RISKS TO THE NORTH AMERICAN BEAVER

FROM EXPOSURE TO THE ALGAL-BLOOM TOXIN MICROCYSTIN

by
Elizabeth Nicole Blauwiekel

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NOMENCLATURE

Algal bloom – a rapid growth of microscopic algae or cyanobacteria in water, often resulting in a colored scum on the surface.

Algicide – any substance which is poisonous to algae.

Acute exposure – contact with a substance that occurs once or for only a short time (up to 14 days)

Bioaccumulation – the accumulation over time of a substance and especially a contaminant (such as a pesticide or heavy metal) in a living organism

Chronic exposure – contact with a substance that occurs over a long time (more than one year).

Cyanobacteria – any of a major group (Cyanobacteria) of photosynthetic bacteria that are single-celled but often form colonies in the form of filaments, sheets, or spheres and are found in diverse environments (such as salt and fresh water, soils, and on rocks).

Cyanotoxins – highly potent toxins produced from cyanobacterial cells.

Gavage – the administration of food or drugs by force, especially to an animal, typically through a tube leading down the throat to the stomach.

Harmful algal bloom (HAB) – harmful algal blooms, or HABs, occur when colonies of algae—simple plants that live in the sea and freshwater—grow out of control and produce toxic or harmful effects on people, fish, shellfish, marine mammals, and birds.

Hepatotoxin – a substance toxic to the liver.

Intrahepatic – within the liver.

Keystone species – a species that produces a major impact (as by predation) on its ecosystem and is considered essential to maintaining optimum ecosystem function or structure.

LD_{50} – the amount of a toxic agent (such as a poison, virus, or radiation) that is sufficient to kill 50 percent of a population of animals usually within a certain time.

LOAEL – the lowest tested dose of a substance that has been reported to cause harmful (adverse) health effects in people or animals.

NOMENCLATURE CONTINUED

NOAEL – the highest tested dose of a substance that has been reported to have no harmful (adverse) health effects on people or animals.

Phosphatases – an enzyme that removes a phosphate group from a protein.

Riparian – land areas along waterways, such as riverbanks and floodplains.

Systemic – affecting the entire body instead of just one part of an organism, effects can be seen in tissues distance from exposure site.

Thresholds – the magnitude or intensity that must be exceeded for a certain reaction, phenomenon, result, or condition to occur or be manifested.

ABSTRACT

My paper is a risk assessment of the effects of the algal-bloom cyanotoxin, microcystin, on the North American beaver, *Castor canadensis*. Microcystins are not only the most common cyanotoxins detected in the Great Lakes, but also in freshwater lakes worldwide. I have specifically focused on the population of beavers on Isle Royale in Lake Superior because cyanobacteria blooms have become more frequent on Isle Royale's inland lakes and have recently begun to appear in the surrounding Lake Superior area. Isle Royale's inland lakes have been gradually warming for decades and the beavers help foster a nutrient-loaded, slow-moving water area for those blooms to occur. Beavers tend to be a keystone species for an ecosystem, and they are generally seen as an indication of good ecosystem health. Isle Royale is isolated far from the mainland with few opportunities for new organisms to immigrate or ecosystem recovery; therefore, I estimated the risks of microcystin to the North American beaver on Isle Royale. I reviewed toxicity, estimated dietary exposure, projected concentrations of the toxin in the Isle Royale lakes for future years, and compared estimated exposures to toxicity thresholds to characterize risks using a risk quotient (RQ) approach. An RQ is the ratio of estimated exposure to an effect threshold to identify if an organism's exposure exceeds the threshold. My results indicate RQs ranged from < 0.01 to 0.02 for 2022. This means that exposures I calculated were less than 2% of the no-adverse-effect level and the beavers are at negligible risk of adverse health effects from microcystin in the lakes on Isle Royale. Projected microcystin concentrations and RQ values for 2030 and 2040 also indicate a similar level of negligible risk.

INTRODUCTION

The North American Beaver

Long thought of as a keystone species among many of the U.S.'s ecosystems, the North American beaver, *Castor canadensis*, is the focal species for my risk assessment. Beavers are important to ecosystems, especially the ecosystem of Isle Royale. Beavers can change the water chemistry, geomorphology, and therefore the species diversity of an area. These dramatic changes have earned them the nickname "ecosystem engineer," which emphasizes their importance to ecological health and stability (Rosell et al., 2005).

A beaver's foraging style generally includes disproportional selection of less-abundant plant species which influences the succession of plant communities towards dominant species. When feeding on trees, they do not consume the whole plant but instead leave behind parts of the fallen trees which can be used as food sources and habitats for smaller organisms. Beavers select early to mid-succession growth flora which promotes forest health by stimulating young growth and keeping the forest in a high stage of productivity (Rosell et al., 2005).

Beavers also dam streams which cause flooding in the upland areas, but also sufficiently slow the drainage to lengthen the time that water flows during the year. This change in kinetic energy and water availability will also alter the erosion potential, help prevent large flooding events downstream, allow groundwater recharge, and greatly increase the area of riparian habitats (Rosell et al., 2005). Riparian zones, compared to the adjacent terrestrial areas, will have nearly twice the number of species that rely on them for food and habitat (Goforth et al., 2001).

Beavers also construct burrows and lodges to avoid predation and they excavate canals for feeding opportunities, both of which will alter the course of the water and sediment. Once unoccupied, the lodges and canals become homes for muskrat, weasel, and other animals that require similar habitats but do not have the same engineering skills as the beavers (Rosell et al., 2005). The trapped sediment upstream, increased organic matter introduced by flooding terrestrial areas, stored food caches created by the beavers, and overall slowed rate of decay due to flow rate decrease all dramatically increase the available oxygen and nutrients in the water. Therefore, the beaver’s actions have a dramatic effect on the productivity and chemistry of the water (Rosell et al., 2005).

Due to the change in flow rate, the beaver can alter the shape of rivers and streams over time. The increase in productivity that coincides with their habits as well as their generalist tendencies as foragers make them an ideal species for ecosystem repair. Ecosystems, such as Yellowstone National Park, have endured periods without their native beavers, and have experienced substantial changes in ecosystem structure and health during that time (Brooks, 2017). Upon returning to the area, the beavers were cited as the reason for the ecosystem’s transition back to wetland-meadow complexes with productive riparian zones from the dry grasslands that had become established (Figure 1) (Brooks, 2017).

The North American beaver is a long-term resident of Isle Royale National Park and has been regularly tracked since the 1960s. Between 1990 and 2008, an extensive study was conducted to estimate the number of beavers in the population (Romanski, 2010). The beavers are rarely found on the outside banks where the island and Lake Superior meet, but rather are found in protected embankments of the inland waters. Here, they feed on both terrestrial and

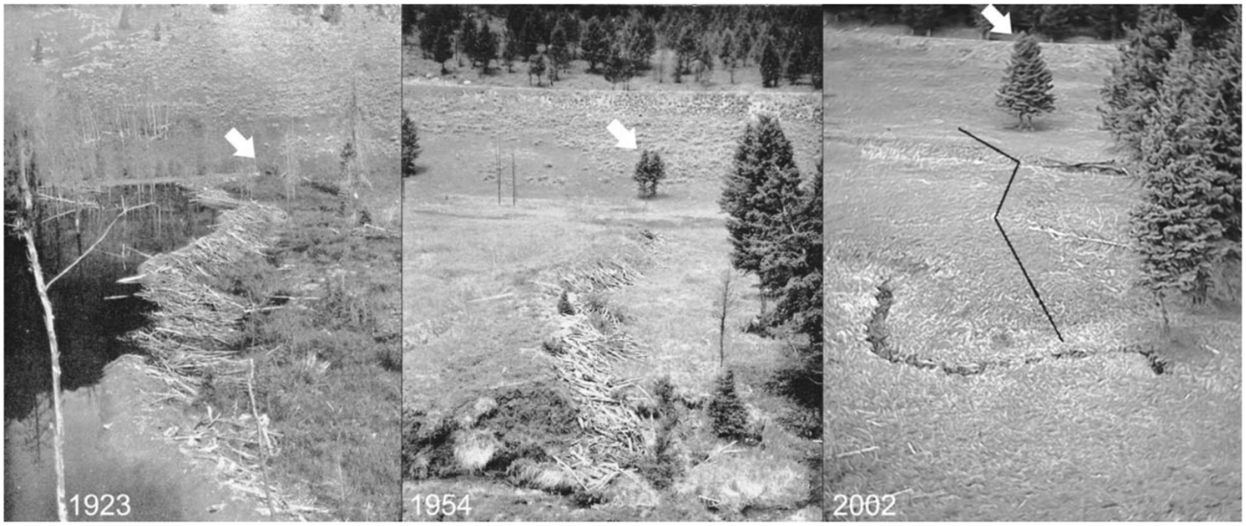

Figure 1: Comparison photos from a hillside at Elk Creek in Yellowstone National Park in 1923, 1954 and 2002. The beaver dam in the first image was not maintained and in the 1954 image you see no more standing water. By the 2002 image the previous riparian landscape is now grassland (Wolf et al., 2007).

aquatic vegetation. In an ecosystem such as Isle Royale where the surface water of inland lakes and streams can freeze during the year, beavers store food for the winter, forage under the ice, and rely somewhat more on terrestrial plants. However, a beaver's diet is approximately 60-80% aquatic vegetation, and that value does not change significantly in the winter (Milligan and Humphries, 2010).

Isle Royale

Isle Royale is a remote island archipelago in the northwest part of Lake Superior. At more than 544 km^2, it was designated as wilderness in 1976 and as an International Biosphere Reserve in 1980 (NPS, 2019). The few cottages and other small resorts of the early 1900s were all eventually transitioned back from landowners to the government beginning in the 1930s

(NPS, 2010). As such, the island has not been subjected to the impact of humans the way the mainland is. The island is 71 km long and 7.7 km wide and has a series of ridges and valleys running lengthwise across it. Although Isle Royale is considered forested, about 7.2% is characterized as lakes, ponds, or wetlands, and the island includes 254 km of streams (Romanski, 2010). The inland lakes of Isle Royale are the focal areas for this paper (Figure 2).

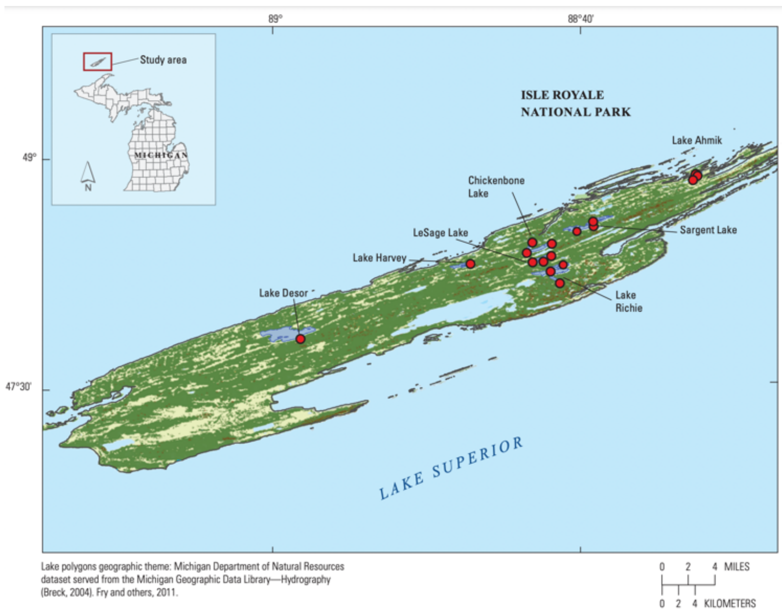

Figure 2: Map of Isle Royale highlighting the lakes that were tested for the presence of the toxin microcystin (Fuller et al., 2017).

At 25 km from the nearest mainland (Peterson et al., 2011), Isle Royale's ecosystem is isolated so native populations of organisms and populations from the mainland do not often mix. These smaller populations and the island's seclusion make the ecosystem more sensitive to ecological disturbances. Warming global temperatures have prevented ice bridges from forming between Isle Royale and the mainland. When present, they allow new species and organisms to be introduced to the island. The most recent ice bridge was in 2019 and the only animal that was tracked to cross the bridge was a single wolf (NPS, 2019).

Isle Royale has a diverse food web for a remote island and is studied most often for the predator-prey relationship of the island's gray wolves (*Canis lupus*) and moose (*Alces alces*). There are 18 confirmed mammal species on the island including beaver, wolf, moose, fox, mink, six species of bats, otter, hare, muskrat, mice, squirrel, marten, and weasel. There are also 245 recorded bird species, multiple species of snakes, turtles, frogs, toads, and salamanders, and 54 species of fish (NPS, 2021). The beavers are a secondary food source for the wolves, and when the numbers of wolves fluctuate, the beaver population responds accordingly (NPS, 2021).

Beavers are an important species in this food web because they are one of a few larger mammals that the wolves can prey on, they provide forage and habitats for the smaller herbivores, and they help maintain the productivity of the land. As beavers tend to search for food nearer to the streams and lakes and they are generalist feeders, they do not directly compete with the other larger herbivores, such as the moose, which can venture farther inland (Rosell et al., 2005).

Algal Blooms

Algal blooms are events in which a local population of algae has grown out of control and becomes a thick mass, or scum, on the water's surface. These blooms are typically blue-green or light green in color and concentrate near the water's surface and shoreline. Within the last few years, Lake Superior, the world's largest freshwater lake, has experienced its first algal blooms. Unlike the smaller Great Lakes, Lake Superior had been thought to be too cold and deep for such blooms to occur, but with the warming of the lake due to climate change, blooms have become more frequent (Oosthoek, 2021).

As algae die, their cells break down and can release toxins they produce into the water. Also, the decomposition process uses up the dissolved oxygen in that area which can result in large "dead zones," or regions that cannot support life. Algal blooms that release toxins are referred to as harmful algal blooms (HAB) (Parker, 2020). Thus far, algal blooms occurring in the main body of Lake Superior have not produced toxins, but toxic algal blooms are common and well-studied in nearby Lake Erie and many other inland lakes in the US. The Isle Royale archipelago contains 44 inland lakes and unlike the surrounding main body of Lake Superior, some of the small inland lakes of Isle Royale have had algal blooms with detectable toxins. Since 2007, the toxin microcystin has been detected in algal blooms in some of the inland lakes of Isle Royale (Fuller et al., 2017). These HAB can have major detrimental effects on any ecosystem, and those effects are easier to track on an isolated island such as Isle Royale.

The algal blooms that produce harmful toxins in the Great Lakes region are typically cyanobacteria, which produce toxins 50% to 75% of the time a bloom is present (Parker, 2020; WHO, 2003). Cyanobacteria bloom and persist in water that is between 15°C and 30°C (although the optimal range is 20 to 25°C), has a pH of 6 to 9, and has available nitrogen and phosphorus (WHO, 2003). In the Great Lakes region, the summer and fall seasons are within the temperature range to experience algal blooms, and there are many areas where runoff into the lakes will provide the necessary excess inorganic nutrients. Light intensity is also a factor for HAB formation, but the cyanobacteria that I focus on in this paper can change their buoyancy in response to light changes, making photosynthesis functional in a larger geographic range (WHO, 2003). Most of the cyanobacteria is found in the shallow, warmer areas of Lake Erie where runoff is more prevalent (Figure 3).

Figure 3: Harmful algal bloom as seen from satellite imagery in Lake Erie (NOAA, 2019)

Microcystin

Microcystins are cyclic peptides and are the most reported cyanobacteria toxin worldwide (WHO, 2020). There are many variants of microcystin that differ by which two of seven amino acids are present as the amino acid side chain. Microcystin-LR is the most common variation with leucine and arginine as the amino acids in the variable position (Figure 4) (Health Canada, 2019). The LR variant is the most toxic and abundant worldwide. In 2006, the U.S. Geological Survey (USGS) surveyed and sampled 23 lakes across the Midwest while they were experiencing algal blooms and found that toxic microcystin was present in 91% of the samples. During this study, the average concentration for microcystin-LR was 104 µg/L which is well above regulatory values for this toxin created by the World Health Organization (WHO) (D'Anglada, 2015).

A major issue with microcystin is its resistance to biological and chemical degradation (Health Canada, 2019). Even after applications of algicide, the concentration of toxin remaining in the water can vary dramatically (D'Anglada and Strong, 2015). Hepatoxins like microcystins are very stable and can remain in bodies of water for weeks after release from the cyanobacteria without breaking down (Machado et al., 2017). Some studies suggest a one-week half-life for microcystin (WHO, 2003), so the toxins can remain in the water even as it seeps into the soils surrounding the lake. The dissolved toxin can be potentially taken up by plant species or ingested by aquatic organisms. Unfortunately, the toxin does not have a color or taste once released into the water, making its detection by any species more difficult (WHO, 2020).

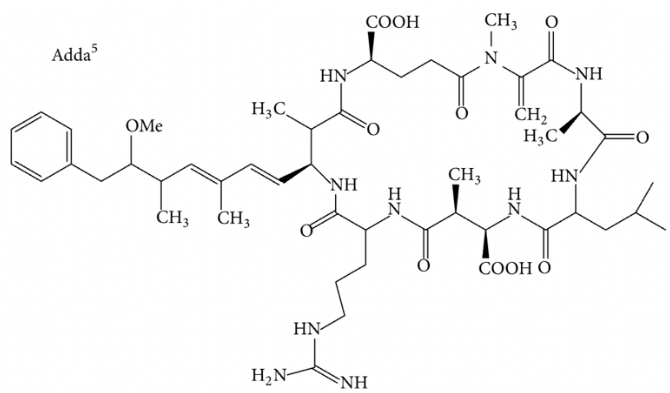

Figure 4: Chemical structure of the hepatoxin microcystin-LR, the most common cyanotoxin detected in the freshwater Great Lakes (Lone et al., 2016).

Microcystins are transported into cells by organic active transport proteins (OATPs). Liver tissue contains high amounts of these proteins thereby classifying microcystin as a hepatoxin, although it can also be found anywhere OATPs are present. Once inside cells, microcystins bind to protein phosphatases which are enzymes that remove phosphate from other proteins (Butler et al., 2009). This is a step involved in many regulatory processes such as cytoskeletal structure, DNA repair, stress response, and cell replication (WHO, 2020). Without properly functioning phosphatases, the cytoskeletal structure of the liver cells will break down and allow blood to flow between them (Figure 5). The toxin can remain in the body tissues for as many as two weeks after ingestion allowing the toxin to spread within the organism. Microcystin also affects the immune system and promotes tumor growth (Fuller et al., 2017).

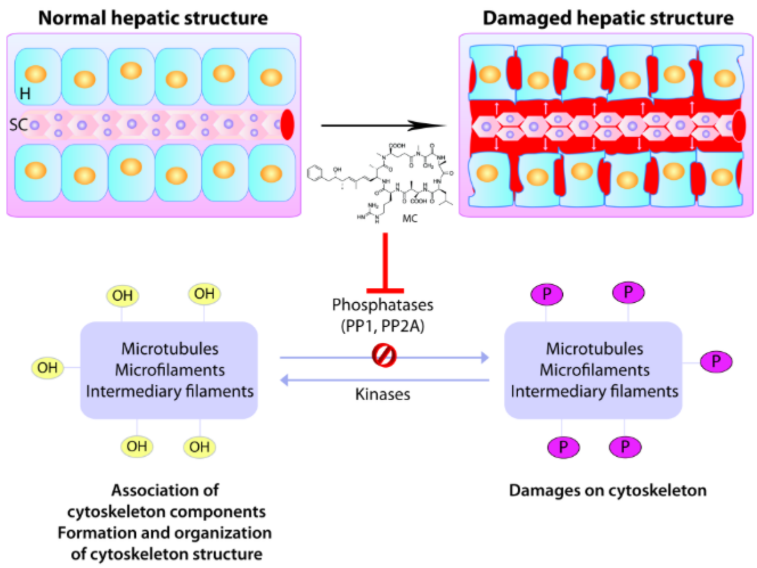

Figure 5: Comparing the functioning of liver tissues with or without infection by microcystin. The toxin binds to the phosphatases and prevents normal functioning which leads to blood flow between cells due to damaged cytoskeletons, among other issues (Menezes et al., 2013).

The cyanobacteria species that produce microcystins are predominantly found in freshwater with anthropogenic nutrients from agriculture or wastewater (WHO, 2020). Phosphorus and nitrogen are the two nutrients of highest concern and abundance when these blooms form. The Environmental Protection Agency (EPA) has released many warnings to the public that during an algal bloom humans, pets, and livestock should not be allowed in the water because adverse health effects, even death, can result if exposed to high concentrations of the toxins (EPA, 2019). Most documented cases of microcystin poisoning are from livestock, birds, or domestic animals and most were fatal (Butler et al., 2009). Microcystin is not volatile, so it will not be airborne other than if the water is sprayed into the air. The toxin has been found in the organs and tissues of fish and mollusks that have been caught in areas with algal blooms, so ingestion of other organisms is also a route of possible exposure (Schmidt et al., 2014; WHO, 2020).

PROBLEM FORMULATION

During visible algal blooms, lakes on Isle Royale have been sampled and found to have toxins such as microcystin dating to at least 2007. During the years 2012 and 2013, the USGS and National Park Service (NPS) conducted a 2-year study on the inland lakes on Isle Royale for the presence of microcystin (Fuller et al., 2017). The study used enzyme-linked immunosorbent assays to test for the toxin and found it to be present in several of the island's lakes (Figure 6).

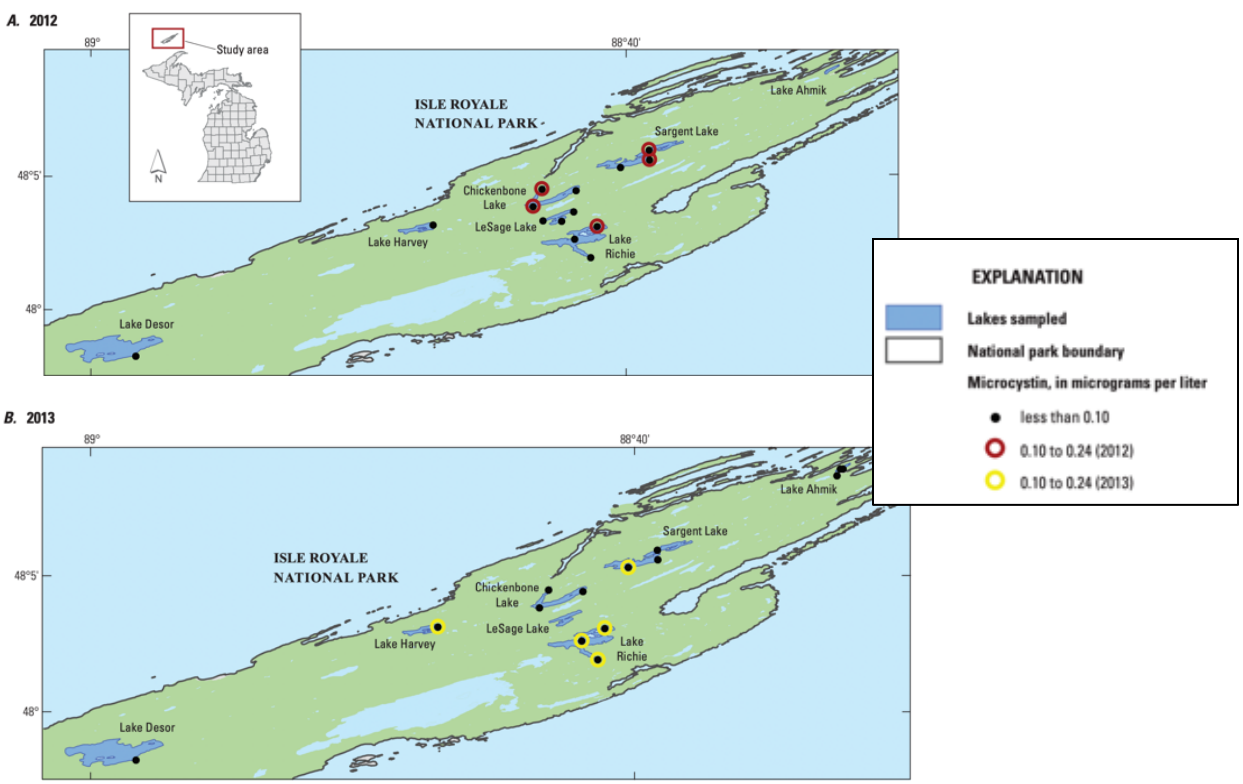

Figure 6: The study areas on Isle Royale for the 2012-2013 lake sampling (Fuller et al., 2017). The map of 2012 (top) and 2013 (bottom) outlining with red and yellow the samples with concentrations ranging 0.1-0.24 µg/L.

The lake study also showed that microcystin can be present even in the absence of a visible algal bloom (Fuller et al., 2017). No comprehensive studies like this have been done on Isle Royale since 2013.

As the beavers of Isle Royale cannot detect the colorless and odorless microcystin toxin, and it can persist in the water for up to two weeks after the algae have gone, ingestion of the toxin could represent a serious risk. The toxic metabolites produced from cyanobacteria can bioaccumulate, or gradually increase in a food chain or individual because they are not released as fast as they are consumed (Lone et al., 2016). This results in even more concern for the island's ecosystem as it opens the possibility of spreading the toxin through consumption of fish, beavers, and other aquatic foragers by the wolves and predatory birds.

The presence of toxins from HABs, such as microcystin, may have long-lasting negative health effects on beavers and could possibly lead to extirpation of the species on the island. Warming temperatures will continue to promote toxic algal blooms as they are now appearing in the much larger and colder Lake Superior (Oosthoek, 2021). In addition, the beavers tend to increase the available inorganic nutrients in the surrounding waters which will only further promote algal blooms. Areas with dams built by beaver colonies show an increase in the nitrogen and phosphorus in the water immediately upstream from the dam due to the change in sediment movement and organic matter present (Lazar et al., 2015).

The purpose of an environmental risk assessment is to estimate current or potential exposure to a toxin and determine the level of exposure that would cause harm to the focal species or area. I estimated risks of the toxin microcystin specifically on North American beavers on Isle Royale. Understanding the levels of the toxin that could have a negative effect on the beavers and what can be done to help mitigate that will be prudent as climate conditions continue to influence the production of HAB in freshwater ecosystems.

HAZARD IDENTIFICATION AND EFFECTS ASSESSMENT

Toxicity and Dose-Response Relationships

The route of exposure of microcystin in mammals is typically by ingestion, and about 7-10% is absorbed in the gastrointestinal tract (McLellan and Manderville, 2017). The toxin will accumulate mainly in the kidney and liver tissues, but it has been detected in the brain, heart, lungs, and reproductive organs of infected individuals. If an organism has acute toxicity to microcystin, it may experience intrahepatic hemorrhages, blood loss within the liver. This spreads the toxin to other cells and can cause widespread damage within the liver and other tissues. Multiple immunotoxicity studies show the toxin causes appetite increase, chronic inflammation, liver cell degeneration, thrombocytopenia, and hepatic congestion (Lone et al., 2016; WHO, 2003). When dosed with an injection of high concentration, mice and rats exhibited liver cell damage within 20 minutes, and the injections were typically fatal within a few hours (Butler et al., 2009).

Red blood cell (RBC) and white blood cell (WBC) counts change with continued exposure to the toxin, and at doses of 37.5 μg/kgBW/day (micrograms per kilogram of body weight per day) it causes breaks in the DNA strands. When treated with a dose of 15 μg/kgBW/day for 2 weeks, mice showed elevated counts of RBC and hemoglobin, but drastic decreases in WBC counts. Lower doses, such as 0.5, 2, and 8 μg/kgBW per day, when given every 48 hours for 30 days caused a decrease in RBCs, WBCs, and hemoglobin. Mice showed an increase in weight of their spleen (Figure 7), DNA fragmentation, lower antibody production, cell apoptosis, and overall immunosuppression (Lone et al., 2016).

Figure 7: A microcystin-LR treatment in mice for 14 days showing splenomegaly, and a significant increase in the weight of the spleen (Lone et al., 2016).

Toxicity and Environmental Thresholds

The toxicity thresholds for my assessment were taken from a laboratory study with mice that were dosed orally by gavage with microcystin-LR. Fifteen male and 15 female mice were dosed with 0, 40, 200, and 1000 µg/kgBW/day for 13 weeks. The highest dose showed negative effects on the liver in all the males and most females. The male and female mice exhibited a 14% and 20% increase in appetite, respectively, but both sexes had a net loss of 7% of their body weight. No treatment-related changes were detected at the lowest dose, which is why 40 µg/kgBW/day was determined to be the no-observed-adverse-effect-level (NOAEL) (WHO, 2003). In another study, mice were given higher doses (750-12000 µg/kgBW/day) of microcystin in their drinking water for up to 1 year to determine chronic exposure and lethal dosages. The higher doses increased significant liver damage and mortality (WHO, 2003). Mortality data from this study are how I determined the LD_{50} threshold of 1000 µg/kgBW/day.

The Michigan Department of Environment, Great Lakes, and Energy (MEGLE) considers an algal bloom harmful if the concentration of microcystin is ≥ 20 µg/L (Parker, 2020). Health Canada established a maximum acceptable concentration (MAC) of 1.5 µg/L (McLellan and Manderville, 2017). The recommended exposure limit to microcystin for a low probability of acute effects to humans is 10 µg/L for brief recreational exposure in freshwater (Fuller et al., 2017; EPA, 2019). In 2015, the EPA established a 10-day health advisory for drinking water concentrations of 0.3 µg/L for children less than six years old. The advisory concentration for six years old and older is 1.6 µg/L (D'Anglada and Strong, 2015). All these values set for recreation and drinking water are for the safety of humans.

EXPOSURE ASSESSMENT

Current and Future Environmental Microcystin Concentrations

To establish a trend for determining if changing lake temperatures would continue to cause HABs, I used a thermal study that was conducted in 2017. Four lakes on Isle Royale were studied with sediment cores and retrospective temperature-depth relationships modeled using satellite imaging (Edlund et al., 2017). Information was collected for two periods, 1962–1986 and 1987–2011, which was then analyzed for statistical trends in water temperatures and thermal gradients. The sediment contained algal pigments which could be reconstructed to determine the presence of algal blooms. As identified by the historical thermal imagery, the average summer shallow-water temperatures of the lakes on Isle Royale have been on a steady incline for decades (Figure 8) (Edlund et al., 2017). Because all four lakes showed their warming trends are similar and steadily rising for the last six decades, the results suggest that temperatures will continue increasing.

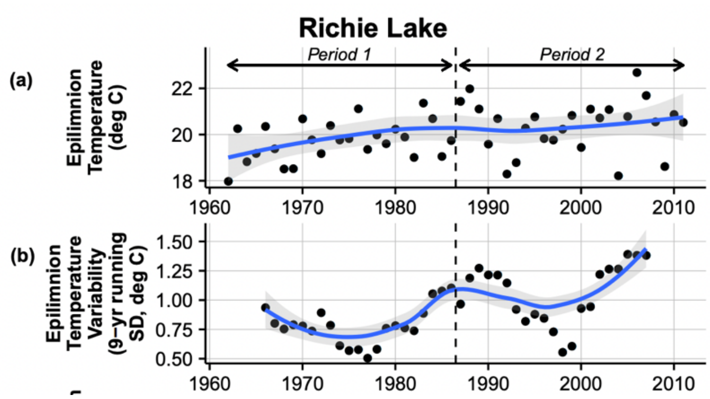

Figure 8: Trends in temperatures for Richie Lake on Isle Royale. (a) shows the average yearly temperature (b) shows the temperature variability from a running 9-year standard deviation. (Edlund et al., 2017).

The 2012-2013 study done on Isle Royale to test for the presence of microcystin in the inland lakes found 11% of the samples in 2012 and 12% of the samples in 2013 had detectable microcystin (Fuller et al., 2017). The 2012 microcystin samples ranged in concentration from less than 0.10 to 0.12 µg/L. The 2013 samples ranged in concentration from less than 0.10 to 0.20 µg/L. Although none of the samples exceeded the EPA's advisory for 10-day health effects on humans, they revealed an increase in concentration in just that one year. For 2012 and 2013, I used the range of concentrations observed to create an average concentration for that year.

As there are no long-term data on microcystin concentrations for Isle Royale, I reviewed studies done with nearby Lake Erie to determine what kind of growth has occurred in the last decade or two. A study done with satellite imagery on Lake Erie was able to determine the size of the blooms in the lake over time, and while they are variable, over many years they demonstrate a linear growth rate (Figure 9) (Ho and Michalak, 2017).

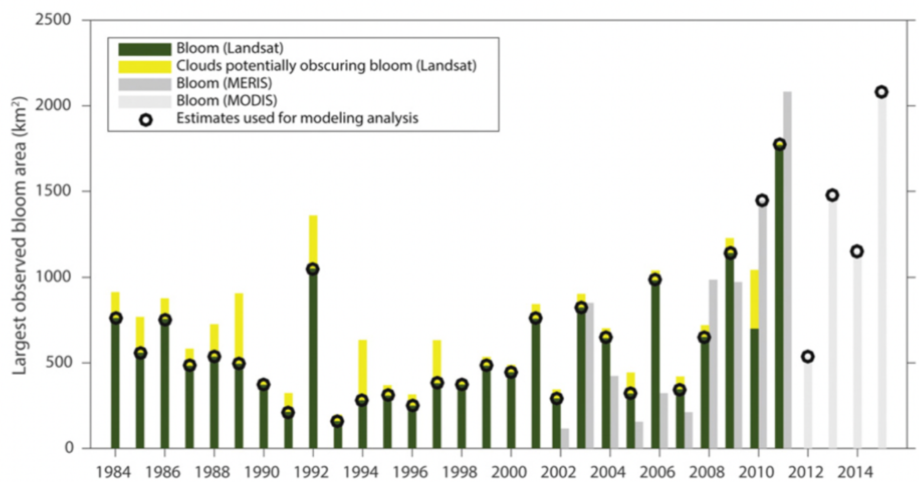

Figure 9: Area (in km^2) of the largest algal bloom observed each year in Lake Erie. The values since 1993 indicate a linear growth for algal bloom sizes for the lake (Ho and Michalak, 2017).

The difference between the toxin concentrations on Isle Royale in 2012 and 2013 was 0.04 µg/L and I used that value as the yearly growth rate to create a similarly linear trend for Isle Royale's lakes. The projected concentrations for the current year, 2022, and future years, 2030 and 2040, are presented in Table 1.

Table 1: Microcystin concentrations in the lakes on Isle Royale for 2012 and 2013 (Fuller et al., 2017), as well as projected concentrations for 2020, 2030 and 2040. Projections are based on linear growth in concentration by year.

Year	Percentage of Lake samples with detectable microcystin	Average concentration of microcystin in samples (µg/L)
2012	11	0.11
2013	12	0.15
2022	N/A	0.51
2030	N/A	0.83
2040	N/A	1.23

Beaver Exposure to Microcystin

Beavers that have access to ponds and lakes and live in a climate with many months of winter, like those on Isle Royale, tend to have a diet that is 60-80% aquatic vegetation (Milligan and Humphries, 2010). I assumed the beaver's exposure to the toxin is through oral consumption in the water during foraging activities. Their water consumption primarily occurs through intake of the aquatic plant portion of their diet. Although the terrestrial plants they consume tend to be near the water and may have toxin uptake due to the toxin's longevity in freshwater, it is unlikely to result in appreciable exposure and therefore will not be considered.

To estimate the concentration of toxin to which the beavers are exposed, standardized by body weight, I used 16 kg as the average mass of an adult (Belovsky, 1984; Romanski, 2010; Jenkins, 1979). I assumed pathways of exposure included both drinking the water while foraging for aquatic plants and the ingestion of aquatic plants which had absorbed the toxin in the water. The total mass of a beaver's diet is made up of 70% aquatic plants on average (Milligan and Humphries, 2010). I used an average mass of vegetation consumed, divided by the average mass of an adult beaver, and found 0.094 kg/kgBW/day as the value for average food intake. Knowing that aquatic plants tend to be about 90% water by mass, the total water ingestion from aquatic food is 0.084 kg/kgBW/day. Data for the volume a beaver drinks in a day are not available, therefore I used the allometric equation for drinking water ingestion for mammals found in the EPA Wildlife Exposure Factors Handbook (EPA, 1993).

$$Water\ Ingestion = 0.099(Wt)^{0.9},$$

where Wt is the average body weight in kg for the animal. Table 2 shows the values of both the water ingested by aquatic food consumption, water ingested by drinking, and total daily water intake.

Table 2: Daily water intake values in kg for a North American beaver. Value derived from the allometric equation used for drinking water ingestion in mammals (EPA, 1993).

Average Mass of Adult Beavers (kg)	Daily Water Ingestion (kg/day)	Daily Water Ingestion (kg/kgBW/day)	Daily Water Intake from Vegetation (kg/kgBW/day)	Total Daily Water Ingestion (kg/kgBW/day)
16	1.2	0.075	0.084	0.159

I used the total daily water ingestion value of 0.159 kg/kgBW/day multiplied by toxin concentration for each year to estimate the daily exposure values (Table 3). The calculated values for daily exposure are far below the threshold value of 40 μg/kgBW/day which was determined to be the NOAEL.

$$Daily\ Exposure = water\ consumption\ \left(\frac{kg}{kgBW\ per\ day}\right)\ x\ lake\ concentration\ of\ microcystin\ (\frac{ug}{kg})$$

Table 3: Daily exposure values in μg/kgBW/day calculated for the North American beavers in Isle Royale for the current year and as lake microcystin concentrations are projected to change for the years 2022, 2030, and 2040.

Year	**Daily Exposure Values (μg/kgBW/day)**
2022	0.0813
2030	0.1323
2040	0.1960

RISK CHARACTERIZATION

Daily exposure values are used with toxicity thresholds to determine a risk quotient (RQ). The RQ is calculated by dividing the estimated daily exposure by toxicity threshold.

$$Risk\ Quotient = \frac{estimated\ daily\ exposure}{toxic\ threshold}$$

The toxicity thresholds are the no-observed-adverse-effect-level (NOAEL), the lowest-observed-adverse-effect-level (LOAEL), and the lethal dose from chronic exposure (LD_{50}). Risk quotients are a ratio, so a value of 1.0 means the exposure is equal to the toxic threshold. The lowest RQ value that the EPA lists as a level of concern is 0.1, but that is only for endangered species (Jones, 2004). RQ values for each year of projected exposure and each of the three toxicity thresholds were calculated (Table 4).

Table 4: Risk quotient values calculated for beavers based on the toxic thresholds for no-observable-adverse-effect-level (NOAEL), lowest-observable-adverse-effect-level (LOAEL), and lethal dose after 1 year of chronic dosage (LD50). BW = body weight.

Year	**Exposure Thresholds (µg/kgBW)**		
	NOAEL (40)	**LOAEL (80)**	**LD50 Chronic exposure (1000)**
2022	0.002	0.001	<0.001
2030	0.003	0.002	<0.001
2040	0.005	0.002	<0.001

Given the conversion of toxicity thresholds from values found on mice to values used for beavers, an additional 10x multiplier is applied to the RQ values to account for uncertainty. This is standard practice for an interspecies extrapolation done in a risk assessment (Table 5).

Table 5: Risk quotient values calculated as above, but with an additional 10x multiplier to account for uncertainty in interspecies extrapolation.

Year	Risk Quotient Values with 10x Interspecies Uncertainty Applied		
	NOAEL	LOAEL	LD50
2022	0.02	0.01	<0.01
2030	0.03	0.02	<0.01
2040	0.05	0.03	<0.01

The RQ values for the NOAEL are 0.02 to 0.05, and for the LOAEL are 0.01 to 0.03 for the years 2022 to 2040. The RQ values show that estimated exposures for 2022 are 2% of the NOAEL, 1% of the LOAEL, and negligible for the LD_{50}. By 2030's projected exposure values, the RQ values suggest exposure will only increase to 3% of the NOAEL and 2% of the LOAEL. Last, for 2040's projected exposure values, the RQ values show us that the exposure will reach 5% of the LOAEL, 3% of the LOAEL, and still negligible for the LD50. All RQ values I calculated for this paper are below the EPA's lowest value of concern and demonstrate the beavers of Isle Royale will likely be at negligible risk for adverse health effects by toxic exposure to microcystin.

DISCUSSION

Uncertainties and Limitations

Toxic effects are inconsistent between studies, and no study has been done directly on North American beavers with microcystin. Since threshold values are determined with many different exposure methods, dosage amounts, and other variable circumstances, many studies cited different thresholds that the mice experienced adverse effects. Those studies were either less comprehensive or not widely cited by health officials, which is why I did not include them in this paper. Because this paper considered thresholds from studies on mice, there is a 10-fold interspecies uncertainty factor applied to the RQ values (Table 5). The 10-fold factor is applied for safety purposes; as conversions between species is not always exact, it's better to have more caution in a risk assessment than too little.

Samples from the inland lakes of Isle Royale were often taken from the areas of the highest recreation traffic. Those shoreline areas would give the surveyors the best idea of risk to people who visited the area or used the lake (Fuller et al., 2017). However, this is not a representation of the entire lake's content, and presumably wildlife in the area would stay farther from the highest trafficked area. The reason these data were useful is that the island is also quite remote even during its busiest tourist season, and the wildlife there have far less human disruption than almost anywhere else. Samples were also taken from at least three locations in each lake to average and account for variability (Fuller et al., 2017). Additional uncertainty arises from the knowledge that microcystin can be present in the water without a visible bloom, but most studies only sample when an algal bloom is visible.

All data collected from the lake temperatures, toxin concentrations, and beaver water consumption are subject to seasonal changes and disturbances. Given the cyclic nature of temperature and the cyanobacteria's relatively small temperature tolerance, there does not exist an opportunity for beavers this far north to experience chronic exposure. They will always have a seasonal break from the algae in the winter. That said, it is still possible to calculate what would be considered a chronic exposure value for them if you consider the value they are exposed to in the algal bloom season divided over the year. I could not find any studies done on toxic exposure that simulated such a situation. Further studies are needed to determine if the toxic effects on an organism are minimized with seasonal relief.

To extrapolate for the toxin concentration that may be present this current year and in future years, I used a linear growth model. The trends shown over long periods of time in nearby Lake Erie suggest a linear growth, and that is the nearest lake with extensive HAB and toxin records. However, deviation values are very high, and it is likely that any given year will experience large changes in values from what is predicted. This also only measured the largest bloom of the year, while all year the algal blooms will vary in size. Using data from the largest bloom was my choice as it is highly conservative for determining risk from the toxin when the amount of toxin is at the highest.

Lastly, this study does not account for bioaccumulation in the aquatic plants with successive blooms in one area. More data is required to determine if the toxin concentration dissipates or grows with each bloom of the season.

Recommended Action

With the RQ values I have calculated for this risk assessment, there is negligible risk and therefore no need for any immediate action. As climate and algal bloom numbers both trend linearly over large periods of time but have occasional major deviations, it is important to continue to test for toxins when large algal blooms are visible. As Isle Royale is often host to scientists and study groups, a "citizen science" program can be implemented that asks for the guests of the island to run these water tests. That would provide the island with assistance from scientists for free as they are already present on the island for research purposes. If the bloom is found to have more than 40 µg/L of toxin, I recommend attempted remediation. Skimming and algicides have shown to be ineffective for removing toxins, even though the water will have visibly less algae. One possible remediation method that has been tested is the use of ultrasonic waves to disrupt cyanobacterial cells (D'Anglada and Strong, 2015). The treatment of the water uses no chemicals and has been able to degrade microcystin-LR in the area local to the sonar equipment. So far, this method is best used for small areas, such as the lakes of Isle Royale, so it would be worth exploring. That said, Isle Royale is known for being unaltered by humans unless deemed necessary for species survival, so given the current projections I have determined in this risk assessment, no action is required.

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