoon, yet maintaining higher than 10 miles/hr velocities. The temperature and relative humidity were very similar on both days.

Table 1. Brevetoxin concentrations in water and air: Collected from North and South transects October 9 and October 10, 1999, from Jacksonville Beach, FL.

Sample	Water µg/L		Air North ng/m^3		Air South ng/m^3	
	PbTx-2	PbTx-3	PbTx-2	PbTx-3	PbTx-2	PbTx-3
10/09/99						
Std Rec	105±5%	100±14%	90±10%	81±10%		
Surf	20	4	51	14	69	24
Dune			55	21	36	0
Park			21	8	14	6
Night (surf)			87	22	49	5
Sea Foam	1900	1200				
10/10/99						
Surf	3.5	0.4	33	0	32	5
Dune			17	14	0	0
Park			0	0	0	

Std Rec = mean and std dev of % of standard recovered from each sample matrix, n=3.

Table 2. Environmental conditions during brevetoxin aerosol sampling on Jacksonville Beach, FL.

Environmental Parameter	October 9, 1999	October 10, 1999
Temperature (° F)	79.3±0.3	79.3±0.5
Relative Humidity (%)	70.0±1.4	71.5±2.0
Wind Speed (miles/hr)	12.8±1.4	7.7±1.3
Maximum Wind Speed (miles/hr)	18.6±2.0	9.4±1.4

These data provide an indication of the distribution of brevetoxins along the beach, from north and south sampling stations and across the beach from near the surf zone across the dunes into the parking lot for each day. Higher amounts of air-borne toxins and greater distance of inland transport were observed on October 9, relative to that on October 10. This corresponded with higher *K. brevis* cell counts in the surf area water of October 9 relative to October 10 ($7x10^5$ cells/L and $7x10^4$ cells/L, respectively). Higher wind speeds also were recorded for October 9 causing more surf, generating more toxin-containing aerosol and transporting the aerosol farther inland. It is also important to note that the night sampling of aerosol toxins exhibited about the same amount and ratio of PbTx-2 and-3 as was observed for the daytime samples.

The amount of brevetoxins recovered from sea water and air samples is consistent with that reported for samples collected previously during 1987 *K. brevis* blooms along the Florida Gulf coast and North Carolina Atlantic coast (Pierce et al. 1989). The Florida Gulf coast bloom had $20x10^6$ cells/L of *K. brevis*, exhibiting PbTx2+3 concentrations of 200 μg/L in water, and 160 ng/m^3 in air. The North Carolina bloom had $5x10^6$ cells/L *K. brevis*, exhibiting PbTx2+3 concentrations of 60 μg/L in water, and 180 ng/m^3 in air. The difference in air-borne concentrations of toxins reflects differences in wind, surf and beach/dune conditions at the different collection sites. High concentrations of brevetoxins recovered from the Jacksonville Beach sea foam samples reflect bubble-mediated transport of brevetoxins from the water column to the sea surface. The toxins became trapped in lipophilic sea foam that was generated in the surf zone.

These results indicate that in addition to toxin-contaminated marine aerosol, sea foam could also serve as a source of irritation and intoxication if it is ingested or inhaled. The results show that brevetoxin concentrations varied from one location to another along the beach and as one moved away from the surf (source of aerosolized brevetoxins). The brevetoxin distribution patterns indicated that localized concentrations of brevetoxins in the surf, breaking waves and wind patterns had an effect on toxin-containing aerosol concentration and distribution over the beach. The concentration of aerosol toxins to which humans were exposed was highest near the surf zone. The intensity was determined by the amount of brevetoxins in the source (surf water), the speed and direction of the wind, the surf conditions generating marine aerosol and the exposure location on the beach.

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