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Toxicol

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## Recreational exposure to microcystins during algal blooms in two California lakes

Lorraine C. Backer<sup>a,\*</sup>, Sandra V. McNeel<sup>b</sup>, Terry Barber<sup>c</sup>, Barbara Kirkpatrick<sup>d</sup>, Christopher Williams<sup>e</sup>, Mitch Irvin<sup>f</sup>, Yue Zhou<sup>f</sup>, Trisha B. Johnson<sup>g</sup>, Kate Nierenberg<sup>d</sup>, Mark Aubel<sup>e</sup>, Rebecca LePrell<sup>a</sup>, Andrew Chapman<sup>e</sup>, Amanda Foss<sup>e</sup>, Susan Corum<sup>h</sup>, Vincent R. Hill<sup>g</sup>, Stephanie M. Kieszak<sup>a</sup>, Yung-Sung Cheng<sup>f</sup>

<sup>a</sup> National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway NE, MS F-57, Chamblee, GA 30341, USA

<sup>b</sup> Environmental Health Investigations Branch, California Department of Public Health, 850 Marina Bay Parkway, Building P, 3rd Floor, Richmond, CA 94804-6403, USA

<sup>c</sup> Siskiyou County Department of Public Health and Community Development, 806 South Main Street, Yreka, CA 96097, USA

<sup>d</sup> Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA

<sup>e</sup> GreenWater Laboratories, 205 Zeagler Drive, Palatka, FL 32177, USA

<sup>f</sup> Lovelace Respiratory Research Institute, Inhalation Toxicology Laboratory, P.O. Box 5890, Albuquerque, NM 87285, USA

<sup>g</sup> Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Chamblee, GA 30341, USA

<sup>h</sup> Department of Natural Resources, Karuk Tribe of California, PO Box 282, Orleans, CA 95556, USA

### ARTICLE INFO

#### Article history:

Received 8 April 2009

Received in revised form 26 June 2009

Accepted 6 July 2009

Available online xxx

#### Keywords:

Aerosol exposures  
Blue-green algae  
Cyanobacteria  
Microcystins  
*Microcystis aeruginosa*  
Waterborne exposures

### ABSTRACT

We conducted a study of recreational exposure to microcystins among 81 children and adults planning recreational activities on either of three California reservoirs, two with significant, ongoing blooms of toxin-producing cyanobacteria, including *Microcystis aeruginosa* (Bloom Lakes), and one without a toxin-producing algal bloom (Control Lake). We analyzed water samples for algal taxonomy, microcystin concentrations, and potential respiratory viruses (adenoviruses and enteroviruses). We measured microcystins in personal air samples, nasal swabs, and blood samples. We interviewed study participants for demographic and health symptoms information. We found highly variable microcystin concentrations in Bloom Lakes (<10 µg/L to >500 µg/L); microcystin was not detected in the Control Lake. We did not detect adenoviruses or enteroviruses in any of the lakes. Low microcystin concentrations were found in personal air samples (<0.1 ng/m<sup>3</sup> [limit of detection]–2.89 ng/m<sup>3</sup>) and nasal swabs (<0.1 ng [limit of detection]–5 ng). Microcystin concentrations in the water-soluble fraction of all plasma samples were below the limit of detection (1.0 µg/L). Our findings indicate that recreational activities in water bodies that experience toxin-producing cyanobacterial blooms can generate aerosolized cyanotoxins, making inhalation a potential route of exposure. Future studies should include collecting nasal swabs to assess upper respiratory tract deposition of toxin-containing aerosols droplets.

Published by Elsevier Ltd.

### 1. Introduction

People and animals are at risk for exposure to toxins produced by cyanobacterial harmful algal blooms (CyanoHABs) in drinking water (Falconer et al., 1983; El

Saadi et al., 1995) or in improperly treated water used for medical purposes such as renal dialysis (Jochimsen et al., 1998; Carmichael et al., 2001). Additional potential exposure sources include contaminated dietary supplements (Gilroy et al., 2000) or fish harvested from lakes with ongoing CyanoHABs (De Magalhaes et al., 2001; Xie et al., 2005; Cazenave et al., 2005; Kann, 2008).

\* Corresponding author. Tel.: +1 770 488 3426; fax: +1 770 488 3450.  
E-mail address: [lbacker@cdc.gov](mailto:lbacker@cdc.gov) (L.C. Backer).

Although outbreaks of human illness associated with Cyanobacteria have been sporadically recorded for decades, information about clinical signs and symptoms from cyanobacterial toxin poisonings is primarily from animal poisonings and laboratory studies (Carmichael and Falconer, 1993). Exposures to cyanobacterial toxins produce a variety of symptoms and illnesses (Hunter, 1998; Falconer, 1998). The primary effects include acute hepatotoxicity, acute neurotoxicity, gastrointestinal symptoms, and respiratory, dermatologic, and allergic reactions. Which, if any cyanobacterial toxins cause respiratory symptoms is not known; however, cyanobacterial hepatotoxins or lipopolysaccharide endotoxins may be associated with the gastrointestinal disturbances (Sykora and Keleti, 1981).

A significant source of cyanobacterial toxin exposure is recreational use of contaminated fresh water bodies because large populations are likely to be exposed and toxins may occur in high concentrations. Initial epidemiologic studies did not find an association between recreational cyanobacteria exposure and adverse health effects (Phillip, 1992; Phillip and Bates, 1992; Phillip et al., 1992). However, Pilotto et al. (1997) reported that persons exposed for more than 1 h to recreational waters containing elevated concentrations of cyanobacteria (>5000 cells/mL) were more likely to report at least one symptom during the 7 days after exposure than were persons exposed to waters that did not contain cyanobacteria (odds ratio = 3.44; 95% confidence interval [CI], 1.09–10.82). In their analysis, Pilotto et al. (1997) excluded people who had recreational water contact or symptoms in the 5 days prior to the interview. More recently, Stewart et al. (2006b) found that persons who used personal watercraft on lakes with high cyanobacteria concentrations (cell surface area > 12.0 mm<sup>2</sup>/mL) were 2.1 (CI, 1.1–4.0) times more likely to report symptoms, particularly respiratory symptoms, than were persons who used their personal watercraft on lakes with low cyanobacteria concentrations (cell surface area < 2.4 mm<sup>2</sup>/mL).

In the United States, there are no regulations defining acceptable levels of cyanobacterial toxins in drinking or recreational waters. The World Health Organization (WHO), Australia, and some European countries have developed guidelines for managing recreational waters with cyanobacterial blooms (WHO, 2003; Chorus, 2005). However, these guidelines were based on cell concentrations rather than on cyanobacteria toxin concentrations, and not all cyanobacterial blooms produce toxins. Data from epidemiologic studies designed to evaluate the associations among environmental cyanobacteria toxin concentrations, human biomarkers of cyanobacteria toxin exposure, and health symptoms are needed to develop more specific recreational exposure guidelines.

In a previous study of recreational microcystin (MC) exposure at a small lake, we recruited 104 study participants from lake visitors planning recreational activities, such as boating and using personal watercraft, that would generate aerosols (Backer et al., 2008). During data collection for that study, MC concentrations within the Bloom Lake water were very low (<2–5 µg/L). Study participants' plasma MC concentrations were all below the limit of detection (0.147 µg/L) for the enzyme-linked immunosorbent assay

(ELISA) (Backer et al., 2008). For this study, we identified lakes with historical blooms producing higher MC concentrations (>100 µg/L) than the Bloom Lake in our initial study. We also added an additional measure of toxin exposure, individual nasal swabs. We collected data over 3 days (Friday through Sunday) when the lakes were most heavily used. Specifically, our primary objectives were to:

- Determine whether MC could be detected in blood samples of people engaged in recreational activities on fresh water lakes during a microcystin-producing algal bloom,
- Assess toxin concentrations from nasal swabs as an improved measure of individual exposure,
- Verify previous findings of MC aerosolization due to recreational activities such as water skiing, boating, and wakeboarding, and
- Collect health-symptom data to generate hypotheses regarding relationships between environmental MC exposures and adverse health effects for future studies.

## 2. Materials and methods

### 2.1. Site selection

We selected two lakes in Siskiyou County, California, that routinely experience prolonged MC-producing algal blooms for this study (Bloom Lakes 1 and 2). Data documenting the development and presence of the 2007 bloom (which involved *Microcystis aeruginosa*) were collected and provided by Susan Corum, Department of Natural Resources, Karuk Tribe of California. The Control Lake, a reservoir in adjacent Shasta County, was selected due to its proximity and public use for similar recreational activities as the Bloom Lakes. Cyanobacterial blooms have not occurred at the Control Lake because it is deeper and cooler.

### 2.2. Environmental samples

Backer et al. (2008) reported the materials and methods applicable to the present study. Briefly, we collected data and recruited study participants over 3 days (Friday through Sunday) in August 2007 when the lakes were most heavily used. We collected water samples in the morning and afternoon, ambient air samples throughout each day, and personal breathing zone samples for Bloom Lake participants during their water-based activities. Specifically, we collected water samples for algal taxonomy and microcystin concentrations and to assess general water quality parameters. We also collected water samples to assess concentrations of some human viruses known to be associated with respiratory illness and that might be important in recreational exposures to ambient aerosols. Finally, we collected ambient air and breathing zone samples to assess MC concentrations.

### 2.3. Water sample collection and analysis

We identified four sampling sites located along the major axis of each Bloom Lake and two sampling sites in

the Control Lake. Each time we collected water samples, we recorded latitude and longitude coordinates using a geographical positioning system (Magellan Map 330X, Magellan, Santa Clara, CA). Water samples were surface grab samples collected twice each day (morning and evening) from four sites in each Bloom Lake. We collected water samples from the Control Lake twice (morning and evening) on one day.

#### 2.4. Water quality parameters

We used a Hydrolab Quanta multi-parameter data sonde (Hach Company, Loveland, CO) to measure general water quality parameters at a depth of 0.15–0.25 m. At the time of water collection, we also recorded ambient environmental conditions (cloud cover, wind speed, wind direction, and air temperature) at the Bloom Lakes.

#### 2.5. Water for algal taxonomy and MC

We collected water samples in capped 10-L carboys that were immersed to 0.15 m before uncapping to avoid collecting surface scum. We thoroughly mixed the large sample volume in a 25-L churn-splitter, dispensed 40 mL into sample vials, immediately preserved these with a final concentration of 0.5–1% Lugols solution, and stored them in a dark, cool environment. We also dispensed four 1-L samples into containers for MC analysis.

#### 2.6. Algal taxonomy

GreenWater Laboratory (Palatka, FL) conducted the algal taxonomy analyses. For each sample, we constructed cleaned Utermöhl counting chambers. Depending on the cell density of the sample, we used settling towers of 5 mL, 10 mL, or 25 mL. We shook the Lugols-preserved samples for a minimum of 60 s to evenly distribute phytoplankton cells, and added the appropriate volume (1–25 mL) to the settling tower. We placed cover glasses on top of the towers, and allowed samples to settle in the dark in a vibration-free location. Minimum settling times were 17 h, 34 h, and 74 h for samples of 5 mL, 10 mL, and 25 mL, respectively.

We enumerated cells using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics (Southern Microscopes, Inc., Mebane, NC). One ocular was fitted with a whipple disc used to define the area of fields to be counted. We did not count specimens that extended beyond the left and bottom edges of the whipple grid. We only counted intact, viable cells as natural units (cells, filaments, colonies). We obtained cell numbers per natural unit by counting the numbers of cells/unit of a minimum of 15–20 cells and calculating a mean value for each taxon.

The goal was to count a total of 400–600 natural units per sample, and we counted a minimum of 10 and a maximum of 50 fields for each sample at both 400× and 200×. We made additional scans of entire slides at 100× to count large and/or rare taxa. We counted a minimum of 400–600 natural units per slide to give a 95% CI of the estimate within ±10% of the sample mean.

#### 2.7. MC analysis for water samples

We used 4-L samples from the 10 L of water collected at each site. To ensure that sampled algae remained alive and at low metabolic states until processing or preservation, we vented the collection bottles and stored them in coolers with ice packs.

We analyzed dissolved and total MC using an MC ADDA polyclonal ELISA (PN 520011, Abraxis LLC, Warminster, PA). We measured extracellular MC concentrations by analyzing dissolved MC in water samples as received, without cell disruption. Total MC concentrations (extracellular and intracellular combined) were determined by ultrasonication of the samples to release the intracellular toxins (Model 300 V/T Biologics Ultrasonic Homogenizer, Manassas, VA). We spiked duplicates of Control Lake samples with 1.0 µg/L MC-LR before ultrasonication and reported the MC values for the water samples as MC-LR equivalents.

We used a Thermo Finnigan LCQ Advantage liquid chromatography–mass spectrometer system (LC/MS) to identify and determine the relative abundance of specific MC congeners present in the positive samples (Thermo Electron Corporation, Madison, WI). The  $[M + H]^+$  ions for the MC congeners LR (995.5 *m/z*), RR (519.5 *m/z*), YR (1045.6 *m/z*), and LA (910.5 *m/z*) provided both specificity and sensitivity. We obtained chromatograms and spectra using the LC/MS system coupled with reversed-phase chromatography using a modified aqueous acetonitrile gradient.

#### 2.8. Evaluation for selected pathogens in water samples

We collected 20-L lake water samples for viral pathogen testing at the same locations and times using the same collection methods as for algal taxonomy and toxin analyses. We concentrated each sample using an ultrafiltration procedure based on Hill et al. (2007) and shipped the ultrafiltration (UF) concentrates (~400 mL each) overnight in chilled coolers to the CDC laboratory in Atlanta, Georgia. We processed the UF concentrates to further concentrate viruses using polyethylene glycol (PEG) precipitation (addition of 8% PEG 8000, 0.3 M NaCl, and 1% bovine serum albumin; incubation overnight at 4 °C, pH 7.0–7.4). Final PEG concentrates were 5.5–14.8 mL in volume. We simultaneously extracted DNA and RNA from 500 µL of each sample (Hill et al., 2007). Final nucleic acid extracts were approximately 100 µL, and we used 20–µL volumes of nucleic acid extract for each 50–µL PCR and RT-PCR assay. We assayed each sample for adenoviruses using a broadly reactive real-time PCR assay (Jothikumar et al., 2005) and a pan-enterovirus real-time RT-PCR assay (AwwarF, 2004).

#### 2.9. Air sample collection and analysis

Cheng et al. (2007) described sampling methods for air-borne particles in our previous study. To collect large ambient air volumes for MC analysis, we used two portable high-volume air samplers (Model TE 5200, Tisch Environmental, Inc., Cleves, OH) containing cascade impactors (Model SA235, Andersen Instruments, Smyrna, GA) to measure total concentration and size distribution of MC-containing particles at the sampling sites. We collected

air samples only on the Bloom Lakes. On Day 1 at Lake 1 and on Days 2 and 3 at Lake 2, we placed one sampler on a boat in the middle of the lake to collect air-borne MC above the lake surface and another on the downwind shoreline. Samplers operated at a 1.2 m<sup>3</sup>/min flow rate. Cellulose filters (Filter Paper 41, Whatman International Ltd., Maidstone, UK) were used for stage substrates and backup collection of the samplers. The air-sampling boat remained stationary near one of the water sample collection sites. Large volume air samplers operated approximately 6 h each day, beginning at 11:00 a.m. or noon and terminating at 5:00 or 6:00 p.m.

### 2.10. Study population

The Centers for Disease Control and Prevention's (CDC) Institutional Review Board and the California Health and Human Services Agency's Committee for the Protection of Human Subjects approved this study protocol.

During the three study days in August 2007, we recruited a convenience sample of adults and children older than aged 12 years at the selected lakes. We invited lake visitors to participate in the study if they planned activities that would likely generate aerosols or that might cause them to swallow lake water (i.e., waterskiing, using personal watercraft, swimming, or wading), and visited the selected lakes during the 3 days of the study. Study participants gave written, informed consent, and we obtained written parental consent for participants younger than aged 18 years. Participants provided pre- and post-water activity nasal swabs and questionnaire responses as well as a single post-water activity blood sample. Questionnaires solicited demographic data, a range of current health symptoms, and previous recreational water exposures. Participants completed a similar health and water exposure-related questionnaire through a telephone interview ~7–10 days after the lake exposure.

### 2.11. Study participant sample collection and analysis

#### 2.11.1. Personal air samples

We assessed participants' air-borne MC exposure using individual personal air samplers (IOM Inhalable Dust Sampler, SKC Inc., Eighty Four, PA) connected to a battery-operated pump (Leland Legacy pump, SKC Inc.). If the participant planned activities on a boat or beach, we placed the sampler at the lapel near the breathing zone. If participants were in a recreational group, we placed a single sampler on their boat. A 25-mm cellulose filter (Filter Paper 41, Whatman International Ltd., Kent, UK) was the collection substrate, and the sampling flow rate was 10.6 L/min.

#### 2.11.2. Nasal swabs

The nasal swabs were self administered under the supervision of study personnel. Each participant used a cotton-tipped swab to collect secretions inside one nostril before exposure, and another cotton-tipped swab to collect secretions inside the same nostril after exposure. Swabs were wrapped in foil then frozen for storage and shipment.

#### 2.11.3. MC analysis in air samples and nasal swabs

Before extraction, we spiked a set of blank filters and swabs with known concentrations of MC standards. Filters and nasal swabs obtained from the study sites were equilibrated to room temperature before extraction. We extracted the whole cotton of a swab and the whole filter of a personal sampler. For high-volume samples, we extracted a 5.8-cm<sup>2</sup> piece cut from the primary filter and a 4.3-cm<sup>2</sup> piece cut from the backup filter. We placed the sample or standard in a glass vial, added 1 mL of phosphate-buffered saline, and placed the vial on a circular rotator (Roto-Torque<sup>®</sup> Low Speed #10; Cole-Parmer Instruments, Vernon Hills, IL) for 10 min. After rotation, we used the extract for analysis of total MC by using ELISA (ELISA kits for MC-LR, Production #520011; Abraxis LLC, Warminster, PA).

#### 2.11.4. Blood sample collection and analysis

We collected blood samples for unbound MC analysis. We collected whole blood into heparinized tubes that were centrifuged immediately. We separated and froze the resulting plasma. The method for plasma MC extraction was developed based on previous extractions conducted on plasma and liver samples (Carmichael et al., 2001; Hilborn et al., 2005; Yuan et al., 2006). We used sterile-filtered serum derived from human male AB plasma (Sigma-Aldrich, St. Louis, MO) as a negative analytical control in conjunction with control samples received from the field. We thawed plasma samples in cold water before analysis. We added 300 µL of plasma sample to 1100 µL of 100% HPLC grade acetone, vortexed it, and placed it in the freezer at –20 °C for a minimum of 20 min in 1.5-mL centrifuge tubes. We centrifuged the samples at 20,000 × g for 20 min and retained the supernatant. We resuspended the pellet in 1200 µL of acetone, allowed to cool at –20 °C for 20 min, centrifuged as before, and pooled the resulting supernatant with the previously extracted portion. We spiked duplicates of approximately a third of the samples after deproteinization at a concentration of 1.0 µg/L MC-LA (one of more than 40 MC congeners). We used nitrogen gas at 60 °C (TurboVap LV evaporator, Caliper Life Sciences, Hopkinton, MA) to blow the samples to dryness, reconstituted them in 300 µL deionized water (18 MΩ), and analyzed them via ELISA without dilution.

We used an MC direct competitive monoclonal ELISA (PN 522015, Abraxis LLC, Warminster, PA) to detect MC in plasma samples. The monoclonal kit exhibited less interference than the polyclonal kit due to its high specificity and reduced reactivity with matrix components inherent in plasma. We generated standard curves using MC-LA. We verified the concentration and adjusted it using a molar extinction coefficient of 36,500 L/mol/cm at 238 nm. We prepared and analyzed all samples and MC standards in an aqueous medium. A method detection limit of 1 µg/L was determined with the use of spikes and negative plasma controls.

#### 2.11.5. Data analysis

We used SAS/STAT software Version 9.1 (SAS Institute, Cary, NC) for statistical analyses. We used the Wilcoxon Signed Rank Test (Sokal and Rohlf, 1981) to compare pre- and post-exposure nasal-swab data. For each study participant in each time period (before exposure, after exposure, and about one week later), we summed

**Table 1**

Weekly water quality data collected on Bloom Lakes during the summer of 2007 (Data provided by Linda Prendergast of the Pacific Corporation and Susan Corum from the Department of Natural Resources, Karuk Tribe of California).

Lake	Sample site location		Date of collection	Depth of collection (m)	Chl-a <sup>a</sup> (µg/L)	TKN <sup>b</sup> (mg/L)	NH <sub>3</sub> (mg/L)	NO <sub>3</sub> /NO <sub>2</sub>	TP <sup>d</sup> (mg/L)	O-PO <sub>4</sub> <sup>e</sup> (mg/L)	DOC <sup>f</sup> (mg/L)	TSS <sup>g</sup> (mg/L)	Water temp (°C)	Sp. Cond. <sup>h</sup> (mS/cm)	DO <sup>i</sup> (mg/L) (% Sat)	pH	Secchi depth (m)
	Latitude (N)	Longitude (W)															
1	41-58.764	122-19.99	7/31/2007	0.5	64.0	1.50	ND <sup>c</sup>	0.06	0.18	0.14	5.1	11	*	*	*	*	*
2	41-56.052	122-26.10	7/31/2007	0.5	19.0	1.70	ND	ND	0.14	0.06	4.8	8	*	*	*	*	*
1	41-58.932	122-19.694	8/8/2007	1.0	19.8	1.10	0.04	0.33	0.20	*	*	*	22.1	0.157	7.8 (98.1)	9.24	2.07
2	41-56.330	122-25.930	8/7/2007	1.0	112	1.40	0.01	0.01	0.15	*	*	*	24.0	0.152	12.2 (159.0)	9.89	0.93
1	41-58.764	122-19.99	8/15/2007	0.5	227	*	*	*	*	*	*	*	*	*	*	*	*
2	41-56.052	122-26.10	8/15/2007	0.5	44.0	*	*	*	*	*	*	*	*	*	*	*	*
1	41-58.932	122-19.694	8/23/2007	1.0	168	1.50	0.02	0.08	0.24	*	*	*	21.4	0.151	13.1 (159.5)	9.55	0.81
2	41-56.330	122-25.930	8/22/2007	1.0	134	1.97	0.03	0.01	0.22	*	*	*	25.0	0.164	16.3 (213.8)	10.0	*
1	41-58.764	122-19.99	8/28/2007	0.5	105	1.30	ND	0.07	0.21	0.18	7.5	20	*	*	*	*	*
2	41-56.052	122-26.10	8/28/2007	0.5	35.0	1.40	ND	ND	0.17	0.15	7.3	12	*	*	*	*	*

\* Not analyzed.

<sup>a</sup> Chlorophyll-a.

<sup>b</sup> Total Kjeldhal nitrogen.

<sup>c</sup> Not detected (below the limit of detection).

<sup>d</sup> Total phosphorous.

<sup>e</sup> Orthophosphorous.

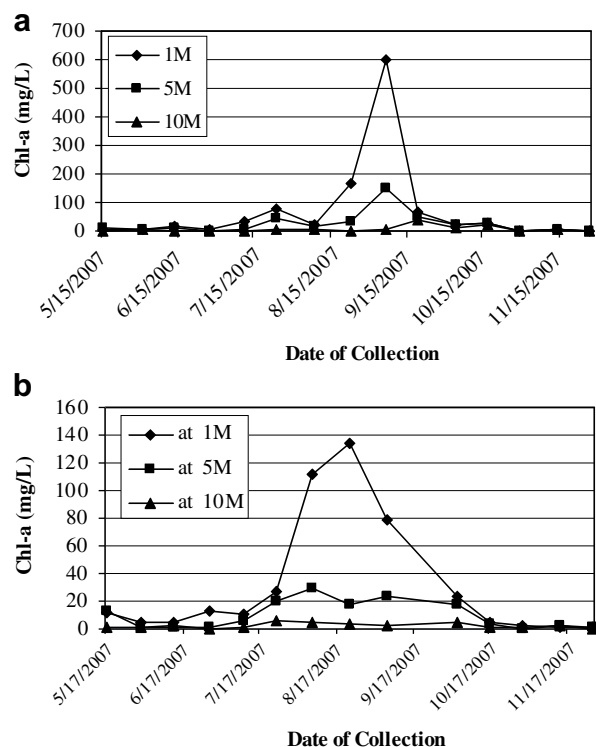
<sup>f</sup> Dissolved oxygen.

<sup>g</sup> Total suspended solids.

<sup>h</sup> Specific conductance.

<sup>i</sup> Dissolved oxygen, percent saturation.





**Fig. 1. a.** Chlorophyll-a (Chl-a) concentrations on Bloom Lake 1 from May to November 2007. Water samples were collected at 3 depths and analyzed for chlorophyll-a (Chl-a). Data provided by Susan Corum, Department of Natural Resources, Karuk Tribe of California. **b.** Chlorophyll-a (Chl-a) concentrations on Bloom Lake 2 from May to November 2007. Water samples were collected at 3 depths and analyzed for chlorophyll-a (Chl-a). Data provided by Susan Corum, Department of Natural Resources, Karuk Tribe of California.

individual health symptoms and then dichotomized to represent any vs. no symptoms. We used the Fisher exact test (Sokal and Rohlf, 1981) to examine whether participants in the exposed group reported having symptoms more frequently than those in the control group.

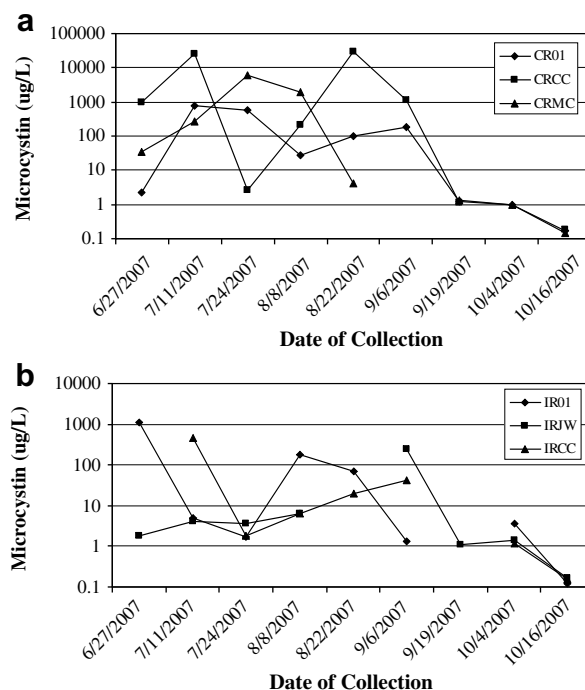
### 3. Results

#### 3.1. Water samples

We collected a total of 28 samples and tested for water quality parameters, algal taxonomy, MC concentrations, and adenoviruses and enteroviruses. We collected eight water samples from Bloom Lake 1 on August 17, eight samples from Bloom Lake 2 on both August 18 and 19, and 4 samples from the Control Lake on August 18. We split 14 of the 28 water samples for additional MC analysis by the U.S. Environmental Protection Agency (EPA) Region 9 Laboratory (Richmond, CA) and 12 of the 28 samples for additional MC analysis by the California Department of Public Health Sanitation and Radiation Laboratory Branch (data not shown).

#### 3.2. Water quality parameters

Water-chemistry data collected independently by the Karuk Tribe of California's Reservoir Monitoring Program



**Fig. 2. a.** Microcystin concentrations on Bloom Lake 1 from June to October 2007. Water samples were from three sites: CR01 from open water near the study sample Site 1, and CRCC and CRMC from shoreline recreational areas. Data provided by Susan Corum, Department of Natural Resources, Karuk Tribe of California; microcystin analyses conducted by U.S. EPA Region 9 Laboratory using the Enviroligix™ ELISA kit. **b.** Microcystin concentrations on Bloom Lake 2 from June to October 2007. Water samples were from three sites: IR01 from open water near the study sample Sites 5 and 9, and IRJW and IRCC from shoreline recreational areas. Data provided by Susan Corum, Department of Natural Resources, Karuk Tribe of California; microcystin analyses conducted by U.S. EPA Region 9 Laboratory using the Enviroligix™ ELISA kit.

and PacifiCorp (the local hydroelectric project manager) are presented in Table 1. During the summer of 2007, both Bloom Lakes had high chlorophyll-a concentrations throughout the month of August, and bloom conditions persisted for at least 36 days. Chlorophyll-a data further suggest that Lake 1 may have been more productive than Lake 2 but both water systems demonstrated high algal biomass potentially capable of producing high toxin concentrations. Nutrient data showed that total nitrogen and total phosphorus concentrations were relatively high, however, inorganic forms were occasionally depleted by algal uptake.

Additional data provided by the Karuk Tribe of California's Reservoir Monitoring Program (Fig. 1a and b) clearly showed that chlorophyll-a concentrations (at 1 m) started to increase in early July and did not dissipate until November 2007. Chlorophyll-a concentrations at a depth of 5 m paralleled the concentrations at 1 m and both peaked in September, although the 5-m peak was considerably lower than the 1-m peak. During this same period, MC analysis performed by U.S. EPA Region 9 Laboratory (Fig. 2a and b) using the Enviroligix™ ELISA kit demonstrated that MC was detectable from June through October in open surface waters at Bloom Lake 1 (CR01), and concentrations were relatively high (>10 µg/L) from July to early

**Table 2**

Microcystin concentrations (average of morning and afternoon samples), water quality parameters (average of morning and afternoon samples), and weather parameters measured on consecutive days at Bloom Lakes 1 and 2 and a Control (non-bloom) lake. We collected samples from four locations on each Bloom Lake and two locations at the Control Lake.

Water quality parameter (units)	Sample site	Bloom Lakes												Control lake	
		Lake 1				Lake 2									
		Day 1				Day 2				Day 3				Day 2	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dissolved microcystins concentration ( $\mu\text{g/L}$ )		10	3.9	4.9	2.5	4.0	3.4	6.9	5.4	1.6	1.8	3.4	4.7	<LOD <sup>a</sup>	<LOD
Total microcystins concentration ( $\mu\text{g/L}$ )		194	37.3	83.3	14.5	26.3	15.7	357	172	63.5	23.5	23.6	159.5	<LOD	<LOD
Water temperature ( $^{\circ}\text{C}$ )		22.3	23.0	23.1	22.3	23.0	24.1	23.4	23.6	22.2	22.7	23.4	23.0	25.5	25.6
pH		9.5	9.5	9.5	9.2	9.5	9.6	9.5	9.7	9.4	9.7	9.8	9.7	8.2	8.2
Dissolved oxygen ( $\text{mg/L}$ )		13.0	14.2	14.3	10.8	12.9	14.1	13.4	13.7	11.5	13.8	16.6	14.7	8.0	8.0
Specific conductivity ( $\mu\text{mhos/cm}$ )		149	152	153	156	176	178	179	182	176	176	182	183	125	124
Secchi depth (m)		0.6	1.0	0.9	1.8	1.2	1.2	1.0	0.8	1.8	1.4	0.9	1.0	2.4	3.4
<i>Weather</i>															
Air temperature ( $^{\circ}\text{C}$ )		28.0	28.6	29.2	29.4	26.1	25.8	26.7	23.6	23.4	23.4	22.2	21.1	*	*
Cloud cover (%)		*	*	*	*	32	43	50	55	38	30	18	15	20	20
Wind speed (mph)		7.2	5.3	7.6	4.2	5.3	4.0	4.4	4.1	7.2	5.8	6.1	7.3	*	*
Wind direction ( $0\text{--}360^{\circ}$ )		278	310	270	252	225	175	202	270	212	230	208	308	*	*

\*Not recorded.

<sup>a</sup> LOD = Limit of Detection ( $0.15 \mu\text{g/L}$ ).

September 2007. MC concentrations in Bloom Lake 2 (IR01; Fig. 2a) were much more variable but did exhibit the same basic temporal pattern. On seven different occasions, MC concentrations at shoreline recreational areas (CRCC/CRM and IRJW/IRCC) were  $\geq 1000 \mu\text{g/L}$ .

We measured dissolved (extracellular) MC in Bloom Lake water at concentrations from  $<2 \mu\text{g/L}$  to  $>10 \mu\text{g/L}$  (see Table 2). Concentrations of total MC (i.e., dissolved extracellular and intracellular) were an order of magnitude higher, ranging from  $15 \mu\text{g/L}$  to  $>350 \mu\text{g/L}$ . MC-LA was the dominant congener present in the water samples collected during this study. MC was not detected in any of the Control Lake samples. Table 2 also contains the ranges of water quality parameters and weather data collected over the 3-day study period.

### 3.3. Algal taxonomy

**Table 3**

Densities of potentially toxigenic cyanobacteria in water samples from four sampling sites on each of 3 study days at Bloom Lakes 1 and 2.

Potentially toxigenic cyanobacteria (PTOX) (cells/mL)	Genera	Species	Lake 1				Lake 2							
			Day 1				Day 2				Day 3			
			Sites				Sites				Sites			
			1	2	3	4	5	6	7	8	9	10	11	12
	<i>Aphanizomenon</i>	<i>flos-aquae</i>	1663	16,634	12,475	12,475	329,348	ND	89,822	259,487	174,654	212,912	349,309	259,487
	<i>Coelosphaerium</i>	<i>cf. dubium</i>	ND	ND	ND	ND	ND	ND	500	ND	ND	ND	ND	ND
	<i>Microcystis</i>	<i>Wesenbergii</i>	ND	40,390	57,700	40,390	101,552	101,552	2,094,039	184,640	39,236	66,932	60,008	628,930
	<i>Microcystis</i>	Sp.	90,261	519,741	309,954	833,738	229,229	123,529	1,089,322	603,327	71,812	242,021	251,004	790,112
	<i>Planktolyngbya</i>	<i>cf. microspira</i>	28	15,878	ND	ND	1059	ND	ND	ND	ND	ND	ND	ND
	<i>Pseudanabaena</i>	<i>Galeata</i>	ND	ND	ND	ND	16,331	ND	ND	ND	ND	ND	1361	ND
	<i>Pseudanabaena</i>	<i>Mucicola</i>	ND	ND	ND	ND	174,201	ND	743,983	ND	57,160	45,970	185,088	464,536
	<i>Cyanophyte filament</i>	Sp.	7258	ND	ND	ND	1815	8710	ND	21775	96	48	5444	ND
Total PTOX (cells/mL)			99,211	592,642	380,129	886,603	853,535	233,791	4,017,666	1,069,229	342,958	567,949	852,214	2,143,314

ND = Not detected.

We assessed algal taxonomy in 12 water subsamples from morning collections at 4 sampling sites in each Bloom Lake. The range of phytoplankton concentrations was  $\sim 100,000\text{--}2,000,000$  cells/ml. The predominant phytoplankton present were *Microcystis* spp. (too few taxonomic characteristics could be observed to allow identification of individual *Microcystis* spp.), followed by *Aphanizomenon flos-aquae*. We observed lower concentrations of other potentially toxin-producing species, including *Pseudanabaena* spp. Table 3 contains a summary of the taxonomic analyses.

### 3.4. Concentrations of selected pathogens in water samples

We collected twenty-eight 20-L lake water samples for viral pathogen testing (24 from the Bloom Lakes and 4 from the Control Lake). We did not detect adenoviruses or enteroviruses in any water sample. Based on published

**Table 4**

Mass and air concentrations of microcystins collected on each study day using personal samplers carried by exposed study participants or placed on their boats. In the latter situation, participants shared the same air pump values ( $N = 44$ ).

Study day	Study lake	Mean sampling time (min) <sup>a</sup>	Mass of microcystins on filter (ng)			Air concentration of microcystins (ng/m <sup>3</sup> )		
			Mean	Standard deviation	Range	Mean	Standard deviation	Range
1	1	85.7	0.4	0.74	<LOD <sup>b</sup> –2.9	0.4	0.75	0.00–2.9
2	2	121.2	0.3	0.15	<LOD–0.7	0.1	0.23	0.00–0.8
3	2	122.4	0.3	0.18	<LOD–0.5	0.2	0.17	0.00–0.4

<sup>a</sup> The air pump flow rate was 0.0106 m<sup>3</sup>/min.

<sup>b</sup> LOD = Limit of detection (0.1 ng). For values <LOD, we used LOD/√2 (0.07) in calculations.

detection limits for these assays, the testing protocol used in this study had detection limits of ~1000–4000 adenovirus gene equivalent copies (GEC) per 20-L water sample and 200–600 enterovirus plaque-forming units (PFU) per 20-L sample. Thus, these data indicate that adenoviruses and enteroviruses were not present at substantial concentrations in the Bloom Lakes.

### 3.5. Air samples

We collected six air samples at the Bloom Lakes using high-volume impactor samplers—three mid-lake and three shoreline samples. We detected MC in ambient air only on Day 1 at Bloom Lake 1 and only from the shoreline sampler. The average air concentration was 0.052 ng/m<sup>3</sup>. The high-volume impactors collected particles of two size distributions, with mean mass aerodynamic diameters of 0.23 μm and 2.64 μm.

We collected 44 personal air samples. MC concentrations in personal samples ranged from the limit of

detection, 0.1 ng/m<sup>3</sup>, to 0.4 ng/m<sup>3</sup>. Table 4 contains a summary of the mass and air concentrations of MC in these samples.

### 3.6. Study population

We recruited 81 persons at the Bloom Lakes (exposed group) and 7 persons at the Control Lake (unexposed group). Approximately 90% of the persons approached at the two Bloom Lakes agreed to participate in the study; of those who refused, most were fearful about providing a blood sample. By contrast, ~5% of the persons approached at the Control Lake agreed to participate, stating that they planned multi-day recreational activities on the lake, were not interested in participating, or feared the blood-collection procedure. Of the exposed group, seven persons participated in at least some of the study activities on 2 consecutive days.

Within the exposed group, participants ranged in age from 12 years to 75 years. Seventy-three (90%) were white and 8 (10%) were of another race; 72 (89%) were non-Hispanic. Thirty-nine (48%) of the exposed participants were women. Unexposed participants ranged in age from 28 years to 53 years. All seven unexposed participants were white or non-Hispanic, and 5 (71%) were women.

### 3.7. Exposure to bloom and non-bloom recreational waters

Possible sources of exposure to cyanobacterial toxins for study participants before and during our study are presented in Table 5. During the study, participants in the exposed group reported time spent in or on the water ranging from 0 min to 360 min and the average was (mean ± standard deviation) 109 min ± 79 min. Forty-eight (59%) of the exposed group reported that they had put their head underwater, and 27 (33%) reported swallowing water, during the study period. Participants in the unexposed group reported time spent in or on the water ranging from 80 min to 360 min and the average was (mean ± standard deviation) 102 min ± 102 min. Four (57%) of the unexposed group reported that they had put their head underwater, and 1 (14%) reported they had swallowed water, during the study period.

As with our previous study (Backer et al., 2008), participants in the current study reported more symptoms during the 7 days before the study than either during the study or during the 7–10 days after the study period. For

**Table 5**

Number of study participants reporting activities associated with exposure to the Bloom Lakes before and during our study.

Time frame	Activity	Number of participants (%)	
		Exposed	Controls
During the year before the study	Boating	68 (84%)	0
	Swimming	68 (84%)	0
	Fishing	27 (33%)	0
	Eating fish they caught	27 (33%)	0
	Used a blue-green algae dietary supplement	2 (2%)	1 (14%)
During the week before the study	Visited Bloom Lakes	29 (36%)	0
	Visited other nearby lakes or rivers	26 (32%)	3 (43%)
	Went to private pools or spas	17 (21%)	1 (14%)
During the study period	Swimming	55 (68%)	4 (57%)
	Boating	10 (12%)	7 (100%)
	Tubing or wakeboarding	12 (15%)	1 (14%)
	Riding personal watercraft	5 (6%)	0
	Wading	5 (6%)	0
	Fishing	12 (15%)	0



**Table 6**

Microcystin concentrations on nasal swabs collected from exposed and unexposed groups on each study day. Results are the total response of the test (baseline interference or baseline interference plus mass of microcystin) from pre- and post-exposure swabs collected from the same nostril.

Study day	Number of participants	Response (ng) <sup>a</sup>			
		Pre-exposure		Post-exposure	
		Mean (standard deviation)	Range	Mean (standard deviation)	Range
<i>Exposed group</i>					
1	18	0.2 (0.1)	<LOD–0.5	0.6 (1.0)	<LOD–4.1
2	59	0.2 (0.2)	<LOD–1.6	0.6 (0.8)	<LOD–5.0
3	4	0.9 (1.4)	<LOD–3.3	0.9 (1.0)	<LOD–2.4
<i>Unexposed group</i>					
2	7	0.2 (0.1)	<LOD–0.5	0.2 (0.1)	<LOD–0.4

<sup>a</sup> Limit of detection = 0.1 ng.

example, during the week before the study, 27 (33%) participants in the exposed group reported upper respiratory symptoms, 7 (9%) reported dermal symptoms, and 24 (30%) reported other symptoms (earache, headache, abdominal pain). By contrast, immediately after study activities, 15 (29%) participants reported having upper respiratory symptoms, 5 (6%) reported dermal symptoms, and 5 (6%) reported other symptoms. None of these differences in reported symptoms was statistically significant ( $P > 0.10$ , McNemar exact test).

For the seven exposed people who repeated study activities, self-reported symptoms were similar on both days. One participant reported “other skin condition” (i.e., not itchy skin, red skin, or rash) before and after participating in study activities on the second day but not the first.

### 3.8. Plasma samples

We did not detect unbound MC in any of the plasma samples. MC-LA spike recoveries for plasma samples ranged from 77% to 119%, with an average recovery of 97%. Spikes administered prior to deproteinization were lost due to interactions with the plasma matrix.

### 3.9. Nasal swabs

Results of nasal-swab evaluations by study day and exposure group are presented in Table 6. For the 81 exposed participants, the baseline or pre-exposure response (i.e., level of interference for the test) on the nasal swabs was  $0.2 \text{ ng} \pm 0.4$  (mean  $\pm$  standard deviation). The post-exposure response (representing baseline interference plus mass of MC) for the exposed group was (mean  $\pm$  standard deviation)  $0.6 \text{ ng} \pm 0.8$ , indicating substantial variability among the samples. For each participant, the difference between post-exposure and pre-exposure responses was interpreted to be the amount of toxin on the swabs and was (mean difference  $\pm$  standard deviation)  $0.4 \text{ ng} \pm 0.1$ . This difference was significant (Wilcoxon signed rank test  $P < 0.001$ ). Study participants who enrolled on Day 3 had been at the lake for the weekend (i.e., a day or two before volunteering to be in the study); thus, we were able to detect MC (i.e., a response to the test above the interference baseline) on their pre-exposure nasal swabs (see Table 6).

For the seven exposed study participants who took part in study activities on 2 consecutive days, the test responses for the swabs were as follows (mean  $\pm$  standard deviation): Day 1 pre-exposure ( $0.1 \pm 0$ ); Day 1 post-exposure ( $0.9 \text{ ng} \pm 0.45$ ); Day 2 pre-exposure ( $N = 2$ ) ( $0.3 \text{ ng} \pm 0.30$ ); and Day 2 post-exposure ( $0.4 \text{ ng} \pm 0.38$ ). For the seven unexposed participants from the Control Lake, the response on nasal swabs did not change as a result of participating in water-based activities (see Table 6).

## 4. Discussion

The success with which epidemiologic studies can assess the impact of environmental contaminants relies on the ability to accurately assess exposure and appropriately identify and quantify associated biological effects. In this study, we continued our assessment of exposures to MC during recreational activities on lakes with ongoing cyanobacterial blooms and included analysis of nasal swabs as part of the exposure assessment.

As in the previous study, we detected potentially toxigenic cyanobacteria, predominantly *Microcystis* spp. and *A. flos-aquae*, in lakes used for various recreational activities. We identified extracellular MC in Bloom Lake water at concentrations  $>10 \mu\text{g/L}$  and total MC concentrations of  $15\text{--}357 \mu\text{g/L}$ . We also detected MC in aerosols collected by personal air samplers and high-volume ambient air samplers at low concentrations ( $<3 \text{ ng/m}^3$ ). Although we can accurately analyze environmental samples, the cyanobacterial cell densities and MC concentrations in water and aerosol samples were highly variable and not necessarily correlated. For example, the highest concentration of dissolved MC ( $10.3 \mu\text{g/L}$ ), one of the highest concentrations of total MC ( $194 \mu\text{g/L}$ ), and nearly the lowest cell concentrations of *Microcystis* spp. (approximately 90,000 cells/mL) occurred simultaneously at Site 1 on Day 1 (see Tables 2 and 3). By contrast, the highest concentration of total MC ( $357 \mu\text{g/L}$ ) and the highest concentration of *Microcystis* spp. ( $>1,000,000$  cells/mL) occurred simultaneously at Site 3 on Day 2. In addition, we were only able to detect MC in the air with the on-shore high-volume air sample on study Day 1, and all high-volume air samples collected in mid-lake were below the limit of detection. Finally, the daily mean concentrations of MC in personal air samples did not

correlate with the concentrations of *Microcystis* spp. cells, dissolved MC, or total MC in the Bloom Lake water.

The absence of correlations among cell densities, water MC concentrations, and air MC concentrations is consistent with other studies examining human environmental water- and air-borne exposures to algal toxins (Backer et al., 2005; Fleming et al., 2005). It is not likely that these findings result from measurement error. For example, for microcystins in water, the average recovery for MC-LR standard and spike samples was 90% and the standard curve  $r^2$  values were greater than 0.99 when ELISA was used. For microcystins on air filters and nasal swabs, the standard curve  $r^2$  values were 0.998 and 0.987, respectively, when ELISA was used.

The water quality data (see Tables 1 and 2) are useful in assessing potential exposures. For example, chlorophyll-a concentrations  $>10$  mg/L indicate an increased probability of cyanobacterial bloom development (Walker, 1985; Downing et al., 2001) and, therefore, can be an indicator for potentially high toxin production, depending on algal composition. The chlorophyll-a data indicate that bloom conditions persisted for at least 36 days during the summer of 2007. These data further suggest that Lake 1 may be more productive than Lake 2; however, both water systems demonstrate high algal biomass and are therefore potentially high toxin-production environments. *M. aeruginosa* and *A. flos-aquae* blooms are naturally variable in their distribution due to the buoyant nature of cells and their tendency to aggregate into colonies/bundles. Buoyancy creates wind-driven accumulations near impediments while aggregation causes adherence of colonies/bundles in low energy environments and dense surface layer formations. In this study the largest formations were observed in the morning before winds developed and in embayments where waterways narrowed. Later in the day when wind and wave action increased, we observed cell accumulations near the shoreline.

Chlorophyll-a and MC concentrations in samples collected near the surface were high during blooms on the study lakes. Sub-surface (1 m) chlorophyll-a and MC levels were also high during those times, indicating the presence of cells and toxin at that depth as well. Nutrient data show that total nitrogen and total phosphorus concentrations were relatively high, but that inorganic forms were depleted occasionally to low levels due to algal uptake. *Microcystis* spp. do not form heterocysts and therefore require an external source of nitrogen to flourish. Although these reservoirs are formed by main stem dams on a relatively large river system with both high natural and anthropogenic nutrient loading from upstream, the large blooms also require the calmer, warmer, and longer water retention-time conditions in reservoirs created by the dams. On the second night of this study, a heavy rain occurred that disturbed bloom formations on Day 3. Following the rain, Secchi depth readings, an index of water clarity, were reduced, indicating that the bloom had dispersed. However, nutrient flushing from the event may have ultimately extended the bloom and increased toxin production beyond the study period.

Data presented in Fig. 1a and b clearly show that chlorophyll-a concentrations (at 1 m) started to increase in early

July 2007 and did not dissipate until November. Fig. 2a and b demonstrate detectable MC concentrations present from June 2007 through September or October, thus providing a potential toxin-exposure period of  $\sim 5$  months. In our study, environmental measurements (i.e., cell counts and toxin concentrations in the water) of these highly variable harmful algal blooms were not well correlated and thus are not likely to be good estimates of human exposure. High densities of cyanobacterial cells can be observed visually during intense blooms, however, CyanoHABs are typically patchy, even in small areas such as bays and coves in small lakes. Thus, cyanobacterial cell and toxin concentrations can be significantly different from one site to another, particularly in open water. MC concentrations in undisturbed water should be well correlated with cell densities because MC are endotoxins, and 90–95% of the toxin is found intracellularly in healthy cyanobacterial populations. However, the presence and concentration of MC in aerosols would be dependent on wind speed and direction as well as on cell concentrations. For example, in our study, MC concentrations in dense wind- and current-concentrated blooms along the shoreline were periodically very high ( $\sim 1000$   $\mu\text{g/L}$ ). In addition, *Microcystis* spp. produce heavy mucilage, and cells aggregate into colonies of various sizes. Wind turbulence disrupts surface accumulations and causes colonies to break apart, distributing them more randomly in both the horizontal and vertical water planes and, if the winds are strong enough, creating aerosols. Similarly, recreational activities would disaggregate colonies and generate toxin-containing aerosols.

In our previous studies of recreational exposure to cyanobacterial toxins (Backer et al., 2008; Cheng et al., 2007), we collected data using personal breathing zone monitors as a measure of exposure. However, depending on the size of the aerosol droplets, this may not be an accurate measure of the internal dose. For example, in this study, the aerosol droplets showed a bimodal size distribution with mass median aerodynamic diameters (MMAD) of 0.23  $\mu\text{m}$  and 2.64  $\mu\text{m}$ . This is consistent with the results from our previous study in which the aerosol droplets from one exposure day had MMAD of 0.4  $\mu\text{m}$  and 6.51  $\mu\text{m}$  (Cheng et al., 2007).

To improve exposure assessment, we took the additional step of determining whether toxin is deposited in the upper respiratory tract by using swabs to collect toxin directly from participants' nasal mucosa. We measured the amount of toxin in nasal swabs using an ELISA method. The mean level of interference on the pre-exposure swabs from Bloom Lake participants and both pre- and post-exposure swabs from Control (non-bloom) Lake participants was approximately 0.2 ng (level of detection (LOD) = 0.1 ng). It is not likely that MC occurred in the nasal secretions of these persons before doing their water-related activities. We assume that there were matrix interferences with the ELISA that will have to be addressed in future studies, perhaps through an additional sample clean-up step using solid-phase extraction. Also, we previously reported that aerosol particles of the size we observed (MMAD of 0.4–6.51  $\mu\text{m}$ ) typically remain in the upper respiratory tract (Schlesinger, 1985). More recent information from Scanlan et al. (1999) indicated that particles of this size can reach the alveoli.

Based on our results and this information, toxin deposition in the nose may represent only part of the total exposure. Our study was not able to determine whether sufficient aerosolized MC reached the lower respiratory tract for it to be absorbed through the lungs and appear in the blood.

This assay for toxin measurement on nasal swabs was not quantitative because we did not control the area of nasal mucosa swabbed nor the amount of mucus obtained. However, assuming individual participants collected their swab samples in a consistent manner, we examined the data semi-quantitatively by comparing individual pre- and post-exposure swabs. Although the amounts of toxin present on the swabs were small (maximum 3.3 ng), on average, significantly more toxin was identified on post-exposure than pre-exposure swabs. An average adult inhales 25 L (0.025 m<sup>3</sup>) of air per minute during light exercise (ICRP, 1994). In our study, the average concentration of aerosolized MC was ~0.3 ng/m<sup>3</sup> and the average exposure time was 109 min. Thus, on average, we would expect that an adult study participant inhaled 0.8 ng MC. This calculated value is of the same order of magnitude as the mean increase in amount of toxin on the post-activity nasal swabs compared with the pre-activity nasal swabs for the exposed group (i.e., 0.39 ng). Thus, our results suggest that MC inhaled in aerosols generated by recreational activities deposit in the upper respiratory tract, specifically in mucus secretions lining the nasal mucosa.

There is limited information from animal studies available for comparison with our data. Benson et al. (2005) examined the toxicity of MC-LR in mice after inhalation exposure. The investigators exposed mice to ~260 µg MC/m<sup>3</sup> for 0.5–2 h each day for 7 days and observed treatment-related microscopic lesions in the nasal cavities of mice in the groups exposed for longer times. While these results suggest that the nasal cavity may be the primary site of response to inhaled MC, these experimental doses are many orders of magnitude greater than those we have documented in our study participants.

Abraham et al. (2005a,b) assessed the effects from exposures to toxin-containing aerosols on asthmatic sheep. In preliminary work with MC, the investigators found that 100 breaths of 1000 ng MC/ml given on each of 4 consecutive days induced airway hyper-responsiveness (Dr. William Abraham, personal communication, July 2007). These results were similar to treating asthmatic sheep with 20 breaths containing up to 10 pg/mL brevetoxin (PbTx-2 or PbTx-3) (Abraham et al., 2005b). However, as with the work of Benson et al. (2005), the MC doses were many orders of magnitude higher than concentrations we detected in aerosol samples collected during recreational activities. In this study, we examined post-exposure plasma specimens for detectable levels of MC. As in our earlier study (Backer et al., 2008), we did not detect MC in the plasma. However, we noted during the analysis that samples spiked before protein precipitation exhibited a significantly reduced recovery of purified MC-LA when analyzed using ELISA. Thus, MC could be present in the blood, but may not be quantifiable in plasma samples because they are bound to some protein component of the blood such as albumin. Also, MC are hepatotoxins with a strong affinity for binding in the liver, thus they may be

sequestered in liver tissue and not measurable in plasma. There are additional possible explanations for these results. MC concentrations in the mucus may be too low for the toxins to be absorbed, or sufficient time may not have passed between exposure and blood sampling to allow absorption through nasal mucosa. Finally, it is possible that MC detoxification occurs in the nasal mucosa, lungs, or liver, thus eliminating most of the parent compounds.

It may be useful to explore other potential biomarkers of exposure to MC. Although fraught with potential interactions, changes in liver enzyme concentrations in serum may be useful in assessing low-level environmental exposures. It is also possible that only a small percentage of absorbed MC occurs unbound in the blood, and we are currently developing a method to evaluate bound MC in blood samples. Benson et al. (2005) detected alterations in serum protein profiles in mice exposed by nose inhalation to MC doses of 260–265 µg/m<sup>3</sup> for 7 days, suggesting that serum protein profiling may be another method to assess human MC exposures.

The second important component of environmental epidemiologic studies is an accurate measure of the health outcome. Based on anecdotal reports and earlier studies (Pilotto et al., 1997; Stewart et al., 2006a), we hypothesized in this and our previous study (Backer et al., 2008) that exposure to aerosolized MC during recreational activities in lakes with *M. aeruginosa* blooms would result in increased frequencies of self-reported acute dermal or respiratory symptoms over baseline. Some study participants reported throat and skin irritation after being in the bloom-affected waters. However, these are common symptoms with myriad causes and only a few participants reported such symptoms. Thus, we were not able to demonstrate differences in symptom reporting between exposed and unexposed participants, nor were we able to examine associations between reported symptoms and environmental measurements (cyanobacterial cell concentrations, water and air MC concentrations, or other water quality parameters).

Self-reported health-symptom data had limited value in assessing acute exposures to low environmental concentrations of MC in our studies. There are several possible reasons for this. First, the symptoms most likely to be reported (respiratory or dermal irritation) are common symptoms associated with exposure to other environmental contaminants and with infections. Our sample sizes may not have been large enough to detect differences between exposed and unexposed groups if they existed. Second, the data may have been biased in a way we cannot control in our analyses. For example, the presence of CyanoHABs may adversely affect communities that rely on local water bodies for tourism, or may restrict community access to popular recreation areas. Individual responses to epidemiologic survey questions could be biased by personal views about the risks and benefits (recreation vs. a cough) associated with these environmental exposures. In both of our studies, there was local interest in minimizing the perceived impact of CyanoHABs, either to preserve property values or to prevent limiting access to popular local lakes, and participants may have responded to questions about symptoms in a way that would support their personal view.

We noted that many of our study participants visited the lakes for several days. In addition, their nasal swab results suggested that MC concentrations may increase with repeated exposures. Therefore, we examined whether Day 1 Bloom Lake participants might serve as a naïve study population. We compared the symptoms reported by this group before exposure with: symptoms reported by this group after exposure, symptoms reported by the Day 2 Bloom Lake group before exposure, and symptoms reported by the Day 2 Bloom Lake group after exposure. There were no significant increases in symptoms identified by these comparisons. Further, more symptoms were reported by the Day 1 group before exposure than by the Day 2 group either before or after exposure. These results reinforce the suggestion that self-reported symptoms are a poor indicator of acute health effects associated with these exposures, either because they are commonly reported and are difficult to associate with a particular environmental exposure or because these responses are easily intentionally biased by study participants.

It may be useful to repeat these studies with a larger population to further explore associations between recreationally exposed individuals and both temporary and longer-term upper respiratory and dermal irritation. Alternatively, a study population with allergenic hypersensitivity or an underlying medical condition, such as asthma, could be evaluated to determine if such low-level CyanoHAB exposures pose an increased risk to sensitive subgroups. Public health decision-makers would benefit from knowing if there is a sensitive subpopulation they should target for messages about avoiding exposures to aerosolized CyanoHABs.

## 5. Summary and conclusion

We attempted to enhance our understanding of the public health impact of recreational exposures to lake water during a CyanoHAB. We demonstrated that there were measurable MC concentrations in the water and documented an inhalation route of exposure with deposition of aerosolized MC within the nasal cavity. We hypothesize that inhaled cyanotoxins may subsequently be absorbed into the body through either upper or lower airway mucosal surfaces. However, we did not demonstrate a detectable internal MC dose as measured by plasma toxin analysis or a significant increase in self-reported acute symptoms after exposure. It is likely that healthy persons will not have adverse acute effects from periodic exposures to MC in aerosols generated by water-based recreational activities in lakes with patches of toxin-producing blooms. However, these healthy persons clearly are exposed to potent hepatotoxins. We also hypothesize that other potent CyanoHAB toxins, such as anatoxin a or cylindrospermopsin may also be incorporated into aerosols, inhaled and deposited in the body, presenting other, potentially synergistic, health risks. In addition, it is possible that swimming and other water-based activities that result in swallowing water present a higher risk for adverse health effects from ingesting cyanobacterial cells and extracellular toxins in the water. Thus recreational exposure to CyanoHAB toxins remains a public health concern. Future studies of

aerosolized cyanobacterial toxin exposure should include improved measures of exposure and improved assessments of adverse health outcomes. It is also important to investigate potential susceptible populations that may respond more vigorously to these exposures such as persons with underlying respiratory disease.

## Acknowledgments

The authors would like to acknowledge the efforts and dedication of the following people who helped to make the study possible: Lori Copan, Kathleen Fitzsimmons, DeShan Foret, Judy Grether, Sharon Lee, and Svetlana Smorodinsky from the California Department of Public Health; Lenee Adams, John Fitzgerald, Terri Funk, Brenda Harris, Brian Parodi, and Shelley Slabaugh from the Siskiyou County Department of Public Health and Community Development; Linda Pendergrast, PacifiCorp; Susan Corum, Karuk Tribe; Karen Kirner, Mote Marine Laboratory Summer Intern Program; and Mathew Barber, Alastair Brown, and Bill Ebel.

## Conflicts of interest

The authors Lorraine C. Backer, Sandra V. McNeel, Terry Barber, Barbara Kirkpatrick, Christopher Williams, Mitch Irvin, Yue Zhou, Trisha B. Johnson, Kate Nierenberg, Mark Aubel, Rebecca LePrell, Andrew Chapman, Amanda Foss, Susan Corum, Vincent R. Hill and Yung-Sung Cheng declare that there are no conflicts of interest and the Lorraine C. Backer, Trisha B. Johnson, Vincent R. Hill, and Stephanie M. Kiesak declares that the findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

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