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### **Characterization of Red Tide Aerosol on the Texas Coast**

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#### Abstract

The Gulf of Mexico red tide, caused by the dinoflagellate *Karenia brevis* (= *Gymnodinium breve*), occurs almost annually and has adverse economic and health effects. Exposure of people to sea spray containing aerosolized brevetoxins (PbTxs, polyether brevetoxins produced by K. brevis) causes irritation of the eyes, nose, and throat. Anecdotal reports suggest that exposed individuals can experience respiratory irritation and exacerbation of existing respiratory illnesses. There has been no systematic study of human exposure to red tide aerosols. In the fall of 2000, during a red tide episode on the Gulf Coast near Corpus Christi, Texas, we sampled at the Marine Science Institute (MSI) at Port Aransas on 25 October. Between 26–27 October we sampled at the Texas State Aquarium (TSA) near Corpus Christi. Two Hi-Vol samplers equipped with a filter and a five-stage impactor gave low concentrations of PbTxs, requiring us to develop methods to improve the minimum detection limit. An LC/MS/MS technique was used combining an HPLC and the API 365 MS/MS. PbTx-2 and PbTx-3 were detected at the TSA sampling location; however, PbTx was not detected in the samples from the MSI. The concentration of PbTx-2 was 1.5-4.9 ng m<sup>-3</sup> but was much lower for PbTx-3. The ratio of PbTx-2 to PbTx-3 was  $8.7 \pm 5.2$ . During the highest exposure period (26-27 October), PbTx-6 was also detected. No one reported respiratory symptoms at the MSI, whereas at the TSA, several field study workers reported symptoms including nose and throat irritation, and itchy skin. A high-volume impactor was used to aerodynamically classify the particles into different size fractions. PbTx-2 was detected in all samples taken at the TSA; however, PbTx-3 was detected only between 26–27 October when the PbTx concentration was high. The mass median aerodynamic diameter (MMAD) was 7-9 mm with a relatively narrow size range (geometric standard deviation [GSD] about 1.6). In this study, much lower airborne concentrations of PbTx, 1.6–6.7 ng  $m^{-3}$  were reported, along with a few incidents of upper respiratory symptoms. Although the number of seven workers was too small for statistical analysis, the reported symptoms were consistent with no to low exposure at the MSI and detectable exposures at the TSA. This suggests that at lower environmental concentrations of about 2-7 ng m<sup>-3</sup>, exposure to PbTx could result in upper respiratory symptoms. This is consistent with the particle size measurement.

#### Introduction

Red tides in the Gulf of Mexico are commonly formed by the fish-killing dinoflagellate, *Karenia brevis* (= *Gymnodinium breve*). Red tides off the Florida Gulf coast are almost annual events (Kirkpatrick *et al.*, in press). Environmental aerosol samples collected during red tides in Florida and North Carolina in 1987 (Pierce *et al.*, 1989; 1990) showed high concentrations of three brevetoxins (PbTx-2, -3, and -5), but measurements of particle size for the red tide aerosol were not reported. During two red tide events in 1999, PbTx levels in air and seawater were measured while conducting personal interviews and pulmonary function tests on people

before and after visiting Florida beaches (Backer *et al.*, 2003). During moderate and high exposure periods,  $36 \text{ ng/m}^3$  and  $80 \text{ ng/m}^3$ , respectively, of PbTx were detected in the air. Lower respiratory tract symptoms (*e.g.*, tightness of chest, wheezing, and shortness of breath) were reported by 8% of the people having no/low exposure, 11% with moderate exposure, and 28% with high exposure. Upper respiratory symptoms (eye and throat irritation, nasal congestion, and cough) were also increased in the moderate and high exposure groups.

In the fall of 2000, there was a red tide event near Corpus Christi, Texas. Field study teams monitored the air and water levels of PbTxs and particle size distribution. In addition, personal samples for PbTx air concentrations were obtained from a few researchers to assess exposure and response, and respiratory symptoms were noted.

#### Methods

Two locations on the Gulf coast near Corpus Christi, Texas were studied. Air samplers were set up at a waterfront location at the Marine Science Institute (MSI), University of Texas at Austin, Port Aransas, and samples were taken from 0900–1600 on 25 October. After 1600 the sampling equipment was moved to the Texas State Aquarium (TSA) where respiratory symptoms were reported. Discolored water was seen adjacent to the TSA, and was verified as a bloom of *K. brevis*. Air samples were collected from 1800 on 26 October and continued through 1600 on 27 October.

Two high-volume air samplers (Model G2000H, Andersen Instruments, Smyrna, GA) were placed about one meter from the water to collect large quantities of material for analysis. One was used to collect suspended particles on one filter substrate for total aerosol concentration, whereas the second sampler housed a five-stage, high-volume cascade impactor (Model SA235, Andersen Instruments). Glass fiber filters ( $20 \text{ cm} \times 25 \text{ cm}$ ) were used for the collection substrate (Whatman EPM2000; Maidstone, UK). The sampling flow rates were 1220 and 1390 L min<sup>-1</sup> for the filter and impactor sampler, respectively. Personal exposure levels were measured by three volunteers who each wore a personal sampler (IOM Inhalable Dust Sampler, SKC, Inc., Eighty Four, PA) connected to a battery-operated pump (Hi Flow Sampler, Gillian Instrument, Wayne, NJ). The sampling flow rate was 2 L min<sup>-1</sup>, controlled by a rotameter in the sampling pump.

Filters from high-volume filter and impactor samplers were extracted with methanol. The extract was diluted and analyzed for PbTxs by an LC/MS technique using an HPLC (SIL-DAD vp, Shimadzu Co., Kyoto, Japan), coupled with the API 365 MS/MS (Applied Biosystems Inc., Foster City, CA) run in the positive ion mode. Using PbTx standards provided by UNCW, we established standard curves for PbTx-2, -3, -6, and -9. The personal sampling filter collected small amounts of PbTx, which were analyzed by a competitive ELISA (Naar *et al.*, 2002) based on the specific activity of the goat anti-PbTx antibody.

#### Results

Air concentrations of PbTxs determined by the high-volume filter samples are listed in Table 1. PbTx-2 and -3 were detected in the TSA sampling location; however, PbTx was not detected in the samples from the MSI. The concentration of PbTx-2 was between 1.5-4.9 ng m<sup>-3</sup>, but the concentration of PbTx-3 was much lower. The ratio of PbTx-2 and -3 was  $8.7 \pm 5.2$ . In the highest exposure period (26–27 October), PbTx-6 was also detected. No one reported respiratory symptoms at the MSI whereas at TSA, several field study workers reported symptoms including irritation in the nose and throat, and itchy skin.

A high-volume impactor was used to aerodynamically classify the particles into different size fractions. The PbTx concentration collected in each impactor stage was summed to give the

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total air concentrations as listed in Table 1. The air concentrations from both samplers were similar. The two samplers were located within about 20 meters of each other. PbTx-2 was detected in all samples taken at the TSA; however, PbTx-3 was detected only from 26–27 October when the PbTx concentration was high. This indicated that after fractionating the particles by size, there is not enough PbTx-3 in any size category to be detectable. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of these size distributions are listed in Table 2. The results show large particles with a mean size 7–9 mm and a relatively narrow size range (GSD about 1.6).

Personal exposure levels of PbTx were measured with personal samplers. Three personal samplers were used on 25 October and 26 October during the daytime sampling period. The total PbTx concentration was 4–18.3 ng m<sup>-3</sup>. PbTx concentrations varied among the three persons by a factor of 2.0–3.9 respectively, for the two sampling days. All three volunteers were working near the waterfront, but they were not at the same location and were involved in different activities. The variability of personal samples may reflect the different activity patterns of the workers. The personal samples (ELISA analysis) represent the total PbTx concentration and were generally higher than those reported by environmental samples (Table 1).

#### Discussion

Red tide events in the Gulf of Mexico have been historically reported along the western coast of Florida and can occur nearly annually. Red tides along the Texas coast are much less frequent. A previous study of recreational exposure to airborne PbTxs in Florida (Backer et al., 2003) showed that airborne concentrations between 36–80 ng m<sup>-3</sup> correlated to increased reports of both upper respiratory symptoms (eye irritation, nasal congestion, throat irritation, and cough) and lower respiratory symptoms (chest tightness, wheezing, and a shortness of breath). In this study, much lower airborne PbTx concentrations between 1.6-6.7 ng m<sup>-3</sup> were reported, along with a few reports of upper respiratory symptoms (throat irritation, nasal irritation, and itchy skin) and no reports of lower respiratory symptoms. Although the number of workers observed was too small for statistical analysis, the reported symptoms were consistent with no/low exposure at the MSI and detectable exposures at the TSA. This suggests that at lower environmental concentrations of about 2–7 ng m<sup>-3</sup>, exposure to PbTx could result in upper respiratory symptoms. This lower level of airborne PbTx concentrations could be detected using a more sensitive LC/MS technique. The detection limit could be further lowered <1 ng m<sup>-3</sup> when we improve the extraction technique. Our measurements of particle size distribution with the impactor samplers are the first time that particle size of PbTx was reported. The MMAD was between 7–9 mm, a relatively large size for inhaled ambient particles. Fine particles below 2.5 mm were not detected. Inhaled particles of this size would be deposited in the upper respiratory tract (nasal, oral, and pharyngeal area) (ICRP, 1994; Yeh et al., 1996), and subsequent respiratory irritation could result from the presence of the particles themselves or from toxins associated with the particles. Inhaled particles would also be deposited on the face and exposed skin, causing the skin to itch.

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# Table 1

Total air concentration (ng m<sup>-3</sup>) of brevetoxins obtained by the high-volume impactor and filter samplers at Marine Science Institute (MSI) or Texas State Aquarium (TSA).

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			E	lter Sampl	er	Imp	actor Sam	pler
Location	Date	Sampling Time	PbTx-2	PbTx-3	PbTx-6	PbTx-2	PbTx-3	PbTx-6
ISM	10/25/00	7.60 hr	0.00	0.00	0.00	0.00	0.00	0.00
TSA	10/25-26/00	15.17 hr	1.50	0.13	0.00	1.49	0.00	0.00
TSA	10/26/00	8.58 hr	2.00	0.14	0.00	4.02	0.00	0.00
TSA	10/26-27/00	15.83 hr	4.89	0.67	1.09	5.86	0.92	0.00
TSA	10/27/00	6.28 hr	1.69	0.84	0.00	2.77	0.00	0.00

#### Table 2

Aerodynamic particle size distributions of high-volume impactor samples.

Date	Sampling Time	MMAD mm	GSD
10/25-10/26	15.17 hr	9.0	1.62
10/26/00	8.58 hr	8.2	1.57
10/27/00	6.28 hr	7.0	1.60