The Effects of Cyanobacteria Bloom Exposures among Visitors to Eagle Creek Reservoir

Abstract

This report outlines the recorded and possible adverse health effects due to cyanobacteria bloom exposures. Cyanobacteria blooms form under certain conditions, including low water flow, low water levels, a low nitrogen to phosphate ratio, increased retention time, and a high pH. Acute symptoms of exposure to hepatotoxic cyanotoxins include allergic dermal reactions, abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia, elevated liver enzymes in serum, hay fever symptoms, dizziness, fatigue, and eye irritations (World Health Organization, 2014, p. 8). There have not been many studies to determine the adverse health effects due to chronic exposure to cyanobacteria, but the toxins are suggested to be a promoter of liver cancer development (Funari & Testai, 2008, p. 110). This report, describes a correlational study conducted in Eagle Creek Reservoir in order to determine the extent of adverse health effects, which are listed above, of visitors to Eagle Creek Reservoir in Indianapolis, Indiana.

Introduction

Cyanobacteria are a group of algae that contains toxins that can cause serious health effects in humans and animals. In Indiana, two species of cyanobacteria, Cylindrospermopsis raciborskii, and Microcystis, are found in reservoirs and rivers, namely Eagle Creek Reservoir (Lembi, 2013). Citizens Water, a division of Citizens Energy Group, is responsible for testing reservoirs that feed into its water treatment plants. Citizens Water’s website explains that Eagle
Creek Reservoir is susceptible to algal blooms due to its shallow water, limited water movement, and increased sunlight penetration (Citizens Energy Group, 2014).

It is important to understand the conditions needed for the growth and survival of the cyanobacteria in order to prevent and remediate blooms of these organisms. *Cylindrospermopsis raciborskii* and *Microcystis* thrive in waters that have low flow, low water level, low nitrogen to phosphate ratio, increased retention time, and a high pH (National Oceanic & Atmospheric Administration Center of Excellence for Great Lakes and Human Health, 2013). *Cylindrospermopsis raciborskii* is also capable of producing the toxin cylindrospermopsin, and *Microcystis* produces microcystin-LR.

The true risk to humans from these cyanobacteria is when the cells in an algal bloom die. High concentrations can be found in the surrounding water (Funari & Testai, 2008) when the cells collapse due to death or treatment with algaecides. Funari and Testai (2008) also report that microcystins can persist in the environment for a period lasting 21 days up to 2-3 months, while cylindrospermopsin has a half-life of 11-15 days (Funari & Testai, 2008, p. 99).

In order to control algal blooms, agencies, including the World Health Organization (WHO) and the United States Environmental Protection Agency (EPA), have created recommendations for concentration limits in drinking water systems and response strategies to handle harmful algal blooms. WHO has developed a provisional guideline for cyanotoxins in drinking water with a maximum concentration of 1 microgram microcystin-LR per liter of water (Backer, 2009, p. 27). The best methods of removing cyanotoxins during treatment processes include ozonation (Hoeger et al., 2002) and use of ultraviolet radiation (Hitzfeld et al., 2000). It is important to monitor cyanotoxins levels in drinking water and recreational sources in Indiana to ensure the public’s safety.
Toxicological Assessment

Acute Exposures in Humans

The cyanotoxins cylindrospermopsin and microcystin-LR are produced by the cyanobacteria *Cylindrospermopsis* and *Microcystis* and have been linked to a number of acute and chronic adverse health effects in humans. Cylindrospermopsin is a protein synthesis inhibitor (Funari & Testai, 2008), while microcystin-LR inhibits protein phosphatases (Honkanen et al., 1990). According to WHO, individuals have reported the following symptoms after exposure to algae blooms containing *Cylindrospermopsis raciborskii* and *Microcystis* in recreational waters: allergic dermal reactions, abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia, elevated liver enzymes in serum, hay fever symptoms, dizziness, fatigue, and eye irritations (World Health Organization, 2014, p. 8). A 1999 WHO document also indicates that microcystin-LR is a specific liver poison in mammals that can potentially lead to death due to liver hemorrhage or liver failure (WHO, 1999, p. 2). These are only the acute symptoms that occur as a result to an acute, low-dose exposure to cylindrospermopsin and microcystin-LR.

There are a number of cases throughout the world where individuals have been exposed to high concentrations of cyanotoxins that result in death. In 1988, an epidemic of 2,000 cases of gastroenteritis were reported after the construction of a dam in Bahia, Brazil. 88 of these cases, which were mostly children, resulted in death, and the cyanobacterium that was responsible for this outbreak was a *Microcystis* strain (Teixera et al., 1993). Both microcystin and cylindrospermopsin were identified as the agent responsible for an outbreak of severe hepatitis at a Brazilian hemodialysis center in Caruaru, Brazil. Investigators collected samples from the
water source and found an average cyanobacteria cell count of 24,500 cells per milliliter (WHO, 1999, p. 7, & Carmichael et al., 2001, p. 663). Researchers were able to obtain blood sera from affected and control patients and liver tissue samples from deceased patients. Lab results showed that the microcystin concentration in the sera samples was up to ten nanograms per milliliter, and liver tissue samples had concentrations ranging from 0.03 to 0.60 grams microcystin-LR per kilogram of liver tissue (Jochimsen et al., 1998, p. 876). This outbreak was responsible for 100 cases of acute liver failure among the patients, which resulted in 50 of these patients dying. Patients were exposed to microcystins through their dialysis treatments, which made the patients’ symptoms more severe since the exposure occurred through intravenous injection of microcystin-contaminated water.

Li et al. conducted a study in 2011 among children living in the Three Gorges Reservoir Region in China and found significantly elevated levels of AST and ALP in children highly exposed to cyanotoxins compared to low exposed children (Li et al., 2011, p. 1486).

**Chronic Exposures in Humans**

To date, there have not been any studies conducted to assess the adverse health effects of a chronic exposure to microcystin-LR and cylindrospermopsin in humans. There is a suspected association between exposure to these cyanotoxins and liver cancer in a region in China. A high incidence of hepatic tumors in this population is thought to be the result of consuming raw, or untreated, water contaminated with microcystin (Funari & Testai, 2008, p. 110), but Funari and Testai note that the individuals in this population were also exposed to Aflatoxin B1, also a known hepatic carcinogen, in their food source. The presence of Aflatoxin B1 confounds the association between these cyanotoxins and hepatic tumors.
Animal Data

Fawell et al. (1994) derived LD_{50} doses among mice, which is the dose that kills 50% of animals in a study. Fawell et al.’s study dosed mice through the intraperitoneal route with pure microcystin-LR, and the true LD_{50} value is believed to be in the range of 25 to 150 micrograms of microcystin-LR per kilogram of body weight. Fawell et al. also administered doses through gavage in other mice and found an oral LD_{50} of 5,000 micrograms of microcystin-LR per kilogram of body weight.

A study published in 1997 was able to determine the LD_{50} dose for cylindrospermopsin in Swiss Albino male mice. The doses administered to the mice range from 10 to 300 milligrams dry weight of cyanobacteria per kilogram of live mouse weight (Hawkins et al., 1997, p. 344-345). After 24 hours, the LD_{50} was found to be 52 milligrams of cylindrospermopsin per kilogram of body weight (Hawkins et al., 1997, p. 345). Hawkins et al. (1997) also found that after 7 days, the LD_{50} was 32 milligrams of cylindrospermopsin per kilogram of body weight (p. 345).

To date, microcystin-LR and cylindrospermopsin have only been evaluated for carcinogenicity in mice and rats. Ito et al.’s 1997 study found that when mice were given a dose of 20 micrograms per kilogram of body weight of microcystin-LR through intraperitoneal injection 100 times over 28 weeks, the mice developed nodules in the liver that were 5 millimeters in diameter (Ito et al., 1997). The same study found that when 80 micrograms per kilogram body weight of microcystin-LR was administered orally, there were not any nodules in mice livers. Another study published in 1999 administered repeated intraperitoneal injections of microcystin-LR induced tumor promotion in rat liver initiated with diethylnitrosamine (Fujiki &
Suganuma, 1999, p. 151). Ito et al. (1997) and Fawell et al. (1994)’s studies support that microcystin-LR has hepatotoxic characteristics in mice.

Apart from the acute toxicity shown in Fawell et al.’s 1994 study, the researchers also reported findings for a subchronic exposure in male and female mice. Male and female mice were given orally administered doses of microcystin-LR at 0, 40, 200, or 1,000 micrograms per kilogram of body weight per day for thirteen weeks (Fawell et al., 1994). The mice given the highest dose of 1,000 micrograms per kilogram of body weight per day showed liver changes that included chronic inflammation, focal degeneration of hepatocytes and hemosiderin deposits (Fawell et al., 1994). Based on these doses, Fawell et al. found a no observable adverse effect level, or NOAEL, of 40 micrograms per kilogram of body weight per day for the liver inflammation, focal degeneration of hepatocytes and hemosiderin deposits. Fawell et al.’s 1994 study shows that more research is needed in order to better characterize the risk from exposure to microcystin-LR in humans.

**Developmental Effects of Microcystin-LR in Mice**

Fawell et al.’s 1994 study also evaluated the possible developmental effects of microcystin-LR in female mice. Female mice were given daily doses of microcystin-LR by gavage at dose levels of 0, 200, 600, or 2,000 micrograms per kilogram of body weight (Fawell et al., 1994). They observed that the 2,000 microgram per kilogram of body weight was associated with maternal mortality. Also at the highest dose, they found that the fetuses were underweight and showed skeletal ossification but there were no fetal deaths. Fawell et al. (1994) found a NOAEL for developmental toxicity at 600 micrograms per kilogram of body weight per day.
Dose-Response Relationship

There is evidence to suggest that the higher the dose of cyanotoxins a person is exposed to, the severity of the symptoms also increase. A significant trend of increased occurrence of diarrhea, vomiting, flu-like symptoms, skin rashes, mouth ulcers, fevers or eye or ear infections was associated with increased duration of water contact and increased cyanobacteria cell density in the water (Pilotto et al., 1997). After excluding individuals who had recreational water contact in the five days preceding the interview, it was determined that those who spent more than sixty minutes of contact with the water with/or cyanobacteria cell density was greater than 5,000 cell per milliliter were 3.44 times more likely to have symptoms listed above of cyanotoxin exposure than those who were unexposed (OR=3.44, CI 1.09-10.82, p=0.004) (Pilotto et al., 1997).

Hazard Identification and Exposure Assessment

How are humans exposed?

Humans are exposed to cyanotoxins through consumption of drinking water, ingesting water during recreational water activities, and dermal contact. Table 1, modified from Stone and Bress (2007), shows the exposure potential in three levels based on the recreational activity. Swimming, diving, water skiing, jet skiing, and wind surfing all have a high exposure potential through ingestion and/or inhalation. Fish consumption, canoeing, rowing, sailing, kayaking, and motor boating have moderate exposure potentials through ingestion, inhalation, and dermal pathways. Catch and release fishing has a low exposure potential through the dermal route of exposure.
Table 1. List of primary exposure pathways of concern for cyanotoxins during recreational activities

<table>
<thead>
<tr>
<th>Exposure Potential</th>
<th>Recreational Activity</th>
<th>Primary Exposure Pathways of Concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Swimming/Wading</td>
<td>Ingestion</td>
</tr>
<tr>
<td>Moderate</td>
<td>Canoeing</td>
<td>Inhalation/Dermal</td>
</tr>
<tr>
<td></td>
<td>Kayaking</td>
<td>Inhalation/Dermal</td>
</tr>
<tr>
<td></td>
<td>Motor Boating (cruising)</td>
<td>Inhalation</td>
</tr>
<tr>
<td></td>
<td>Rowing</td>
<td>Inhalation/Dermal</td>
</tr>
<tr>
<td>Low/none</td>
<td>Fishing</td>
<td>Dermal</td>
</tr>
</tbody>
</table>

What levels have been measured in humans?

Serum samples from the patients who died from cyanotoxin exposure at the hemodialysis center in Caruaru, Brazil were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. CDC found microcystin contents of up to 10 nanograms per milliliter in sera and a range of 0.03 to 0.60 grams microcystin-LR per kilogram of liver tissue (Jochimsen et al., 1998).

What levels have been measured in environmental settings in Indiana?

In Indiana, Citizens Water, a division of Citizens Energy Group, is responsible for taking water samples to assess water quality at Eagle Creek Reservoir in Marion County, Indiana (Citizens Energy Group, 2014). Citizens Energy Group reported a testing sample collected on August 23, 2013 and analyzed on August 28, 2013 of Cylindrospermopsin concentration of less than 0.050 ppb through an ADDA ELISA test (IDEM, Cylindrospermopsin Report, 2013, p. 3). An ADDA ELISA test is specifically designed to react to proteins found microcystins through binding with Adda moiety and has a detection level as low as one nanogram cyanotoxin per milliliter of water (Enzo Life Sciences, Inc., 2014). One nanogram per milliliter of water is equal to 1 part per billion when converted. There are also ADDA ELISA kits for cylindrospermopsin
(Abraxis, 2014). Citizens Energy Group also tested for Microcystin concentration and found a concentration of less than 0.150 ppb (IDEM, Microcystin Report, 2013, p. 3). Based on the product description of the ADDA ELISA test, the microcystin and cylindrosporin levels found by Citizens Energy Group’s reporting were below detectable limits.

**Study Design**

A correlational study is being proposed to examine the effects of exposure to cyanotoxins through recreational water activities at Eagle Creek Reservoir in Indianapolis, Indiana. My study population will include Indiana residents who use Eagle Creek Reservoir for recreational water activities. Participants will be enrolled between the week of June 15 and August 31, as these months are known to produce conditions that are ideal for cyanobacteria blooms to grow in, and this is also the testing period the Indiana Department of Environmental Management and Citizens Energy Group uses. Participants will be eligible for the study if they do not have any history of liver conditions or diseases including hepatitis, liver failure, liver transplant or a history of alcoholism. Participants will also be excluded if they have had any of the following symptoms 5 days before their first interview: allergic dermal irritations, abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia, elevated liver enzymes in the serum, hay fever symptoms, dizziness, fatigue, or eye irritations. Residents whose drinking water is sourced from Eagle Creek Reservoir will also be included in the study under the same inclusion and exclusion criteria as the recreational visitors.

In order to correlate the concentrations of cyanobacteria to health outcomes present in other studies, water samples will be taken from Eagle Creek Reservoir on a daily basis. ADDA ELISA tests will be used to assess cyanobacteria concentrations in Eagle Creek Reservoir. Water sample records will also be obtained from Citizens Energy Group to compare our daily
testing results against Citizens Energy Group’s records. If cylindrospermopsin and microcystin-LR concentrations are below detectable limits, we will concentrate the toxins in water to improve quantitation. Citizens Energy Group will also be able to provide information on residents who receive their drinking water from Eagle Creek Reservoir. To measure recreational exposures to cyanobacteria, I will conduct a survey among the visitors to Eagle Creek Reservoir. The survey will ask questions regarding activities like fishing, boating, swimming, diving, kayaking, rowing, and canoeing. The survey will also assess the duration of water contact each visitor had during his or her recreational activities. This survey can be found on page 14. We will take blood samples from participants to run a panel of tests to determine elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT). Blood samples will also be analyzed to assess if concentrations of either cyanotoxin are present. The results from the tests will be correlated with the concentrations of cylindrospermopsin and microcystin-LR found in Eagle Creek Reservoir. A correlation between elevated hepatic gamma-glutamyltransferase activity and drinking water from a source contaminated with cyanobacteria was noted in a study conducted by Falconer et al. in 1983 (Codd et al., 2010, p. 411). In addition to performing logistic regression tests comparing symptom occurrence to cylindrospermopsin and microcystin-LR concentrations in water, we will also calculate chi-squares in order to quantify the strength of the statistical significance of our findings.
Risk Characterization

What federal and state agencies regulate this hazard?

In Indiana, the Indiana Department of Environmental Management (IDEM), the Indiana State Department of Health (ISDH), the Indiana Department of Natural Resources (DNR), and the Board of Animal Health (BOA) work together to provide information and testing on cyanobacteria blooms in Indiana’s recreational waters. Because Eagle Creek Reservoir is a Citizens Water (a division of Citizens Energy Group) Reservoir, IDEM does not test that body of water, but Citizens Energy performs testing to monitor cyanobacteria blooms (IDEM, 2014).

What are the current exposure limits for this hazard?

According to the Blue-Green Algae website, Indiana uses a 6 parts per billion of microcystin toxin as a warning level, while the World Health Organization (WHO) recommends a warning level of 20 parts per billion (IDEM, Blue-Green Algae, 2014). Indiana also uses Ohio’s recommendation of 5 parts per billion for cylindrospermopsin toxin in recreational waters (IDEM, Blue-Green Algae, 2014). For cell density counts, Indiana uses WHO’s recommendation of 100,000 cells per milliliter as a high risk count alert (IDEM, Blue-Green Algae, 2014).

Susceptible Populations to Consider In Risk Characterization

There are a few groups of individuals that are uniquely sensitive to cyanotoxin exposures. Children are more susceptible to adverse health effects from cyanotoxin exposure (North Carolina Division of Public Health, 2013) than adults are because they consume more water in proportion to body weight than adults (World Health Organization, 1999, p. 4). Because many cyanotoxins are hepatotoxic, WHO also identifies any individual with hepatitis, liver cirrhosis, toxic liver injury from other sources, or kidney damage as susceptible populations (WHO, 1999,
As was seen in the case of the hemodialysis center in Caruaru, Brazil, dialysis patients are more at risk because large volumes of water are administered intravenously (Jochimsen et al., 1998). According to the North Carolina Division of Public Health, dogs are also a susceptible non-human population because the cyanobacteria will cling to their fur and, in turn, ingested when the dogs clean themselves (North Carolina Division of Public Health, 2013). These groups of individuals need to be included when posting warnings about cyanobacteria blooms in recreational waters.

Control Measures to Reduce Exposure to Cyanotoxins

A single framework for cyanobacteria bloom monitoring is not used for the United States. Instead, some states have developed their own frameworks to monitor cyanobacteria blooms. Oregon and Vermont have developed frameworks that Indiana should adopt in developing its own framework for cyanobacteria bloom monitoring. Oregon uses two mechanisms in order to determine if warnings should be posted at a recreational water site (Stone & Bress, 2007). The first mechanism is to identify the existence of visible scum in the water that primarily consists of cyanobacteria; this leads to a prompt warning posted at the recreational water site. The second mechanism relies on determining the cell density of toxigenic cyanobacteria. If the cell density reaches 100,000 cells per milliliter, warnings are posted until the cell density falls below that limit. Oregon’s framework is an example of a lenient monitoring framework.

Vermont’s monitoring framework for cyanobacteria blooms is much more conservative than Oregon’s. Vermont implemented a five-tier surveillance program to reduce exposure to cyanotoxins and blooms in recreational waters (Stone & Bress, 2007, p. 140). Officials begin with qualitative sampling that increases in frequency once blooms have developed. If toxic cyanobacteria are found, officials proceed to quantitative sampling twice a month until
cyanobacteria cell densities reach 2,000 cells per milliliter. Once that threshold is met, weekly sampling is performed until cell densities reach 4,000 cells per milliliter, which would lead to weekly sampling until microcystin concentrations reach six micrograms per liter of water. If concentrations exceed the six micrograms per liter of water limit, public access to recreational water bodies halts. Only when microcystin concentrations fall below six micrograms per liter is access to recreational water bodies opened again to the public. Vermont’s framework uses conservative measures to minimize human exposure to cyanobacteria blooms. Based on the potential for adverse human health effect for cyanotoxins, Vermont’s framework should be the primary example that Indiana uses when developing its own monitoring framework.

Further recommendations for a remediation plan will be based on the findings from our proposed correlational study in Eagle Creek Reservoir. If we find high levels of cylindrospermopsin and microcystin-LR in the water and in study participants’ blood samples, stronger guidelines should be implemented, including immediate closure of public beaches along the reservoir and closure of public access ramps for boats.
Questionnaire

Please answer the following questions to the best of your ability.

1. Gender: Male / Female

2. Age (in years): ____

3. County of Residence:

4. If you have had any of the following symptoms in the past 5 days, please place a checkmark next to the symptom:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>X</th>
<th>Symptom</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic Skin Irritation</td>
<td></td>
<td>Abdominal Pain</td>
<td></td>
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<tr>
<td>Nausea</td>
<td></td>
<td>Vomiting</td>
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<tr>
<td>Diarrhea</td>
<td></td>
<td>Sore throat</td>
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<tr>
<td>Dry Cough</td>
<td></td>
<td>Headache</td>
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<tr>
<td>Blistering of the Mouth</td>
<td></td>
<td>Atypical Pneumonia</td>
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<tr>
<td>Hay Fever Symptoms</td>
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<td>Dizziness</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
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<td>Eye Irritations</td>
<td></td>
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</tbody>
</table>

5. Have you ever had a history of any of the following liver conditions or diseases?
   - Hepatitis: Yes / No
   - Liver failure: Yes / No
   - Liver transplant: Yes / No

6. Do you have a history of alcoholism?
   - Yes / No

7. Which of the following activities did you participate in today?

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes / No</th>
<th>Activity</th>
<th>Yes / No</th>
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<tbody>
<tr>
<td>Fishing</td>
<td></td>
<td>Boating</td>
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<td>Kayaking</td>
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<td>Canoeing</td>
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<tr>
<td>Rowing</td>
<td></td>
<td>Swimming</td>
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</tbody>
</table>

8. Please estimate to the best of your ability how much time (in hours and minutes) you spent doing each of the activities from Question 7.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Hours</th>
<th>Minutes</th>
</tr>
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<tbody>
<tr>
<td>Fishing</td>
<td></td>
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<td>Boating</td>
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<td>Kayaking</td>
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<td>Canoeing</td>
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<tr>
<td>Rowing</td>
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<tr>
<td>Swimming</td>
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