Public Health Risk Associated with Recreational Exposure to the Algal Toxin Microcystin in Western Washington Lakes

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Introduction:

Freshwater cyanobacteria are ubiquitous gram-negative prokaryotes inhabiting aquatic systems around the world (Codd, Morrison, and Metcalf 2005). Cyanobacteria are a diverse group, with some classification systems estimating 2,000 different species. Also known as blue-green algae, cyanobacteria lack a membrane bound nucleus and reproduce asexually. Like true algae, the majority of species are autotrophic, contain chlorophyll-a and photosynthesize (Zurawell et al. 2005). Cyanobacteria often dominate the phytoplankton community in freshwater environments during warm months due to their ability to tolerate wide ranges in salinity and turbidity (Funari and Testai 2008). This seasonal dominance has led to an increase in discoloration of freshwater bodies due to suspended cells and surface scums known as cyanobacterial blooms. Species comprising cyanobacterial blooms may produce a variety of bioactive compounds, including toxins that may present a health risk to humans through exposure by way of contaminated drinking water or contact during recreational activities. A toxin producing bloom is known as a harmful algal bloom (HAB). Blooms of cyanobacteria occur worldwide causing a range of ecological, economic and quality of life issues (Sinclair et al. 2008). The presence of cyanobacterial blooms can affect the value of recreational waters and may reduce recreational uses of water bodies (Oliver and Ganf 2000).

In Washington State, the number of harmful algal blooms in lakes has increased over the past 25 years (Hardy 2008). These blooms are comprised of species capable of toxin production, and concentrations above state recreational guidance values for certain toxins have been observed (SWM 2010). Both human and animal illnesses associated with water contact during HABs have been reported in Washington (Hardy 2008). At present, the extent of bloom impacts on recreational activity in western Washington is not well defined. Indeed, gaps exist in assessing the risk of exposure to blooms of cyanobacteria in human populations (Funari and Testai 2008). In order to assess associated risks to human health with increased bloom activity in Washington lakes, knowledge is needed regarding patterns of exposure as they relate to the presence of algal blooms.

Cyanobacterial Blooms

The proliferation of cyanobacteria into bloom stage occurs under certain environmental conditions. Algae blooms may appear as green mats and sheens on the water surface (surface bloom) or large specks distributed throughout the water column (Chorus and Bartram 1999). Surface blooms are largely the result of cyanobacterial species with the ability to control buoyancy. Many species of cyanobacteria possess gas vacuoles which have a hydrophobic inner surface and a hydrophilic outer surface (Chorus and Bartram 1999). Cyanobacteria use vacuoles to control their buoyancy in water allowing them to position at depths most favorable for growth. Surface bloom severity is dependent on cyanobacterial growth rates as well as the pre-existing concentration of cells within the water column. Surface blooms form from the upward movement of existing cyanobacteria from lower in the water column (Oliver and Ganf 2000). Once at the surface, algal bloom distribution may not be uniform across the lake. Surface bloom location may be influenced by wind, concentrating blooms along shores or within coves (WHO 2003).

A variety of environmental factors contribute to the growth of HAB's including light availability and intensity, nutrient content and temperature. Maximum growth rates vary by environmental conditions and phytoplankton species. Cyanobacteria have the ability to harvest light between 500 and 650 nanometers (green-yellow-orange) due to the additional pigments of allophycocyanin, phycocyanin and phycoerythrine. These additional pigments give cyanobacteria an advantage over many other phytoplankton species (Cohen-Bazire and Bryant

1982). Eutrophic conditions, specifically the ratio of phosphorous to nitrogen are known to be advantageous to cyanobacterial growth. Total nitrogen to phosphorous ratios of less than 29 have been observed to be most favorable to cyanobacteria (Smith 1983; Jeppesen et al. 2005). Seasonal variability in growth rates is due in part to temperature variations. Maximum growth rates for diatoms and green algae occur at lower temperatures than for cyanobacteria (Chorus and Bartram 1999). Maximum growth rates for bloom-forming cyanobacteria have been observed at temperatures exceeding 25 degrees Celsius (Roberts and Zohary 1984).

Products of Harmful Algal Blooms

When environmental conditions favor bloom formation, cyanobacteria can produce taste and odor issues, clog filtration systems and contribute to fish kills due to depleted dissolved oxygen and release of ammonia by decaying cells (Zurawell et al. 2005). Acute toxicity is not associated with the compounds responsible for aesthetic issues including the most common, geosmin and 2-methylisoborneol (MIB) (Sinclair et al. 2008). However, several species of cyanobacteria also produce a range of toxins as secondary metabolites (Funari and Testai 2008). These natural toxins have a variety of adverse health endpoints and have been observed to be more harmful to mammals than to aquatic biota (Chorus and Bartram 1999). Diversity exists among the toxin producing species, and several species may produce more than one toxin (Table 1).

Table 1. Toxin producing genera of cyanobacteria (adapted from Chorus and Bartram, 1999)

Genera	Toxins	Target organ in mammals
Microcystis, Anabaena, Planktothrix, Nostoc	Microcystins	Liver
Nodularia	Nodularin	Liver
Anabaena, Planktothrix, Aphanizomenon	Anatoxins	Nerve synapse
Cylindrospermopsis, Aphanizomenon	Cylindrospermopsins	Liver
Anabaena, Aphanizomenon, Cylindrospermopsis	Saxitoxins	Nerve axons
All genera	Lipopolysaccharides	Irritant affecting all exposed tissue

Cyanobacterial toxins can be categorized by their health endpoint in terrestrial mammals; lipopolysaccharides, cytotoxins, neurotoxins and hepatotoxins are among the groups of greatest concern. Lipopolysaccharides (LPS) are a component of cell walls and are common in all species of cyanobacteria. They may produce skin irritation and gastrointestinal illness if ingested (Codd, Morrison, and Metcalf 2005). When inhaled, LPS are known to initiate inflammatory response and are increasingly associated with respiratory illnesses including asthma and chronic obstructive pulmonary disorder (COPD) (Stewart, Schluter, and Shaw 2006). In aquatic environments, aerosolized LPS endotoxins can become concentrated to levels that induce illness (Rose et al. 1998). Cytotoxins include cylindrospermopsin, which inhibits protein synthesis and contributes to necrotic damage in various mammalian organs. Neurotoxins include anatoxin-a and the group of saxitoxins. While saxitoxins are responsible for paralytic shellfish poisoning in humans which is largely associated with ingestion of marine shellfish, saxitoxins are becoming increasingly detected in freshwater systems (Humpage 2008). Hepatotoxins include microcystins (MC) and nodularins, known to promote tumor growth as well as acute liver damage (Codd, Morrison, and Metcalf 2005).

The current project focuses on health risks associated with recreational exposure to the hepatotoxin group of microcystins. Microcystins are the most widely studied group of cyanotoxins and the most common toxins found in freshwater algal blooms (McElhiney and

Lawton 2005). Microcystins are cyclic heptapeptides and include over 80 structural variants. All toxic variants of MC contain a hydrophobic amino acid unique to cyanobacteria known as Adda. Adda plays a role in the inhibition of protein phosphatases 1 (PP1) and 2A (PP2A). Protein phosphatase inhibition is the primary mode of toxicity for MC (Humpage 2008). Variants of MC occur by changing two amino acids at positions 2 and 4 or changes in other small side groups (Humpage 2008). The most common variant is MC-LR, containing leucine and arginine at positions 2 and 4. MC-LR is considered one of the most acutely toxic and thus one of the most studied structural variants (Funari and Testai 2008).

Health Endpoints

The mammalian liver is the target organ for MC. As a result, toxicity studies have focused on hepatic injury and mortality as endpoints. High levels of exposure to MC may result in acute intoxication. A series of events including lipid peroxidation, oxidative stress and apoptosis leads to centrolobular toxicity and intrahepatic hemorrhage. Mortality is the result of hypovolemic shock associated with massive hemorrhaging of the liver (Dawson 1998). An event in Brazil caused the greatest known human mortality from MC exposure. Known as "Caruaru syndrome," 76 dialysis patients died after contamination of hemodialysis fluid by the toxin (Azevedo et al. 2002). Persistent low levels of exposure lead to inhibition of phosphatases resulting in hepatic hypertrophy and histopathological evidence of liver injury (Gehringer 2004). Long-term chronic exposures have also been associated with the development of human liver cancer. In China, epidemiological surveys identified the mortality rate from hepatocellular carcinoma for people who drank surface water from ponds or ditches as 100 deaths per 100,000 people versus 20 deaths per 100,000 for people who accessed drinking water from deep wells in two Chinese villages. Upon switching drinking water sources from surface to ground, mortality rates were reduced to levels consistent with control areas (Yu 1995). Surface drinking water sources for one of the villages were monitored between 1992 and 1994. Microcystin concentrations were detected in the surface water suggesting a correlation between microcystin and the high mortality rate from hepatocellular carcinoma in the region (Harada et al. 1996; Yu 1995).

Exposure to cyanobacterial blooms through recreational activities such as swimming has been associated with illness in humans including short-term gastrointestinal distress, respiratory symptoms, fever, vertigo, headache and long-term effects such as liver damage (Codd, Morrison, and Metcalf 2005; Stewart, Schluter, and Shaw 2006; Turner et al. 1990). Deaths of domestic animals including livestock have been the result of hepatotoxicity after exposure to cyanobacterial blooms in Argentina and Australia, including confirmed ingestion of microcystin in Norway and England (Chorus and Bartram 1999). Lipopolysaccharide endotoxins may be responsible for the short-term acute effects described above, but these effects may also be due to a combination of toxic agents. The health effects of MC from recreational exposure have not been well investigated (Funari and Testai 2008).

Toxicity studies have exposed mice and rats to MC via oral gavage and intraperitoneal (ip) injection. A 30 to 167 fold difference in toxicity between these two methods has been observed (Fawell et al. 1999; Yoshida et al. 1997). A sub-chronic 13-week oral toxicity test of MC in mice established a no observed adverse effect level (NOAEL) of 40 µg/kg day^{-1} with liver tissue damage as an endpoint. A tolerable daily intake (TDI) was estimated based on the NOAEL at 0.04 µg/kg/ day^{-1} incorporating uncertainty factors of 10 each for intraspecific species variations, interspecific variations and a less than lifetime test (Fawell et al. 1999).

Less data is available on acute no-effect doses administered orally. Fawell et al. (1999) also conducted acute exposure experiments and observed diffuse hemorrhage in the liver of mice orally exposed to the lowest administered dose of 500 µg/kg. A NOAEL was not derived from this study because effects were observed at the lowest dose. However, Funari and Testai (2008) proposed a derivation of an acute no-effect dose based on a NOAEL observed with mice acutely exposed to MC through intraperitoneal injection. Several studies have reported doses in mice between 25 and 50 µg/kg that did not induce liver damage (Fromme et al. 2000). The approach taken by Funari and Testai (2008) adjusts the doses from ip injection based on a 30-100 fold difference in toxicity by exposure route reported by Fawell et al. 1999. Incorporating this difference by applying a correction factor of 10 to the acute NOAEL of 25 µg/kg yields a conservative estimate of an oral acute no-effect dose of 2.5 µg/kg. Multiplying the acute NOAEL by body weight and the correction factor and applying uncertainty factors of 100 yields an acute oral no-effect dose of 150 µg/person for a 60 kg adult (37.5 µg for a 15 kg child). In the absence of a NOAEL derived from oral administration studies, this technique allows for an estimation of an acute oral no-effect dose (Funari and Testai 2008). An additional strategy of applying uncertainty factors to the oral LOAEL of 500 µg/kg produces an estimated acute oral no-effect dose of 0.05 µg/kg based on 10 fold reductions for intraspecies variability, interspecies variability and an adjustment for using a LOAEL instead of a NOAEL.

Routes of Exposure

Non-recreational exposure to cyanotoxins may occur through ingestion of contaminated drinking water supplies, contaminated water used for medical purposes such as haemodialysis and ingestion of dietary supplements containing concentrated cyanobacteria cells (Codd, Morrison, and Metcalf 2005). The current study is concerned with recreational exposure to microcystin which may occur through direct skin contact from swimming or bathing, inhalation of aerosolized cells, ingestion of contaminated fish tissue by anglers and incidental ingestion of water while swimming. Cyanobacterial cell counts in recreational water and duration of exposures have been correlated with skin irritation through direct contact (Pilotto et al. 1997). However, skin irritation was not correlated with the presence of microcystin. It is likely that skin irritation is due to other compounds present in cyanobacterial cells released when the cells are disrupted during swimming or from being caught within or between layers of a bather's swimsuit and their skin (Chorus et al. 2000).

Measurable levels of microcystin have been found in personal air samples (up to 2.89 ng/m^3) of individuals recreating in lakes experiencing ongoing algal blooms with microcystin concentrations ranging from 10 to 500 µg/L in the water. However, microcystin was below the detection limit (1 µg/L) in blood plasma (Backer et al. 2010). A sample calculation using the mean value of 0.4 ng/m^3 observed by Backer et al. (2010), an assumed respiratory rate during high activity of 0.039 m^3/minute, event duration of 120 minutes and a child body weight of 15 kg yields a potential dose of 0.0012 µg/kg. Low concentrations observed in blood and a low estimated dose received through inhalation suggests a less important exposure route than ingestion.

Toxins may remain in edible tissues of fish after a bloom has dissipated, presenting potential exposure when there is no visible evidence of a bloom (Codd, Morrison, and Metcalf 2005). Several studies have identified MC accumulation in the organs of freshwater fish both experimentally and naturally exposed (0.11 µg/g and 0.13 µg/g in muscle tissue respectively) (Cazenave et al. 2005; Deblois et al. 2008; Ame et al. 2010). Efforts to overcome difficulties in extraction and analysis of MC in a tissue matrix are ongoing. Microcystin accumulation varies depending on the organ with the liver showing highest accumulation, while edible muscle tissue accumulates lower amounts (Martins and Vasconcelos 2009). Although fish meat is typically cooked prior to consumption, the cooking process is not expected to reduce the risk of exposure to MC (Poste, Hecky, and Guildford 2011). A sample calculation using the mean value of 0.13 µg/g MC observed in muscle tissue for fish naturally exposed, a mean consumption rate of 7 g/day for children in Washington State and a child body weight of 15 kg yields a potential dose of 0.06 µg/kg. Based on a low estimated dose, exposure to MC through ingestion of fish is assumed to be of less importance than water ingestion.

Information regarding the absorption of microcystin through the skin is lacking. The likelihood of exposure through absorption has been regarded as low by prior investigations. The large molecular weight and high aqueous solubility of the molecule (Lawton et al. 1995; Rivasseau, Martins, and Hennion 1998) do suggest a low probability of rapid uptake through the skin. However, a United States Army study, which has apparently never been published in peer-reviewed form, investigated in vitro absorption of MC from 50,000 µg/L aqueous solution through human skin and found uptake of up to 0.09 $\mu g/cm^2$ (Kemppainen et al. 1990). While the study methodology was imperfect, whole body flux at that rate for two hours could lead to an internal dose exceeding 80 µg/kg in a child. Assuming proportionality, water concentrations could be 2000 times lower (i.e., 25 µg/L) and still deliver a dose of 0.04 µg/kg/two hr. event. Further research regarding dermal absorption of MC is therefore warranted.

Incidental ingestion of water occurs during recreational swimming (Dufour et al. 2006). Swimming presents a risk when recreational waters contain microcystin producing species of cyanobacteria. In the majority of documented cases of cyanobacteria causing adverse human health effects, ingestion of microcystin via contaminated drinking water was among the most common routes of exposure (Chorus et al. 2000). Human epidemiological studies have provided evidence for adverse effects of chronic ingestion (Harada et al. 1996; Yu 1995). Documented cases of acute exposures to microcystin via ingestion have led to animal poisonings, and a case in 1989 provides evidence of human illness after acute exposure. British army recruits became severely ill after swimming in a lake containing a bloom of *Microcystis* spp. Ingestion of a *Microcystis* toxin was noted with severity of illness seemingly related to total amount of water ingestion (Chorus et al. 2000).

In the absence of well established grounds for assumption of non-negligible dermal absorption, the limited exposure data for MC through inhalation, along with the evidence of ingestion causing acute and chronic adverse health effects, dose estimates presented here are limited to oral (ingestion) exposure.

Detection of Microcystin in Water Samples

Algal toxins may be present in environmental water samples as free toxins dissolved in water or cellular toxins found within intact cyanobacterial cells. Toxins remain largely within intact organisms during blooms and are released into water when the cells break down (Codd, Morrison, and Metcalf 2005). Total toxin concentrations are reported by extracting cellular bound toxins and combining with the free toxins dissolved in water. In order to determine the highest toxin concentrations, sampling is often focused on areas where cells congregate in dense green mats along the shores of lakes and reservoirs to ensure protection of public health.

Sampling from the thickest part of the scum layer where toxin concentrations are highest allows for a conservative risk estimate. A variety of techniques are used to quantify microcystin concentrations from water samples. A technique that is able to identify the greatest number of structural variants should be used in risk assessment in order to produce the most accurate and conservative estimate of toxin concentration. The techniques described below offer varying degrees of quantification; consistent techniques should be used when comparing concentrations.

A key part in the detection process is the toxin extraction from a complex environmental matrix. Extraction methods include acetic acid, methanol, acidified methanol and a mixture of butanol, methanol and water. Aqueous methanol (50-80%) is the most widely recommended extraction method due to its ability to extract MC variants that have a wide-range of polarities (McElhiney and Lawton 2005). Trace amounts of MC are difficult to detect from water samples, requiring sample concentration and clean-up. Pre-concentration methods such as solid phase extraction and immunoaffinity purification are often used to eliminate co-eluting compounds (McElhiney and Lawton 2005; Kondo, Harada, and Ueno 2005). Additionally, quantifying MC using commercially available purified versions of the toxin has produced a great deal of variability, indicating an inaccuracy in the toxin amounts found in the commercial products (McElhiney and Lawton 2005). This uncertainty, along with the small number of known variants for which standards exist, makes the formulation of calibration curves problematic.

High performance liquid chromatography (HPLC) is a widely used method to determine cellular MC concentrations from water samples. Briefly, water samples are filtered using a 0.45 micron filter and the cell mass lysed by freezing. Cells are then extracted using acetic acid, sonicated and centrifuged. The resulting supernatants are applied to methanol conditioned solid phase extraction cartridges, eluted using pure methanol, evaporated and re-suspended in methanol before HPLC analysis (Ame et al. 2010; Cazenave et al. 2005; Amé, del Pilar Díaz, and Wunderlin 2003).

Determining MC concentrations in water samples can be done using a variety of assay techniques. Protein phosphatase inhibition assays exploit protein phosphatase inhibition by MC and have been developed using both PP1 and PP2A (Deblois et al. 2008). Immunoassays such as ELISA require less sample preparation than the analytical techniques. They are also useful if an indication of MC toxicity is all that is required. Sensitivity among variants may differ greatly with ELISA and the strength of this method is dependent on all variants present in a sample being recognized by the antibodies. Since over 80 MC variants exist, this makes estimating the true toxicity from a sample difficult.

Many commercially available ELISA kits show poor cross-reactivity against variants because they use antibodies that have been raised against a specific variant (McElhiney and Lawton 2005). Toxicity is therefore reported in terms of MC-LR equivalence, similar to analytical techniques. As a result, antibodies have been developed based on the Adda amino acid which is found in the majority of MC variants (Fischer et al. 2001). Using antibodies raised against Adda allows for greater detection of a range of MC variants. Commercial ELISA kits are now available using Adda antibodies and are increasingly used for the detection of MC in water samples (Wood et al. 2006; Papadimitriou et al. 2010).

Guidance Values

The World Health Organization (WHO) has established provisional guideline values (GV) for total MC based on the toxicity of the MC-LR variant. A tolerable daily intake (TDI) corresponds to a daily lifetime exposure level that is believed would not result in adverse health effects. The TDI for microcystin, calculated at 0.04 µg/kg day^{-1}, is based on a no observed adverse effect level (NOAEL) of 40 µg/kg day^{-1} oral toxicity in mice. Intraspecific, interspecific and less than lifetime uncertainty factor values of 10 each are applied. The WHO provisional drinking water value has then been calculated at 1 µg/L for a 60 kg human adult with an assumed drinking water consumption of 2 liters per day and an allocation factor of 0.8 to account for oral exposure through media other than drinking water (Chorus and Bartram 1999).

Tiered recreational guideline levels have been established by the WHO based on cyanobacterial cell counts. Levels 1 and 2 correspond to 20,000 and 100,000 cells per milliliter and level 3 corresponds to a scum presence on the water surface. The WHO recommends immediate action to control scum contact at level 3 (Table 2) (Chorus and Bartram 1999). The United States government has no established guideline values for MC in drinking water or recreational water; however several states have adopted their own provisional guideline values. Washington State has set a recreational guidance value for the protection of children in recreational waters at 6 µg/L (Hardy 2008). The guidance value is based on the TDI of 0.04 µg/kg day^{-1} derived by the WHO, a body weight of 15 kg for a child, an assumed incidental ingestion rate of water while swimming of 0.05 L/hour and an average swimming duration of 2 hours per day.

Table 2. Guidance values

Recreational Risk	Cyanobacteria (cells/ml)	MC concentration
Low	20,000	4 µg/L
WA GV[a]		6 µg/L
Moderate	100,000	20 µg/L
High	Visible Scums	> 20 µg/L

[a] Washington State recreational guidance value

The derivation of a reference dose in risk assessment incorporates uncertainty factors to account for unknown differences in sensitivity between humans and test animals (Faustman and Omenn 2008).This technique is employed in the derivation of the TDI for microcystin by the WHO. To provide a conservative recommendation of a safe intake level, it is assumed that humans are more sensitive to a compound than test animals.

Risk Assessment and Management

An unclear view of all the potential health hazards and varying degrees of technical expertise associated with cyanobacteria blooms led to a slow development of risk management strategies (Codd, Morrison, and Metcalf 2005). Different approaches have been proposed focusing on the prevention of blooms at the earliest point possible. One strategy outlined by Codd et al. (2005) suggests the following steps; identification of problem waterbodies, historic issues and management plans; determine priorities based on available resources and potential health impacts; identify control points including short-term and long-term measures to mitigate cyanobacterial blooms; determine economic feasibility of control measures; determine environmental and economic effects of control measures; select control options and incorporate into a structured action plan; implement action plan; monitor the effectiveness of the action plan and revise plan if necessary. This structure is based on the Hazard Assessment Critical Control Point (HACCP) system which has been successfully applied to the food industry for many years.

The HACCP system requires a thorough understanding of all risks in order to identify and monitor critical control points most effectively (USFDA 1997). An approach which integrates both ecological and human health assessments to determine collective risks associated with blooms of cyanobacteria has been proposed. Human activity and environmental factors contributing to cyanobacteria biomass in freshwater systems is first assessed. The effects of cyanobacteria biomass on ecological systems, aquatic biota, wildlife, human health and socioeconomic considerations are then measured (Orme-Zavaleta and Munns Jr. 2008). Human well being may be affected by direct exposure to toxins and ingestion of wild caught fish, reduction in recreational activity, perception of poor water quality, reduced land values, increased water treatment costs and loss of intrinsic value of natural systems. Thus, the overall risk to public health and well being integrates both ecological and human health paradigms.

Assessing exposure associated with recreational activities is one piece of the integrated approach. The potential dose of MC associated with recreational exposure has been examined by the World Health Organization (WHO 2003). Exposure is evaluated based on toxin concentrations corresponding to cyanobacterial cell densities (Table 2). Average intake rates are applied to determine dose per event. Doses are compared to the established TDI (0.04 μg/kg) for microcystin and derived acute no-effect doses (WHO 2003). Deterministic assumptions regarding event durations and body weight are made in the absence of data specific to a population. Information specific to residents in western Washington are needed to estimate doses received and the risk of adverse health effects from exposure to MC through water ingestion.

Microcystins have been observed in certain Washington lakes at concentrations that would lead to doses above the TDI derived by the WHO (SWM 2010). The duration of daily swimming events used in WHO assessments may not apply to populations in Washington due to differences in water and air temperatures, population densities, and differences in recreational patterns. The current project focused on assessing the risk associated with exposure to microcystin through recreational swimming in western Washington lakes.

Doses of microcystin being ingested by users of Washington lakes has not been investigated, nor has the probability of acute health effects occurring associated with these doses. The effect algal issue awareness has on recreational behavior and the subsequent risk associated with event duration has not been established for bodies of freshwater in Washington. This risk assessment addresses exposure based on actual recreational use data from the region combined with the impact of awareness of toxicity problems using the following two steps; first, an exposure questionnaire was administered to assess the frequency and duration of swimming and perceptions and awareness of algae issues among individuals living along western Washington lakes. Second, a two-dimensional Monte Carlo based model was developed with the goals of modeling doses received for comparison with established guidance levels and the probability of receiving a dose of microcystin leading to hepatic injury.

Monte Carlo Modeling

A two-dimensional analysis allows for the characterization of both uncertainty and variability within each variable using nested loops. The inner loop simulated variability by sampling from a defined distribution. The outer loop simulated uncertainty. The outer loop generates new values for each parameter. The inner loop then samples from the new set of values generated by the outer loop, and the process is repeated. Variability and uncertainty are assigned to assumption cells for which a distribution is defined. Assumption cells select a value based on a data set's defined distribution and the associated input parameters (mean, standard deviation, etc.). Model simulations are run for a single cell defined as a forecast. A forecast cell holds an equation that may include values from standard cells or assumption cells. The value of the forecast cell changes with each step in the simulation based on these inputs.

Methods

Exposure Questionnaire Methods

The questionnaire was approved by the Human Subjects Division of the University of Washington. The approval letter is included in Appendix A.

Purpose

A questionnaire was designed to assess perceptions lakeshore residents had about water quality and their reported recreational behavior in the lakes. Information was collected regarding event frequency and duration at the individual level over a four month period from June-September, 2011. For each household, information was collected regarding concerns over water quality and algae issues, how knowledge of water quality and algae issues was obtained, and whether concerns affected recreational behavior.

Information on four water based recreational activities (swimming, fishing, boating and wading/playing) was collected. Swimming represents full immersion, wading/playing represents partial immersion, boating may refer to human powered or motor boats, and fishing may be from a boat, dock or shore.

Differences in activity level based on age, gender and awareness level were investigated. To assess the difference in behavior between adults and children and account for the influence an adult may have on a child living in the same household, swimming frequency and duration data were compared by matching each child with the first adult listed on their household questionnaire.

Populations

The mailer targeted residents living along 11 Washington lakes, eight in Snohomish County and three in King County. Five of the lakes, Lost, Loma, Ketchum, Martha North and Wilderness, were routinely monitored during the summer of 2011 for the presence of algal toxins through a Centers for Disease Control and Prevention (CDC) cooperative agreement to the Washington Department of Health (DoH) that funded a project known as the Regional Examination of Harmful Algae Blooms (REHAB) (Cullen et al. 2009). Additional monitoring was also performed at these lakes when algae blooms were present during the non-routine REHAB monitoring weeks. Non-routine sampling was funded through The Washington State Department of Ecology (Sweeney, Cullen, and Hardy 2010). Bloom based monitoring was also conducted at Steel Lake and Lake Howard. Lake Cochran, Crabapple, Serene and Pine have reported good water quality in the past and were not tested for algal toxins.

The four control lakes have similar characteristics to the lakes that were tested for algal toxins. The potential for algal blooms exists in these lakes though routine monitoring for algal toxins was not performed. All lakes were selected based on the well developed shorelines of residential properties, small surface area and relatively shallow depth (Table 3).

Table 3. Lake characteristics

	Cochran	Crabapple	Loma	Lost	Howard	Ketchum	Martha North	Serene	Pine	Steel	Wilderness
Area (acres)	33	37	23	13	28	25.5	63	45	88	46	67
Avg. depth (m)	7.9	5.5	3.4	7	8.8	3.7	10.1	5.3	6.1	--	6.4
Max depth (m)	16.5	14.9	8.5	13.7	15.2	6.4	21.3	6.7	11.9	7.3	11.6
Docks (#)	--	49	52	43	35	--	79	80	--	--	--

Mailer

1000 mailers were sent to residents living within one property lot of the 11 selected lakes. Property lots and addresses were identified using the Geographic Information System (GIS) software ArcGIS 10 by ESRI. Parcel and hydrology data sets were obtained from King and Snohomish Counties. The parcel data included attribute tables holding information including parcel addresses. Properties within one lot of the 11 lakes were selected and a new data layer was generated based on the selection. The street address, state and zip code columns were extracted from the attribute table.

The mailer was comprised of three pieces: a cover letter, the questionnaire, and an addressed return envelope with postage paid. The complete letters were mailed during the third week of September, 2011. The cover letter described the purpose of the survey and provided information on how to obtain more information about harmful algal blooms in Washington (Appendix B). The questionnaire was informed by prior recreational use surveys conducted in Washington (Costello et al. 2009; Parametrix 2003) (Appendix C). The length of the survey was kept to one page front and back. Financial considerations inhibited the ability to send additional pre-survey and post-survey notifications and follow up.

One mailer was sent per household. Each questionnaire allowed for responses by up to five individuals within each household. Data regarding pets and pet activity, and temporal distribution of recreational activities were collected at the household level.

Questionnaire data analysis

Information from the returned questionnaires was entered into Microsoft Excel. Data was assessed based on individual lake, gender, age and reported algae awareness. Descriptive statistical tables were generated using Excel, while graphs and plots were generated using STATISTICA 10 Academic version. Only behavior of individuals participating in activities that would lead to exposure was included in the summary statistics.

Model Methods

The two-dimensional Monte Carlo model was created in Oracle Crystal Ball which operates using the Microsoft Excel interface. An Excel visual basic macro was used to sort data generated by Crystal Ball and to plot probabilities for risk estimation using tolerance bounds.

Exposure Assessment

Children had higher event durations and intake rates than adults. In addition, children are considered as a high risk population due to their relative risk based on size and body weight. Model scenarios were run for children under age 18 as they are more likely to receive a higher dose than adults. Microcystin concentrations from June through September in Lake Ketchum along with concentrations from September in Lake Cassidy were selected as inputs to the model.

Event duration

Reported event durations for each individual were designated as assumption cells using a custom distribution. The custom distributions for event duration used the reported hours per day for all individuals reporting swimming (column A, Table 4). Assumption cells were then assigned to the months June through September based on whether an individual reported swimming in a given month. A mean and standard deviation were calculated after each step in the simulation based on the assumption cell selections. A final assumption cell for event duration was assigned a lognormal distribution with the mean and standard deviation from the step above as inputs (Table 4). Cells in column B were designated to the uncertainty loop. Cell A in column C was designated to the variability loop.

Table 4. Example of event duration calculation for a given month

A	B	C	
0.5	Custom (A1:A6)	**Mean**	=AVERAGE(B1:Bn)
0.5	Custom (A1:A6)	**SD**	=STDEV.S(B1:Bn)
2	Custom (A1:A6)		
1	Custom (A1:A6)	**June hours/event**	
1	Custom (A1:A6)	Lognormal (Mean,SD) **Cell A**	
n	Custom (A1:An)		

SD, Standard Deviation

Intake rate

Values for incidental water ingestion while swimming for children were obtained from a study by Dufour et al., (2006). Children are defined as being under the age of 18. Values were calculated based on 45 minute swimming events with a mean ingestion rates of 37 mL/event (49 mL/hour) for children. The upper percentile (97^{th}) value was 120 mL/hour. An assumption cell was created for ingestion rate. The assumption cell was given a lognormal distribution with input parameters of the mean and 97^{th} percentile ingestion rates per hour (Figure 1). Cell B was designated to the variability loop.

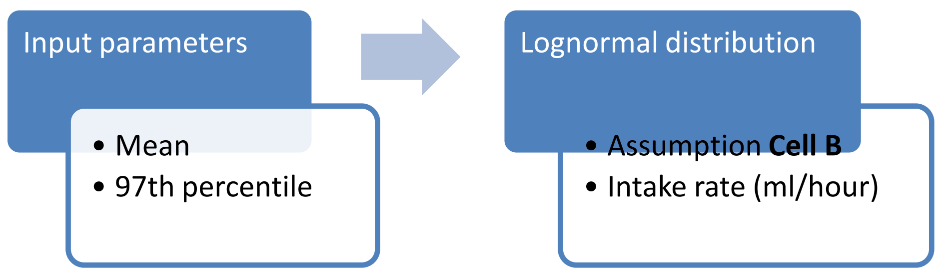

Figure 1. Ingestion rate

Microcystin concentrations

Concentrations of microcystin in recreational waters were obtained through the Washington State Department of Ecology's Toxic Algae Monitoring Program. The Ecology program responds to bloom events throughout Washington, with specific locations varying from year to year depending on bloom activity (Sweeney, Cullen, and Hardy 2010). Microcystin analysis was conducted by King County (WA) Environmental Laboratory using Enzyme-Linked Immunosorbent Assay (ELISA) method (KCEL 2005).

Microcystin concentrations from specific lakes were selected as inputs for the model. Concentrations from Lake Ketchum and Lake Cassidy were used. Lake Ketchum represents a lake with consistent bloom activity and Lake Cassidy represents the highest concentrations of microcystin monitored during 2011. Concentrations were grouped by month, and each cell with a concentration value was designated as an assumption cell using a lognormal distribution with mean and standard deviation parameters generated from the original group of monthly concentration values (Column B, Table 2). A final assumption cell for microcystin concentration (Cell C) was assigned a lognormal distribution with the mean and standard deviation from the step above as inputs (Table 5). Cells in column B were designated to the uncertainty loop. Cell C was designated to the variability loop.

Table 5. Example of toxin concentration calculation for a given month

A	B	C
Raw Values	**June Toxins**	**June µg/L**
CDC/ECY	Lognormal (A2:A6)	Lognormal (Mean, SD) **Cell C**
CDC/ECY	Lognormal (A2:A6)	**Mean**
CDC/ECY	Lognormal (A2:A6)	=AVERAGE(B2:B6)
CDC/ECY	Lognormal (A2:A6)	**SD**
CDC/ECY	Lognormal (A2:A6)	=STDEV.S(B2:B6)

CDC, Centers for Disease Control funded monitoring
ECY, Washington State Department of Ecology funded monitoring
SD, Standard Deviation

Body weight

Event duration data was collected for two age groups, under 18 and 18 years and older. The Washington State Recreational Guidance value for microcystin uses a standard body weight for children of 15 kg (Hardy 2008). This body weight roughly corresponds to the age of 2 years old (USEPA 2011). For the model, body weights for children between 2 and 17 years old were obtained from the Exposure Factors Handbook of the United States Environmental Protection Agency. The number of individuals in Snohomish County by age was obtained through the State of Washington Office of Financial Management which provided a summary of the 2010 census data for the county (WAOFM 2010).

Assumption cells for body weights by age range were given normal distributions based on the mean and 95th percentile for each age group as input parameters (Column C, Table 6).

Each age range was then assigned a probability that a child falls within the range by summing the number of individuals within the range and dividing by the total number of individuals between ages 2 and 17 (column F, Table 6).

A rank was assigned to each age range and correlated with the probability calculations (column E, Table 6). A selection cell was assigned a custom distribution to select a rank based on the probability of occurrence. A final body weight cell used the CHOOSE function in Excel to select the body weight corresponding to the selected rank. This final cell was used as an input in the dose calculation (Cell D). Cells in column C were designated to the variability loop. The selection cell in column E was designated to the uncertainty loop.

Table 6. Body weight calculation

A	B	C	D	E	F
Mean	**95th%**	**BW (kg)**	**Age range**	**Rank**	**Probability**
EFH	EFH	Normal (A2:B2)	2<3	1	0.072
EFH	EFH	Normal (A3:B3)	3<6	2	0.211
EFH	EFH	Normal (A4:B4)	6<11	3	0.353
EFH	EFH	Normal (A5:B5)	11<17	4	0.364
				Selection	**BW (kg) Cell A**
				Custom (E2:F5)	=CHOOSE(E7,F2,F3,F4,F5) **Cell D**

EFH, Child-specific Exposure Factors Handbook of the US EPA; BW, Body Weight

Dose by month

The following equation was used to calculate the dose received for each month. A correction factor (CF) of 1000 was applied to convert ml/hour to L/hour.

$$Dose\ (ug/kg\ day^{-1}) = \frac{Cells\ A * B * C}{Cell\ D * CF}$$

$$OR$$

$$Dose\ (\mu g/kg\ day^{-1}) = \frac{Duration\ \left(\frac{hours}{day}\right) * Intake\ \left(\frac{ml}{hour}\right) * Concentration\ (\frac{\mu g}{L})}{Body\ weight\ (kg) * CF}$$

A sensitivity analysis was performed using Crystal Ball to determine which variables had the greatest effect on dose estimates.

Dose Response Assessment

Acute exposure will be assessed by a study reported by Fawell et al., (1999), where mice were administered single doses of microcystin-LR via oral gavage. For the acute study, three endpoints resulting in liver pathology were categorized; these included centrilobular necrosis, cytoplasmic vacuolation and hepatic hemorrhage. Hemorrhage was broken down into three sub-groups; moderate centrilobular hemorrhage, marked centrilobular hemorrhage and diffuse hemorrhage. For this assessment, total hepatic hemorrhage will be used as the endpoint of interest. Total hepatic hemorrhage is defined as the sum of the three subgroups.

Line of best fit

The acute study administered three doses, with pathology observed at the lowest administered dose of 500µg/kg. Given the limited number of data points, an additional acute oral toxicity study by Yoshida et al. (1997) was included in the dose response assessment. Lethality was the endpoint of interest in the Yoshida study and administered doses were higher than the range in Fawell et al. (1999); however, autopsies revealed the presence of hepatic hemorrhage in deceased animals. Therefore, it was concluded that hepatic hemorrhage occurred for all animals at all dose levels. The combined six data points were entered into Microsoft Excel and a logistic regression was fit to the data with an R^2 value of 0.97 (Figure 2, Table 7). The fit of the log transformed line was significant at the $p<0.05$ level.

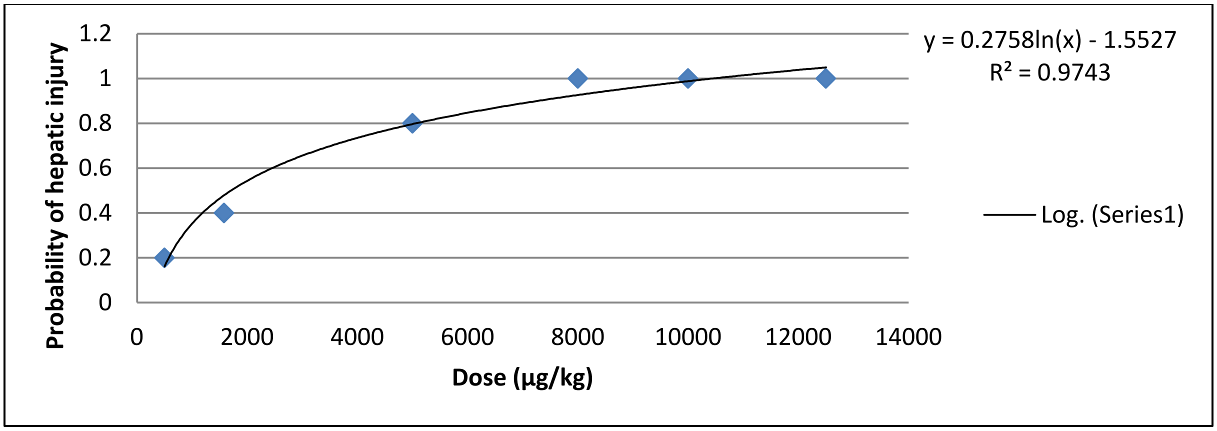

Figure 2. Dose response curve fit

Table 7. Dose response input data

Dose (µg/kg)	Percent response	Source
500	0.2	Fawell et al., 1999
1,580	0.4	Fawell et al., 1999
5,000	0.8	Fawell et al., 1999
8,000	1	Yoshida et al., 1997
10,000	1	Yoshida et al., 1997
12,500	1	Yoshida et al., 1997

A two log correction factor was applied to the line of best fit for the microcystin dose response assessment. Varying the dose response curve by two logs in either direction accounts for species variability including differences among mice (intraspecific) and differences between mice and humans (interspecific).

The original line of best fit crosses the y axis at 278.6; this value represents the dose at which no effect would be observed for this data set. A two log correction in either direction was applied to this value. Maximum and minimum y intercepts associated with the corrected lines were calculated. The slope of the corrected dose response lines remains constant; however the y-intercept was allowed to sweep between the minimum and maximum based on the 2 log correction factor to the x-intercept (Figures 3, 4). Cell E was designated to the uncertainty loop.

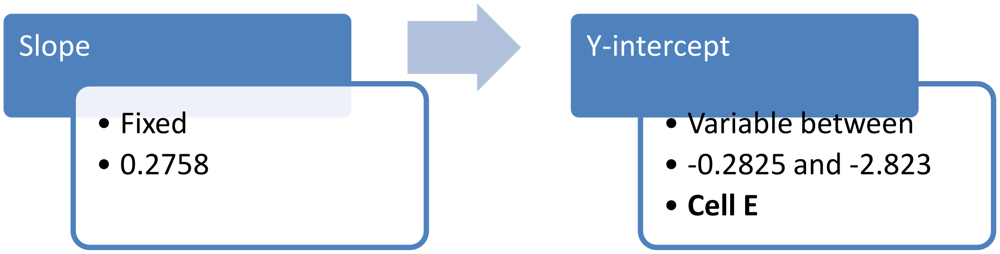

Figure 3.Variables for the dose response line

The log transformed dose response lines are represented in Figure 4, with the central line representing the original fitted relationship represented in Figure 2.

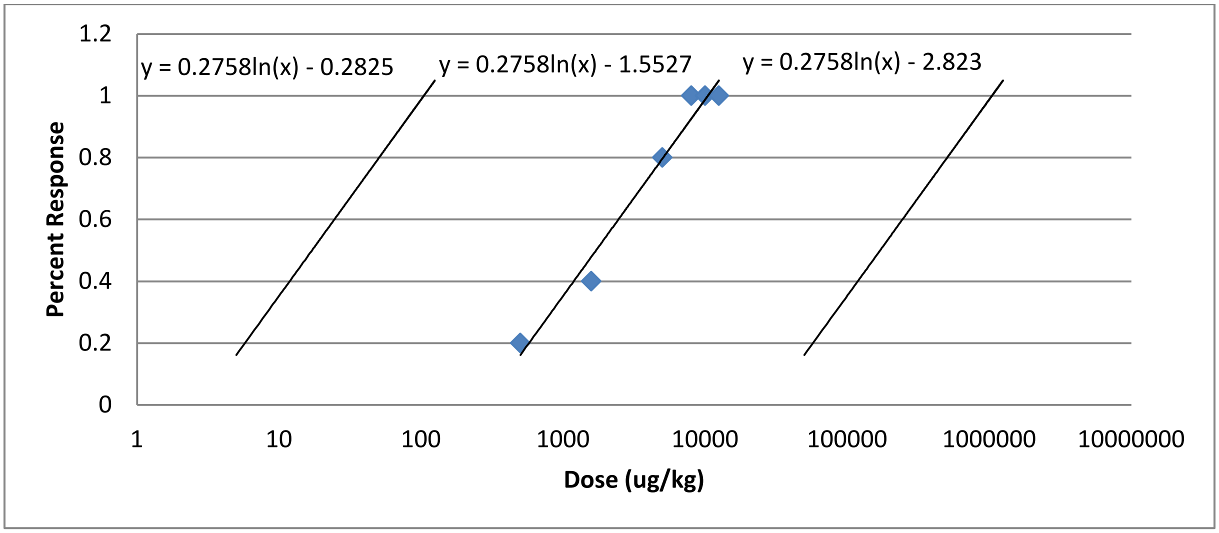

Figure 4. Log transformed dose response line with a bi-directional 2-log correction factor applied

Low dose extrapolation

The current line of best fit does not provide a good estimate of risk below the lowest data point of 500 µg/kg. To estimate the risk for doses below the lowest data point for each curve, a linear extrapolation was performed. A line of best fit is selected with correction factors applied, as noted above. After this selection a series of calculations are made which selects a linear extrapolation. The linear extrapolation represents the dose response curve between the lowest data point on the line of best fit, where $Y_2 = 0.2$, and a selected point on the X axis, where $Y_1 = 0$. The linear extrapolation is then drawn between point (X_2,0.2), and an endpoint between (0,0) and where the selected line of best fit would cross the X axis (X_1,0) (Figure 5). The following steps illustrate how the linear endpoints were calculated; "line of best fit" refers to the semi-log line that was fit to the original data points.

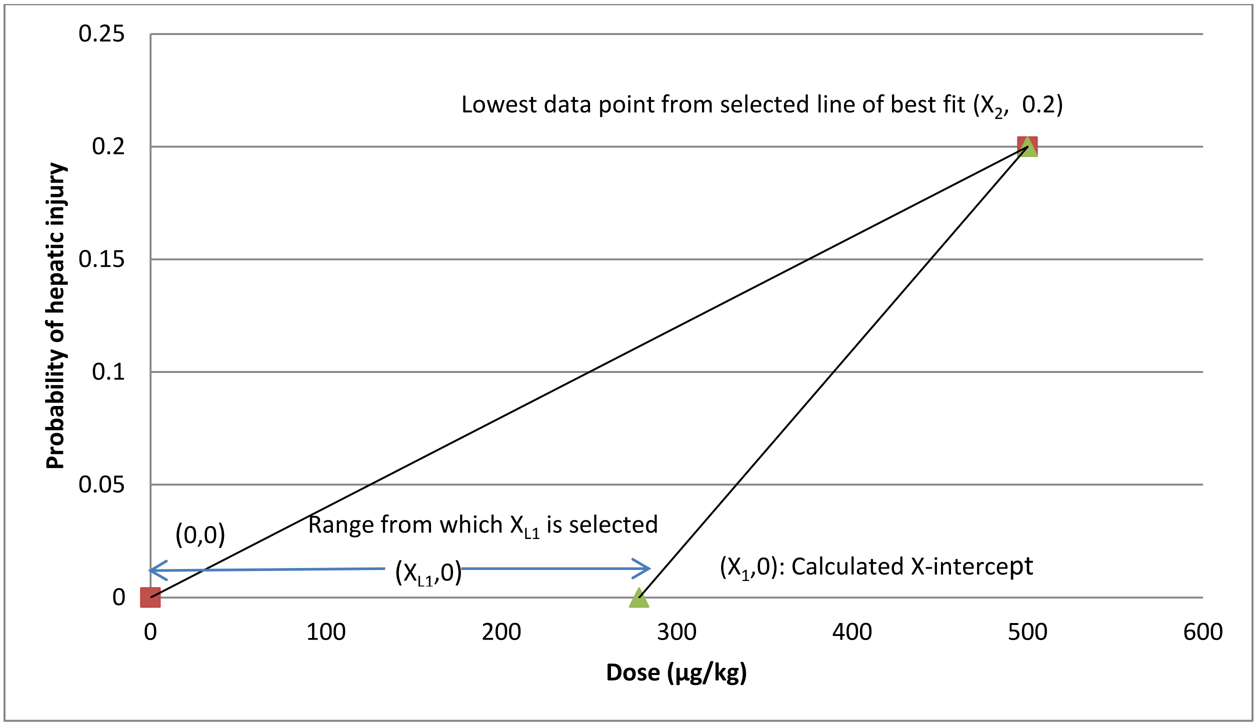

Figure 5. Linear extrapolation

1) Y-intercept (yint) of line of best fit selected as noted above
2) X-value (X_1) when y=0 (Y_1) of line of best fit calculated from y-intercept:

$$X_1 = e^{\left(0-\frac{yint}{m}\right)}$$
$$where\ m = slope$$

3) X-value (X_2) when y=0.2 (Y_2) calculated:

$$X_2 = e^{\left(0.2-\frac{yint}{m}\right)}$$

4) Slope (m_L) of the linear extrapolation calculated:

$$m_L = \frac{Y_2 - Y_1}{X_2 - X_{L1}}$$
$$where\ X_{L1}\ is\ an\ assumption\ cell, uniformly\ distributed, selecting\ a\ value\ between\ 0\ and\ X_1$$

5) Y-intercept of the linear extrapolation ($y_L int$) calculated:

$$Y_L\, int = 0 - m_L * X_{L1}$$

Risk Characterization

The probability of an individual receiving a dose leading to hepatic hemorrhage is a product of the selected dose response line solved for y, where x is the dose.

$$Risk = dose * dose\ response$$

$$Risk = 0.2758 * \ln(dose) - yint$$

where the y intercept varies based on the application of correction factors

$$OR$$

$$Risk = m(dose) + yint$$

where slope and y intercept vary depending on extrapolated endpoints

The following Excel "IF" statements were used to place bounds on the risk probability so that only values between zero and one were delivered. The first statement reads that if the original risk calculation is less than zero, zero shall be used as the result, otherwise use the original risk value. The second statement reads that if the lower bounded risk ($risk^a$) is greater than one, one shall be used as the result, otherwise use the value of $risk^a$.

$$risk^a = IF(Risk < 0{,}0, risk)$$
$$risk^b = IF(risk^a > 1{,}1, risk^a)$$

A final "IF" statement was used to select either the line of best fit or the linear extrapolation below the lowest data point based on the selected dose. The statement reads if the selected dose is greater than the lowest data point (X_2), calculated from the selected line of best fit, then use the line of best fit to estimate the risk. If the dose is less than the lowest calculated data point than use the selected linear extrapolation.

$$Risk = IF\ (dose > X_2\ , best\ fit, linear\ extrapolation)$$

A sensitivity analysis was performed using Crystal Ball to determine which variables had the greatest effect on risk estimates.

Questionnaire Results

Response rates

The distribution of questionnaires and response rates by lake are shown below (Table 8). A total of 216 questionnaires were returned; 11 questionnaires were not included in the results due to insufficient information or unclear responses. The total number of questionnaires from which data was collected is 205, representing a total of 382 individuals.

Table 8. Response rates

Lake	Mailed	Number Returned	Percent Returned	Percent of Total	Individuals Represented
Cochran	43	7	16.3%	3.4%	14
Crabapple	44	6	13.6%	2.9%	20
Howard	102	16	15.7%	7.8%	33
Ketchum	205	30	14.6%	14.6%	40
Loma	70	16	22.9%	7.8%	24
Lost	53	14	26.4%	6.8%	22
Martha North	85	13	15.3%	6.3%	27
Pine	144	36	25.0%	17.6%	80
Serene	118	31	26.3%	15.1%	50
Steel	81	21	25.9%	10.2%	34
Wilderness	55	15	27.3%	7.3%	38
Total	**1000**	**205**	**20.5%**	**100.0%**	**382**

Despite an encouraging response rate, non-response bias may influence the analysis of the questionnaire data as recreational activities among the population of respondents may differ from those who did not respond. Non-response bias may affect the algae awareness and concern data and influence the proportion of households who report activity levels being affected by water quality and algae issues.

In determining the risk associated with microcystin exposure through recreational activity, non-response bias may also affect the risk if non-respondents recreate at levels above or below those who responded. However, considering the nature of the questionnaire, it is likely that individuals who recreate are more likely to respond than individuals that do not recreate.

Individuals that do not recreate are not exposed to microcystin and therefore are not of interest in the risk determination portion of the current study.

Demographics

Of the 382 individuals for whom data was available, 368 reported their gender resulting in a total of 182 males and 186 females. Respondents were also categorized by two age groups, over 18 years of age and not over 18 years of age. Age was reported for a total of 370 individuals, 45 reporting their age as “not over 18” and 325 reported their age as “18 or over".

Temporal distribution of activities

Event frequency and duration data were collected for the period of June through September, 2011; however, respondents were also asked which months out of the entire year they participated in each activity. For all activities, the majority of respondents reported recreating in July and August. Participation in non-immersion activities (fishing and boating) were reported in all months of the year, while swimming and wading/playing were not reported during the months of November through March.

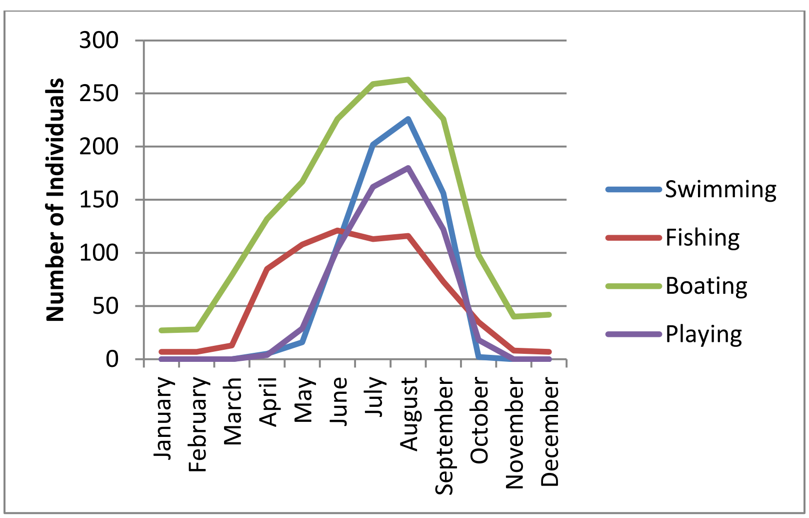

Figure 6. Activities by month

Water quality concerns

Perceptions and concerns regarding the water quality in each lake were collected and reported on the household level (Table 9). It was assumed that members of the same household would have similar awareness levels regarding general water quality and algae issues. 71.7% of all household reported having concerns about general water quality, with 49.8% of households reporting having seen or heard of warnings specific to algae in their lakes.

For all lakes, the majority of general water quality concerns (62.6%) surround aesthetic issues, followed by posted warning signs (29.3%), self-reported algae issues (19%), hearing about water quality problems from neighbor or community meeting (17.7%), nuisance plant growth (8.8%), other issues (6.8%), and health concerns (2.7%). Health concerns included infections and swimmers itch. Other concerns included lakeshore development, animal feces runoff, waterfowl, lakeshore fires, stagnant conditions, oil in the water, and uneducated neighbors. Percentages of concerns by lake are reported in Appendix D.

The majority of households (54.9%) reported their algae awareness being the result of posted warning signs. Mail (37.3%) and email (27.5%) were additional reported sources of information (Table 10).

Table 9. Lake quality concerns (by household)

		Water Quality Concerns		Seen Algae Warnings	
Lake	**Households**	**Count**	**Percent**	**Count**	**Percent**
Cochran	7	2	28.6%	0	0.0%
Crabapple	6	5	83.3%	1	16.7%
Howard	16	10	62.5%	4	25.0%
Ketchum	30	29	96.7%	30	100.0%
Loma	16	14	87.5%	14	87.5%
Lost	14	8	57.1%	1	7.1%
Martha North	13	4	30.8%	1	7.7%
Pine	36	25	69.4%	11	30.6%
Serene	31	18	58.1%	10	32.3%
Steel	21	18	85.7%	17	81.0%
Wilderness	15	14	93.3%	13	86.7%
Total	**205**	**147**	**71.7%**	**102**	**49.8%**

Table 10. Information source for algae specific warnings (by household)

		Email		Mail		Warning Signs		Newspaper		Other[a, b]	
Lake	Total count	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Cochran	0	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Crabapple	1	0	0.0%	0	0.0%	1	100.0%	0	0.0%	1	100.0%
Howard	4	0	0.0%	1	25.0%	1	25.0%	0	0.0%	3	75.0%
Ketchum	30	8	26.7%	15	50.0%	23	76.7%	2	6.7%	3	10.0%
Loma	14	3	21.4%	7	50.0%	10	71.4%	3	21.4%	1	7.1%
Lost	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	100.0%
Martha North	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	100.0%
Pine	11	1	9.1%	1	9.1%	3	27.3%	5	45.5%	5	45.5%
Serene	10	2	20.0%	4	40.0%	3	30.0%	0	0.0%	3	30.0%
Steel	17	9	52.9%	5	29.4%	6	35.3%	3	17.6%	0	0.0%
Wilderness	13	5	38.5%	5	38.5%	9	69.2%	2	15.4%	5	38.5%
Total	**102**	**28**	**27.5%**	**38**	**37.3%**	**56**	**54.9%**	**15**	**14.7%**	**23**	**22.5%**

[a] Friends, neighbors, meetings, county staff, other face-to-face conversation
[b] percents across rows may add to over 100 due to respondents reporting multiple sources

Recreational behavior affected by water quality concerns was reported on the household level including activities affected by all water quality concerns, not just algae specific concerns (Table 11). Across all lakes, households with water quality concerns reported reduced participation in activities with the greatest water contact in the following order: swimming, wading/playing, fishing and boating. Households on Lake Cochran and Martha North reported general water quality concerns, but the concerns did not affect recreational behavior of individuals within households. A similar trend is seen with respect to households reporting they have seen or heard of algae-specific warnings for their respective lake. 61.8% of these households across all lakes reported a reduction in swimming, 58.8% a reduction in wading/playing, 38.2% a reduction in fishing, and 26.5% a reduction in boating.

Households that reported general water quality concerns but did not report seeing or hearing of algae warnings reported a lesser effect on behavior. 43.4% of these households across all lakes reported a reduction in swimming, 30.2% a reduction in wading/playing, 13.2% a reduction in fishing, and 9.4% a reduction in boating. The difference in percentages of households reporting general water quality concerns with and without seeing or hearing about algae-specific warnings can be used as an indicator of the degree recreational behavior is influenced specifically by lake algae issues: 18.4% for swimming, 28.6% for wading/playing, 25% for fishing, and 17.1% for boating.

Table 11. Activities affected by water quality concerns by lake

	WQ Concerns	Fishing		Boating		Wading/Playing		Swimming	
Lake	**Count**	**Count**	**Percent**	**Count**	**Percent**	**Count**	**Percent**	**Count**	**Percent**
Cochran	2	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Crabapple	5	2	40.0%	1	20.0%	3	60.0%	3	60.0%
Howard	10	0	0.0%	0	0.0%	3	30.0%	4	40.0%
Ketchum	29	21	72.4%	15	51.7%	20	69.0%	20	69.0%
Loma	14	7	50.0%	4	28.6%	11	78.6%	12	85.7%
Lost	8	1	12.5%	0	0.0%	2	25.0%	4	50.0%
Martha North	4	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Pine	25	4	16.0%	4	16.0%	8	32.0%	12	48.0%
Serene	18	4	22.2%	2	11.1%	10	55.6%	13	72.2%
Steel	18	3	16.7%	4	22.2%	9	50.0%	10	55.6%
Wilderness	14	4	28.6%	2	14.3%	10	71.4%	8	57.1%
Total	**147**	**46**	**31.29%**	**32**	**21.77%**	**76**	**51.70%**	**86**	**58.50%**

Event frequency and duration for all respondents by awareness

Respondents were asked how many days they participated in each activity between June and September, 2011. Of the 382 individuals represented across all lakes, 69.9% reported boating in their lake, 59.9% reported swimming in their lake, 49.5% reported wading/playing in their lake, and 37.2% reported fishing in their lake. Descriptive statistics for recreational activities by lake, gender and age are shown in Appendices E and F.

No strong pattern was observed between the frequencies of each recreational activity by lake, with the exception of swimming for Lakes Loma and Ketchum for which the mean frequency was far below the rest of the lakes. Lake Ketchum also had the lowest percent of individuals reporting they participated in immersion activities (swimming, wading/playing).

No strong pattern was observed for frequencies of each recreational activity between genders with the exception of the percent of individuals (predominately males) reporting participation in fishing. For age, respondents under the age of 18 were more likely to swim, fish, wade/play and boat than respondents 18 years of age and older. In addition, the average frequency of events for respondents under the age of 18 was higher for immersion activities than older respondents (Appendix E). Respondents were asked how many days between June and September, 2011 on average they participated in each recreational activity. Across all lakes there was a slight rise in mean event frequencies for individuals uninformed of algae issues versus those informed, however this difference was not significant (Figure 7).

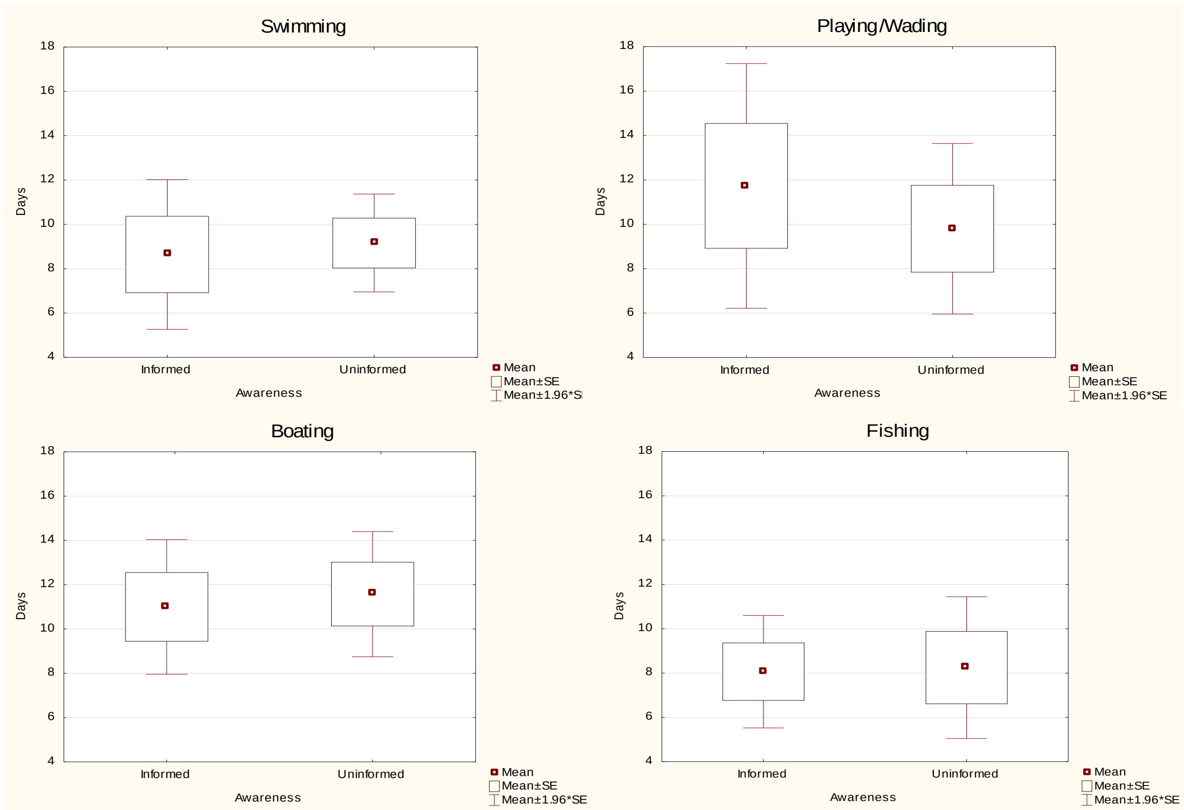

Figure 7. Event frequency by algae awareness

Respondents were asked (on average) how long they participated in a recreational event on a given day between June and September, 2011. Across all lakes there was a slight rise in mean event durations for individuals uninformed of algae issues versus those informed; however this difference was not significant (Figure 8, Appendix F).

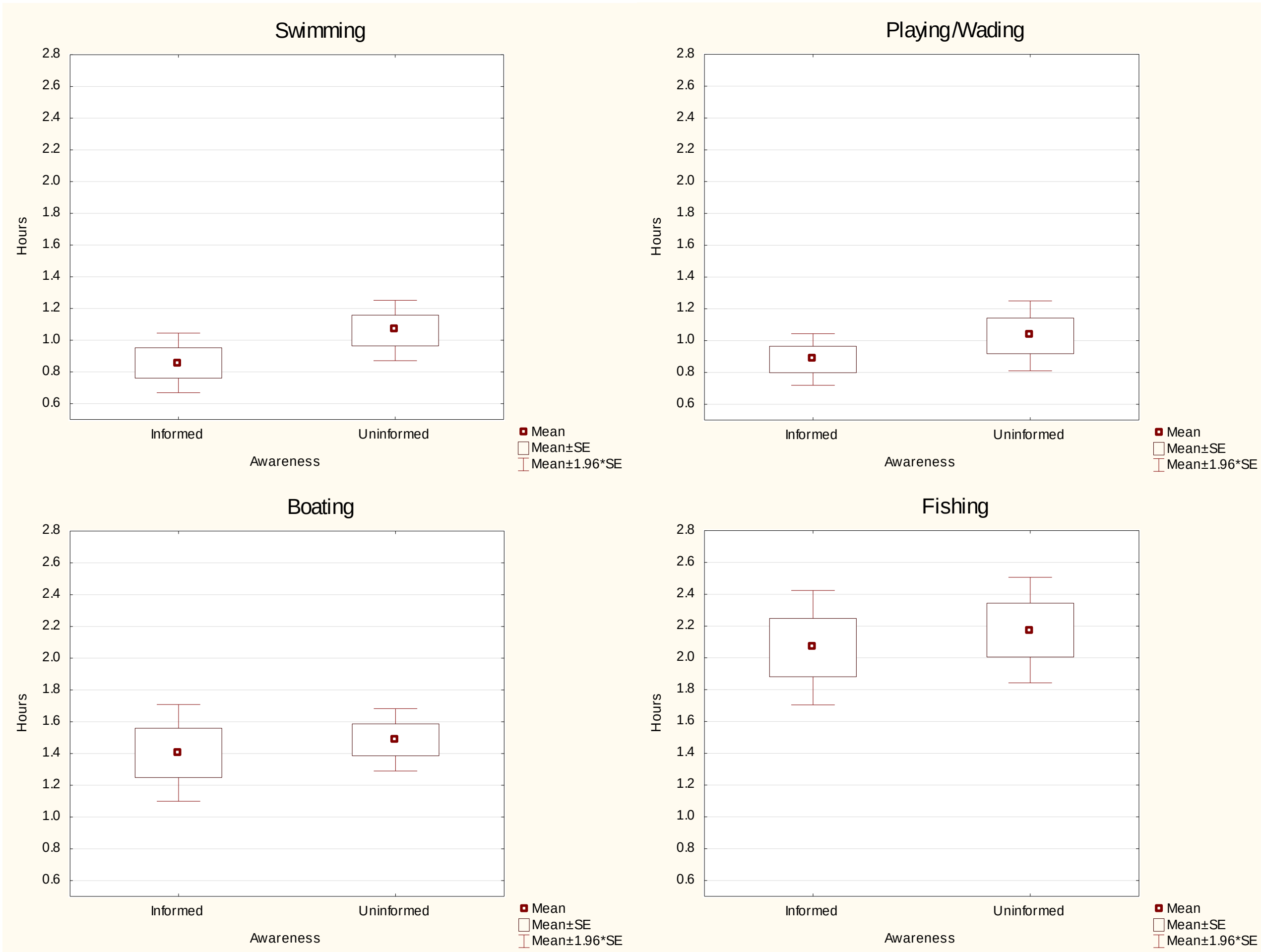

Figure 8. Event duration by algae awareness

Affected activities

Overall, households aware of water quality and algae issues reported reductions in their recreational behavior (43.4% and 18.4% for swimming respectively). This pattern suggests that algae issues in a lake affect whether an individual chooses to swim. Once the choice to swim is made, the event duration is not significantly lessened by the knowledge of algae issues in the lake.

Swimming frequency and duration by age and awareness

Similar to the grouped percentages noted above, swimming frequency and duration were greater for individuals under the age of 18. When separated by household awareness of algae issues, children from households uninformed of these issues had higher rates of frequency and duration than children from households aware of the issues (Figures 9 and 10). The higher swimming rates for individuals under the age of 18 represent an increase in the risk of exposure to microcystin.

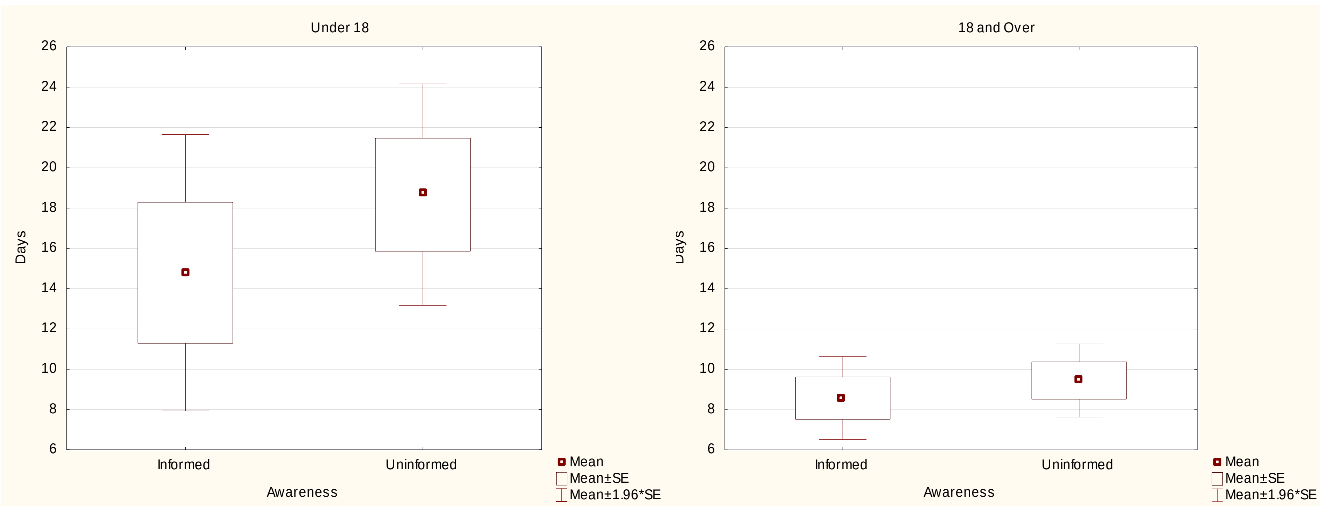

Figure 9. Swimming frequency by age and awareness

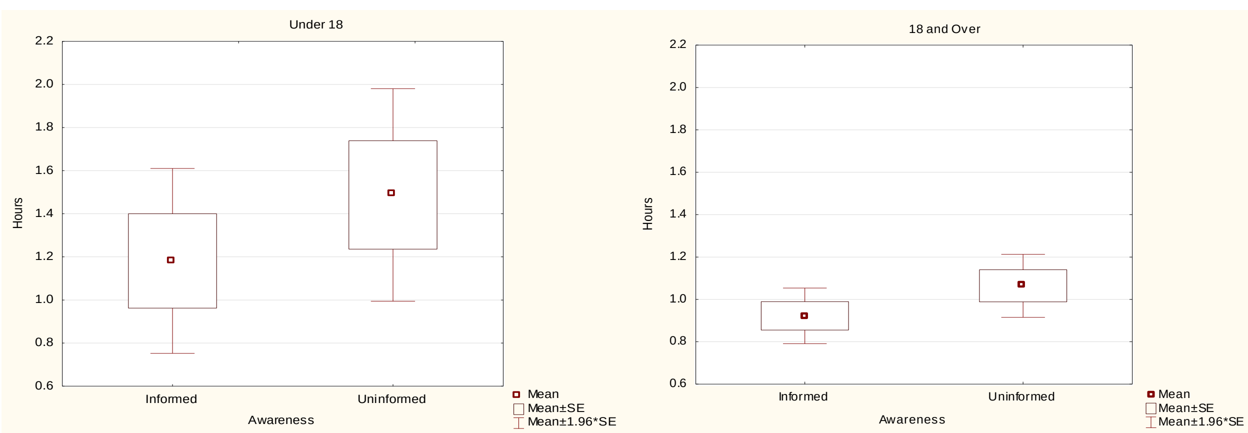

Figure 10. Swimming duration by age and awareness

Fishing

Respondents who reported fishing were asked if they fished from a boat, shore or while standing in water, and about the outcome of their catch. A total of 160 individuals reported fishing in their lake. Summing the reported values exceeds 160 due to respondents reporting multiple fishing locations and outcomes of their catch. Of the nine individuals reporting they fished while standing in water, all nine reported not wearing waders during the activity (Figure 11).

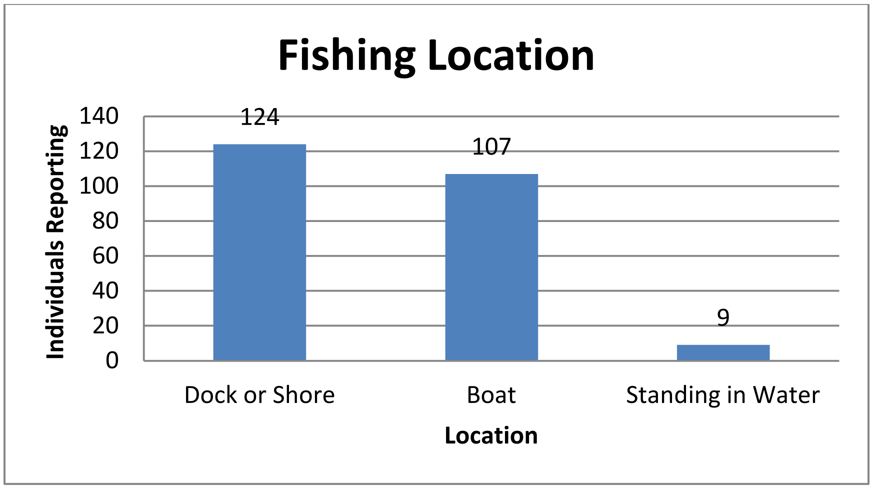

Figure 11. Fishing location

The majority of respondents who fished reported releasing fish they caught or consuming the fish (Figure 12). The most dominant species reported consumed was trout followed by perch and bass. 37% of individuals reported consuming their catch. Assuming fish given away were consumed, this percentage rises to 40%.

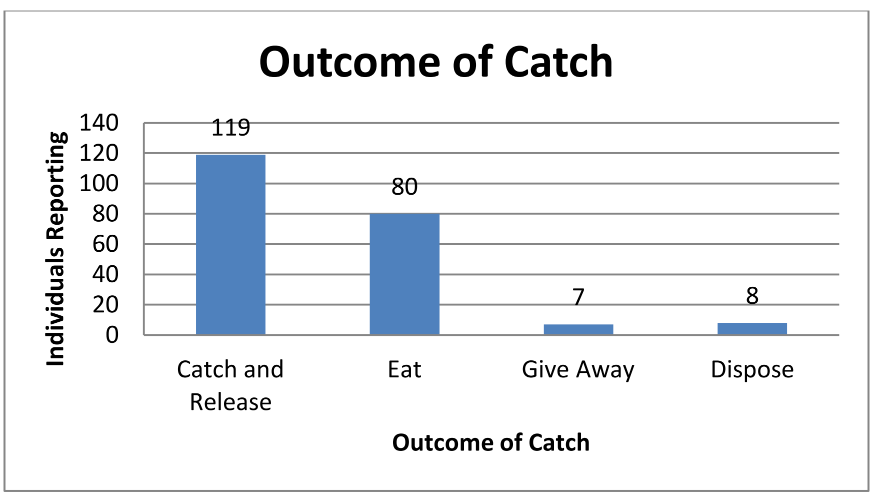

Figure 12. Outcome of catch

Household pets

60 households reported having dogs that drank water from the lake on which the household was located. 27 households reported their dogs drank daily from the lake, 21 reported their dogs drank lake water on a weekly basis and 8 reported their dogs drank water monthly.

Model Results

Microcystin concentrations

Concentrations of microcystin monitored in 2011 and funded through the Washington State Department of Ecology (Ecology) were included as inputs to the model and are represented in Table 12. Ecology-funded monitoring was performed during bloom events and represent the highest concentrations for each month.

Table 12. 2011 microcystin concentrations (μg/L)

Lake	June	July	August	September
Ketchum	44.7	49.8	4.07	19.9
	16.8	3.65		
	4.03	551		
Cassidy				432
				312
				18,400
				1.3
				1.59
				479

Exposure assessment

Doses were calculated as described in the methods section and plotted using the Excel visual basic macro (Figures 13-19). The median (50^{th} %ile) curve represents the best estimate of doses received for a given month based on input data. The lower tolerance limit (LTL) and upper tolerance limit (UTL) are upper and lower bounds on the 5^{th} and 95^{th} percentile confidence limits. The tolerance limits essentially represent confidence limits on the confidence limits. The tolerance limits may be interpreted as follows: there is a 95% confidence that the LTL does not overestimate the 5^{th} percentile confidence limit, and a 95% confidence that the UTL does not underestimate the 95^{th} percentile confidence limit.

Doses were modeled for individuals uninformed of algae issues in their lake. Event duration between individuals informed and uninformed of algae issues did not differ significantly, however a slight increase in average event duration for those uninformed was observed. To see if this had an effect on the dose outcome, July in Lake Ketchum and September in Lake Cassidy were also modeled for individuals informed of algae issues (Figures 18 and 19) and compared to those uninformed. The median dose estimate in July for Lake Ketchum was higher for the informed group (0.08 µg/kg) than for the uninformed group (0.06 µg/kg), while the median dose estimate in September for Lake Cassidy was lower for the informed group (2.46 µg/kg) than for the uninformed group (3.38 µg/kg).

Sensitivity analysis revealed that concentrations of microcystin had the greatest influence on the dose model, followed by event duration, ingestion rate and body weight.

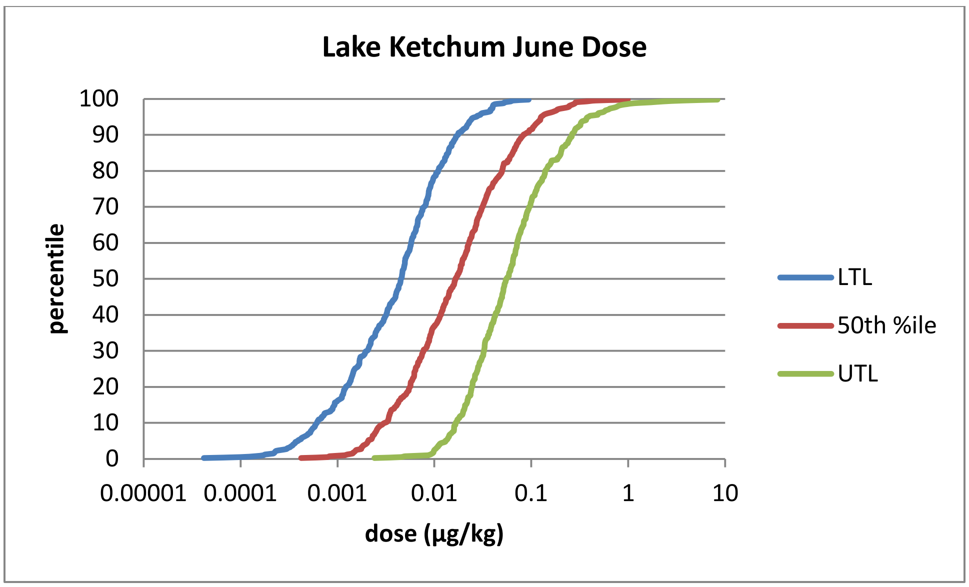

Figure 13. Doses for individuals uninformed of algae issues for June
Dose for 50th percentile (µg/kg): LTL: 0.005, Median: 0.02, UTL: 0.06

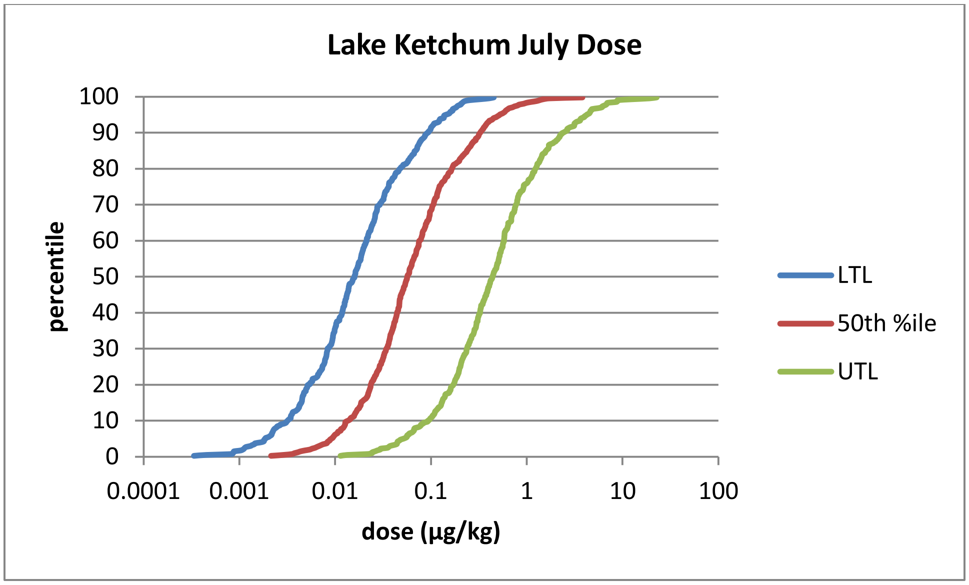

Figure 14. Doses for individuals uninformed of algae issues for July
Dose for 50th percentile (µg/kg): LTL: 0.02, Median: 0.06, UTL: 0.44

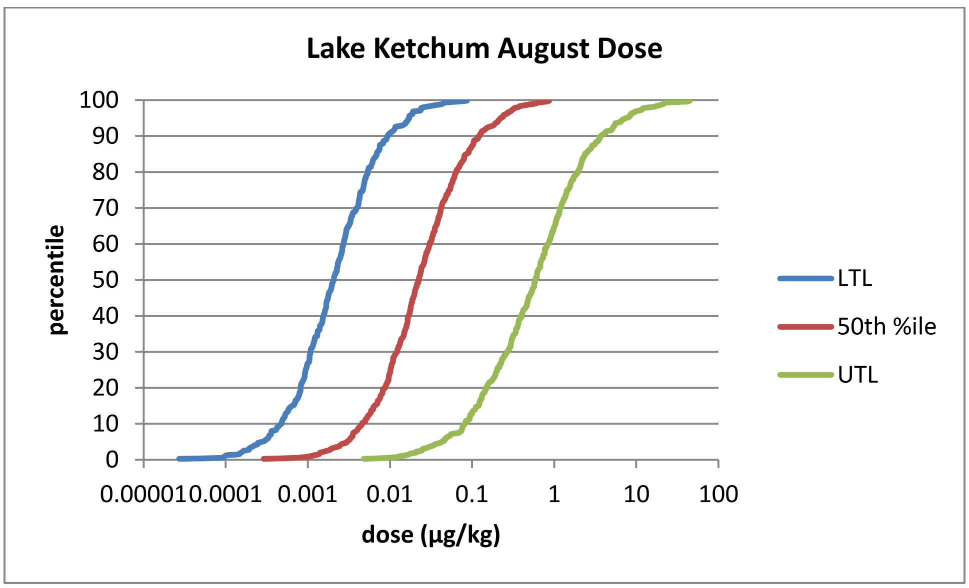

Figure 15. Doses for individuals uninformed of algae issues for August
Dose for 50th percentile (µg/kg): LTL: 0.002, Median: 0.02, UTL: 0.59

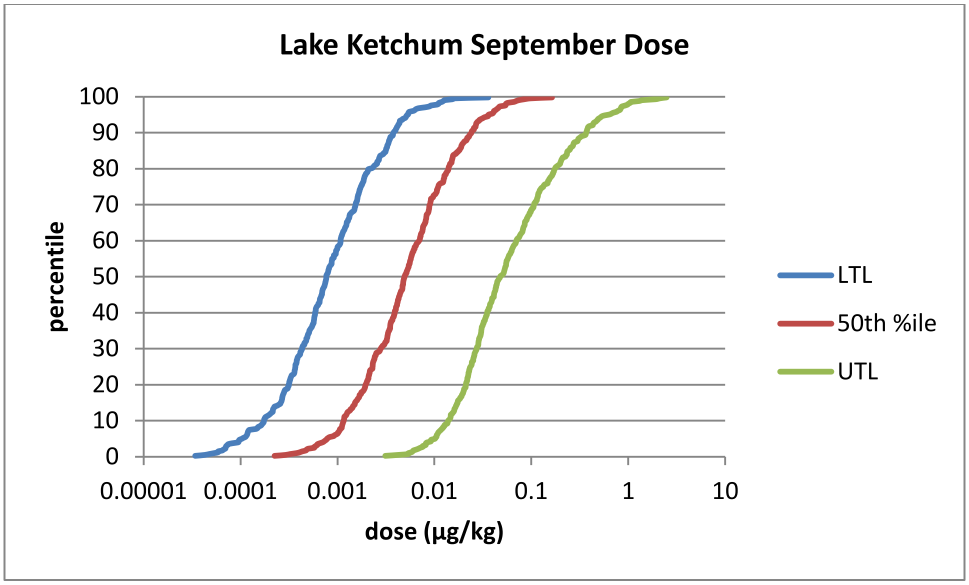

Figure 16. Doses for individuals uninformed of algae issues for September
Dose for 50th percentile (µg/kg): LTL: 0.0008, Median: 0.005, UTL: 0.05

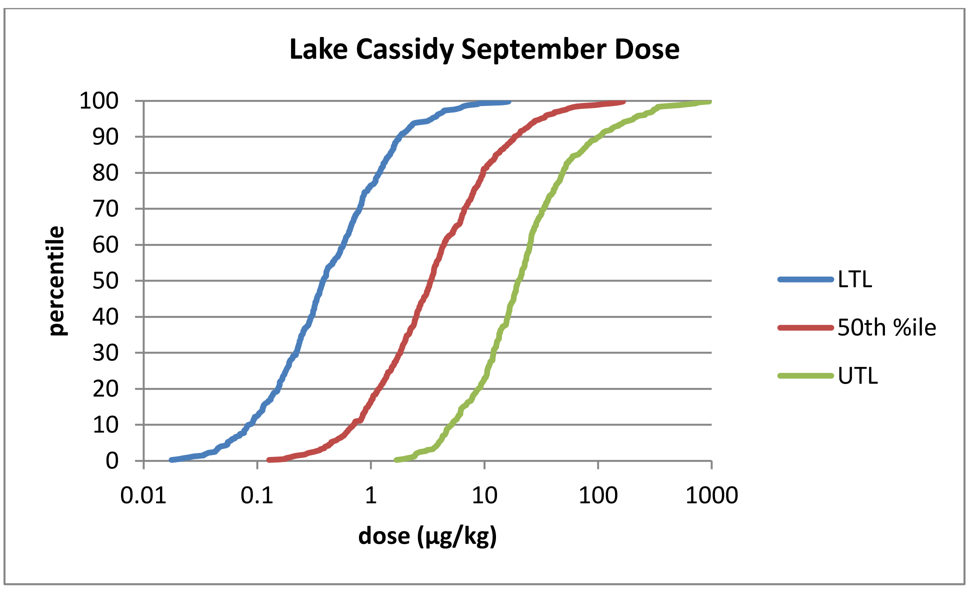

Figure 17. Doses for individuals uninformed of algae issues for Lake Cassidy in September
Dose for 50th percentile (µg/kg): LTL: 0.38, Median: 3.38, UTL: 19.96

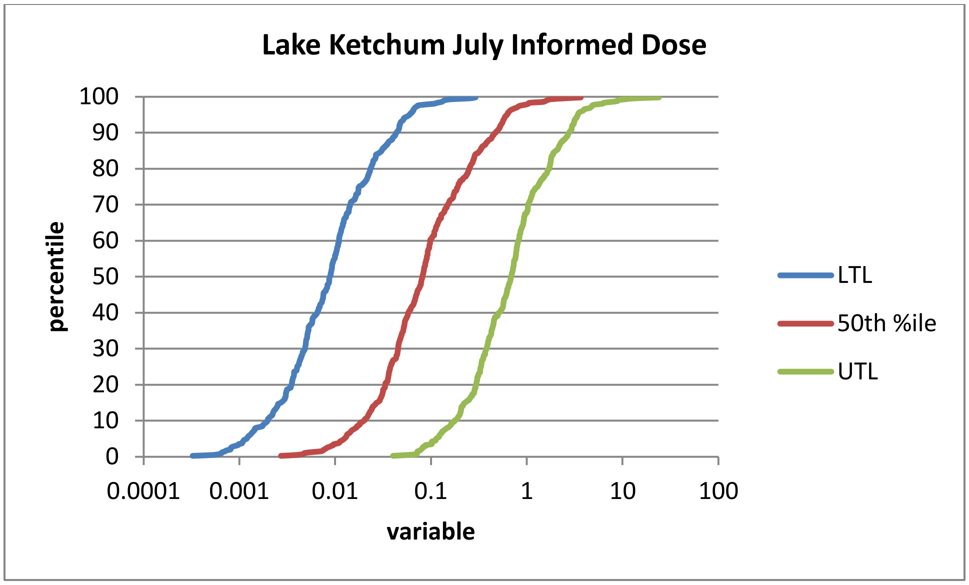

Figure 18. Doses for individuals informed of algae issues for July
Dose for 50th percentile (µg/kg): LTL: 0.01, Median: 0.08, UTL: 0.69

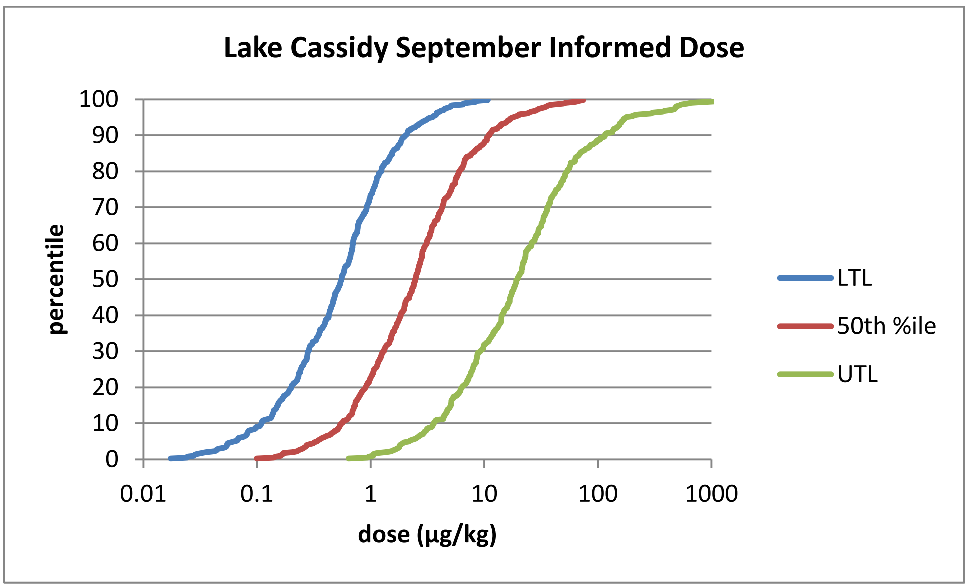

Figure 19. Doses for individuals informed of algae issues for Lake Cassidy in September
Dose for 50th percentile (µg/kg): LTL: 0.56, Median: 2.46, UTL: 19.62

Dose estimates may be compared to derived acute oral no-effect dose levels (0.5 to 2.5 µg/kg) as well as the TDI (0.04 µg/kg day^{-1}). Median dose estimates for individuals at the 50th percentile do not exceed any of the derived no-effect doses in June, August or September

(Figures 13,15,16). In July the median estimate at the 50th percentile exceeds the TDI and the 50th percentile of the UTL approaches the most conservative estimate of an acute no-effect dose, but does not exceed it (Figure 14). The highest concentrations of microcystin were observed in Lake Cassidy in September. Dose modeling run for Lake Cassidy in September yielded median estimates at the 50th percentile of 3.38 µg/kg, above the TDI and derived acute no-effect dose levels (Figure 17).

Risk characterization

Zero risk was modeled for the month of June in Lake Ketchum. Risk for August and September in Lake Ketchum was only modeled for the upper tolerance limit above the 99th percentile. A greater risk range was seen at the upper tolerance limits for July in Lake Ketchum and September in Lake Cassidy (Figures 20 and 21).

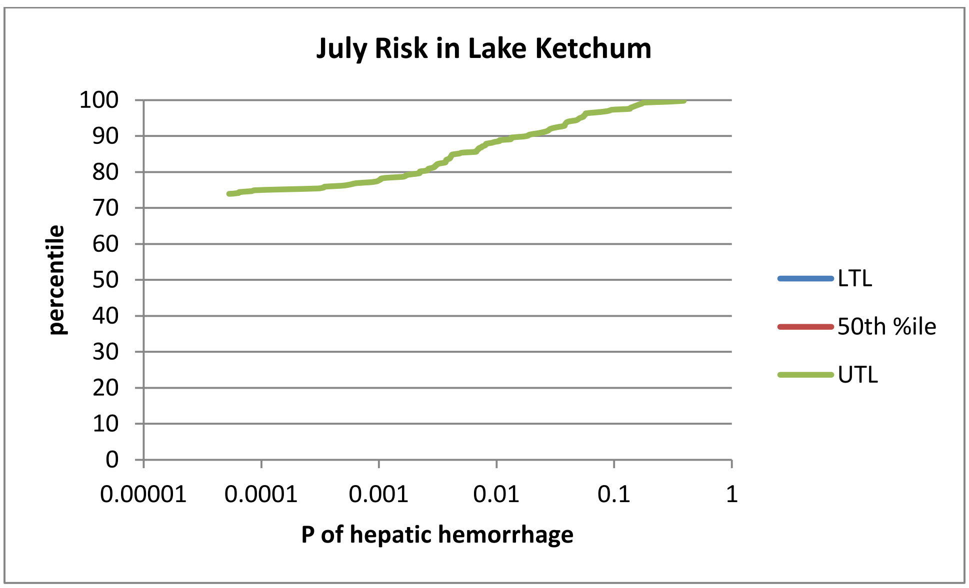

Figure 20. Probability of hepatic hemorrhage in July for Lake Ketchum

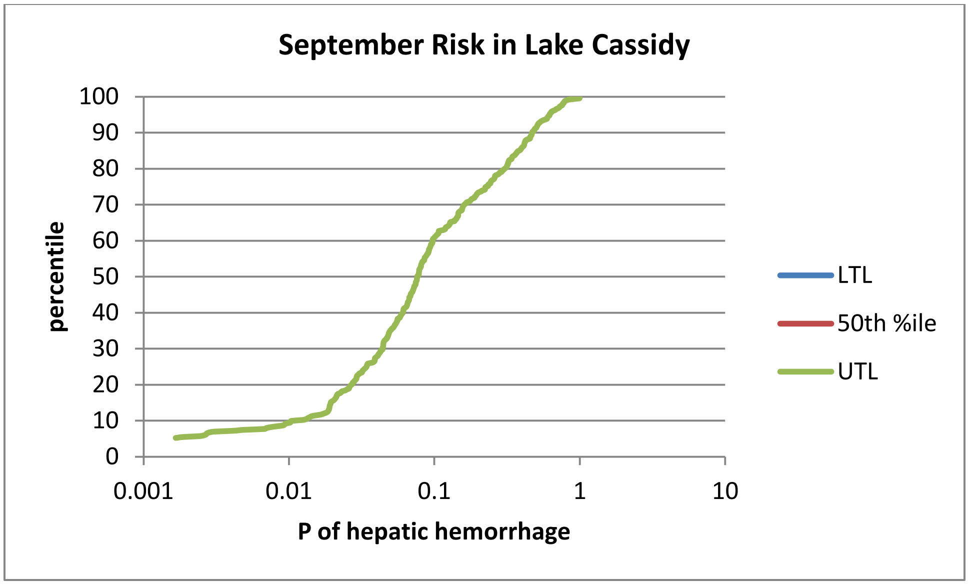

Figure 21. Probability of hepatic hemorrhage in September for Lake Cassidy

Risk was modeled based on a line of best fit with a two log correction factor in either direction for species variability. This uncertainty did not have an effect on the median dose prediction curve; instead the application of correction factors expanded the tolerance limits to encompass a greater region to account for the uncertainty. A lack of dose response data in the region where doses were observed required extrapolation which induced additional uncertainty to the risk model.

The model predicts no risk for most individuals, with the exception of those recreating in July in Lake Ketchum and September in Lake Cassidy. Risk estimation begins above the 73rd percentile for July in Lake Ketchum at a probability of hepatic hemorrhage of 1.1×10^{-5}. For September in Lake Cassidy predictions begin above the 4th percentile at a probability of 1.3×10^{-3}.

Discussion

Questionnaire

Several factors influence response rates of questionnaires administered through the mail and a wide range of response rates among mailed surveys have been observed. Higher rates of response have been associated with the mailing of notification letters prior to the actual survey. Follow-up letters encouraging participation have also increased total response rates. In addition, financial incentives may increase an individual's likelihood of response, and shorter survey lengths have also shown an effect (Shih and Fan 2008; Kanuk and Berenson 1975). An initial response rate of 21.6% falls within the wide range observed.

A non-response on the questionnaire was interpreted as a true zero for frequency or duration of recreational event. Zeros and non-responses were not included in the duration and frequency calculation as they represent zero risk.

Awareness levels are most likely due to mail and email alerts sent out by Snohomish and King Counties to lakeshore residents regarding algal toxin concentrations as well as warning signage posted at public access points along the lakes. Lakes Loma and Ketchum had consistent blooms of algae during the summer of 2011 and had measurable concentrations of the toxin microcystin. The reduced frequency and duration of immersion activities for Lakes Loma and Ketchum provide evidence of aversion to water contact when consistent blooms are present.

Several factors may influence an individual's recreational behavior independent of their awareness of algae issues including socioeconomic status, age, ability to swim and health. These factors were not accounted for in this questionnaire. Rates at which algae blooms occurred in each lake differed, therefore a household reporting awareness of algae bloom may have only encountered a warning once, while another household may have encountered several if blooms persisted throughout the summer.

The 18.6% reduction in swimming due to algae awareness did not coincide with a significant decrease in event frequency or duration between individuals from households informed or uninformed of algae issues. It is unknown whether reported durations and frequencies represent a decrease from background as background rates are not known. Since individuals not reporting swimming were not included in these calculations, the discrepancy between reported reductions and reported frequencies and durations may suggest that certain people are choosing not to recreate at all due to algae; however, those that still choose to recreate do so at rates that are not decreased, despite any awareness of algae issues.

Model

Concentrations of microcystin have exceeded the recreational guidance value of 6μg/L, suggesting that residents living along the shores of lakes experiencing algal blooms are at risk when swimming in the lake while toxins are present. Microcystin sampling occurred at the thickest part of the bloom and concentrations varied greatly by month. The sensitivity analysis reported microcystin concentrations driving the exposure model, with event duration also playing a large role. Since most blooms are heterogeneous and therefore not equally distributed throughout a waterbody, those who recreate in these lakes can avoid areas where blooms are occurring and limit contact with water to reduce exposure to microcystin.

Results of the exposure assessment modeling suggest the possibility of a dose leading to hepatic injury in western Washington lakes when compared to derived acute no-effect doses. The risk of acute adverse health effects associated with swimming is highest when high toxin concentrations occur during warm months (July, September) concurrent with the months when individuals are more likely to swim.

Estimates of doses received in Lake Ketchum varied by month. While the results for July in Lake Ketchum and September in Lake Cassidy exceed the TDI, they represent only doses from an acute event and do not imply this level of intake is happening on a daily basis. Median estimated doses from Lake Cassidy in September suggest that children recreating at levels observed in western Washington lakes are at risk of hepatic hemorrhage from acute exposure when doses are compared to the derived no-effect levels. Little difference is observed between the two awareness levels which is consistent with the lack of significance between the reported event durations.

The limited acute oral dose response data presents a challenge in determining the risk from exposure to microcystin. The LOAEL of 500 µg/L is far above observed doses, and the equation of the line of best fit extends to a zero probability of hepatic hemorrhage at a dose of 278.6µg/kg. This suggests a threshold for adverse effects based on the current acute oral toxicity data. However, a NOAEL was not determined so the actual dose of zero probability is likely less than 278.6µg/kg and a threshold cannot be assumed.

The abrupt beginnings of risk estimation suggest a steep dose response relationship. Studies determining NOAEL through ip administration of microcystin in mice observed a small ratio between concentrations with no effect and severe health effects, supporting a steep dose response relationship for microcystin (Fromme et al. 2000).

The current study brings to light data gaps in acute oral toxicity studies for microcystin. The sensitivity analysis reported the selection of the y-intercept of the line of best fit, which was based on the application of correction factors to the X intercept, was dominant in driving the exposure model. Therefore, the majority influence on risk estimation comes from dose response data that does not encompass the range of doses observed. In order for a more accurate assessment of risk to be performed which incorporates dose response relationships, there is a need for data within the range of observed doses and the need for the determination of a NOAEL. Generation of this data will eliminate the need to extrapolate the dose response curve to estimated dose levels in Washington State.

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Appendix A: Institutional Review Board Approval Letter

PI: Mr. Jesse Billingham
Graduate Student
DEOHS

CC:

RE: HSD study #41425 “Algal toxin exposure assessment in Washington lakes”

Dear Mr. Billingham:

The University of Washington Human Subjects Division (HSD) has determined that your research qualifies for exempt status in accordance with the federal regulations under 45 CFR 46.101/ 21 CFR 56.104. Details of this determination are as follows:

Exempt category determination: 2

Determination period: **8/29/2011 - 8/28/2016**.

Although research that qualifies for exempt status is not governed by federal requirements for research involving human subjects, investigators still have a responsibility to protect the rights and welfare of their subjects, and are expected to conduct their research in accordance with the ethical principles of *Justice, Beneficence* and *Respect for Persons, as* described in the Belmont Report, as well as with state and local institutional policy.

Determination Period: An exempt determination is valid for five years from the date of the determination, as long as the nature of the research activity remains the same. If there is any substantive change to the activity that has determined to be exempt, one that alters the overall design, procedures, or risk/benefit ratio to subjects, the exempt determination will no longer be valid. Exempt determinations expire automatically at the end of the five-year period. If you complete your project before the end of the determination period, it is not necessary to make a formal request that your study be closed. Should you need to continue your research activity beyond the five-year determination period, you will need to submit a new *Exempt Status Request* form for review and determination *prior to implementation*.

Revisions: Only modifications that are deemed “minor” are allowable, in other words, modifications that do not change the nature of the research and therefore do not affect the validity of the exempt determination. **Please refer to the Guidance document for more information about what are considered minor changes.** If changes that are considered to be “substantive” occur to the research, that is, changes that alter the nature of the research and therefore affect the validity of the exempt determination, a new *Exempt Status Request* must be submitted to HSD for review and determination *prior to implementation*.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events or any problem that may increase the risk to the human subjects and change the category of review, notify HSD promptly. Any complaints from subjects pertaining to the risk and benefits of the research must be reported to HSD.

Please use the HSD study number listed above on any forms submitted which relate to this research, or on any correspondence with the HSD office.

Good luck in your research. If we can be of further assistance, please contact us at (206) 543-0098 or via email at hsdinfo@uw.edu. Thank you for your cooperation.

Sincerely,

Katy Sharrock
Human Subjects Review Coordinator
(206) 616-5576
sharrock@u.washington.edu

Appendix B: Cover Letter

Dear Lakeside Resident,

During the summer months Washington State lakes may experience blooms of algae. These blooms appear as dense green specks in the water or green sheens on the surface that look like spilled paint. The algae that produce these blooms also produce toxins that can make you or your pet sick with close or prolonged contact with the water.

Jesse Billingham, a graduate student at the University of Washington is investigating the risk these algae blooms pose to your health. Included in this mailer is a short questionnaire asking about the type and frequency of recreational activities you participate in on lakes. **You will not be identified by your responses, and you will remain anonymous.** This is a single questionnaire with no follow up commitments on your part.

Understanding how residents use Washington State lakes will help determine the risk of exposure to algal toxins and guide future management and outreach practices to safeguard your health. Your participation in the project would be greatly appreciated. An addressed stamped envelope is included for your convenience. For questions about the project, questionnaire or algae blooms in general contact Jesse at the email listed below.

If you would like further information about algae blooms please visit:

Washington State Department of Ecology
www.ecy.wa.gov
Snohomish County (click on Lakes)
www1.co.snohomish.wa.us
Or contact Jesse at
jwb24@uw.edu

Thank you very much for your consideration.

J. Scott Meschke
Associate Professor
Department of Environmental and Occupational Health Sciences
University of Washington

Appendix C: Questionnaire

What Lake do you live near OR recreate in most often? ____________________________

(this will be referred to as "*your lake*" throughout this survey)

In your household who is?	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
Over 18 years old?	Y N	Y N	Y N	Y N	Y N
Male or Female?	M F	M F	M F	M F	M F

Please answer the following questions about yourself and members of your household:

Since June, how many days has each member of your household:	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
Gone fishing in your lake?					
Gone boating in your lake?					
Gone swimming in your lake?					
Waded or played in your lake?					
For a typical day what is the average time (in hours) each person spent:	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
Fishing in your lake?					
Boating in your lake?					
Swimming in your lake?					
Wading/playing in your lake?					

Please check the months in which members of your household participated in the following at your lake:												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Fishing?												
Boating?												
Swimming?												
Wading/playing?												

If you fish, do you fish: (check all that apply)	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
From the shore or a dock?	☐	☐	☐	☐	☐
From a boat?	☐	☐	☐	☐	☐
While standing in the water?	☐	☐	☐	☐	☐
If standing in water, are waders worn?	Y N	Y N	Y N	Y N	Y N
If someone fishes in your lake, what do they do with the fish? (check all that apply)	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
Catch and release?	☐	☐	☐	☐	☐
Eat?	☐	☐	☐	☐	☐
Give away?	☐	☐	☐	☐	☐

Compost, other disposal?	☐	☐	☐	☐	☐
Approximately how many fish caught in your lake did each person eat since June?	**Yourself**	**Person 2**	**Person 3**	**Person 4**	**Person 5**
What species of fish from your lake were caught and eaten? (please list)					

Please answer the following questions about your pets:

How many dogs do you have?				
Do your dogs: (check all that apply)				
Play/wade/swim in your lake?	☐			
Drink water from your lake?	☐			
Since June how often did your dog(s): (circle one)				
Play/wade/swim in your lake?	Daily	Weekly	Monthly	
Drink water from your lake?	Daily	Weekly	Monthly	

Please answer the following questions about your concern for your lake:

	Please Circle Answers
Have you ever had concerns about water quality in your lake?	Yes No
If Yes, what caused your concerns? (circle all that apply)	lake looked dirty bad smell warning signs posted neighbor expressed concern other____________________
If Yes, did those concerns affect you or your household doing the following activities at your lake:	
fishing?	Yes No
boating?	Yes No
swimming?	Yes No
wading/playing?	Yes No
Have you ever heard of or seen warnings about harmful algae in your lake?	Yes No
If Yes, How did you learn about such warnings? (Circle all that apply)	email mail television radio warning signs newspaper other__________

Appendix D: Causes of Water Quality Concerns by Household

	WQ Concerns	Aesthetic Issues (odor & visual)		Warning Signs Posted		Neighbors or Community Meetings		Plant Growth or Milfoil		Algae		Health Concerns[a]		Other[b]	
Lake	Count	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Cochran	2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	100.0%
Crabapple	5	5	100.0%	0	0.0%	0	0.0%	0	0.0%	1	20.0%	0	0.0%	0	0.0%
Howard	10	4	40.0%	0	0.0%	0	0.0%	2	20.0%	1	10.0%	1	10.0%	2	20.0%
Ketchum	29	20	69.0%	24	82.8%	6	20.7%	0	0.0%	7	24.1%	1	3.4%	0	0.0%
Loma	14	9	64.3%	7	50.0%	4	28.6%	1	7.1%	3	21.4%	0	0.0%	0	0.0%
Lost	8	4	50.0%	0	0.0%	2	25.0%	0	0.0%	2	25.0%	0	0.0%	1	12.5%
Martha North	4	3	75.0%	0	0.0%	1	25.0%	1	25.0%	0	0.0%	0	0.0%	0	0.0%
Pine	25	12	48.0%	0	0.0%	4	16.0%	2	8.0%	9	36.0%	0	0.0%	3	12.0%
Serene	18	10	55.6%	0	0.0%	2	11.1%	5	27.8%	1	5.6%	1	5.6%	2	11.1%
Steel	18	15	83.3%	3	16.7%	4	22.2%	0	0.0%	2	11.1%	1	5.6%	0	0.0%
Wilderness	14	10	71.4%	9	64.3%	3	21.4%	2	14.3%	2	14.3%	0	0.0%	0	0.0%
Total	147	92	62.6%	43	29.3%	26	17.7%	13	8.8%	28	19.0%	4	2.7%	10	6.8%

[a] Infections, swimmers itch

[b] Development, animal feces, water fowl, lakeshore fires, general quality, stagnant, oil, uneducated neighbors

Appendix E: Event Frequency

Event frequency (days per June-September) by lake

	Lake	N	Percent Reported [a]	Mean	Min	Max	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	Cochran	12	85.7%	10.83	2	20	6	16	6.38	1.84
	Crabapple	13	65.0%	15.69	3	30	8	25	10.13	2.81
	Howard	22	66.7%	12.36	1	60	3	20	15.25	3.25
	Ketchum	5	12.5%	2.20	1	5	1	2	1.64	0.73
	Loma	8	33.3%	3.50	1	10	2	5	3.07	1.09
	Lost	6	27.3%	10.50	1	25	2	25	11.34	4.63
	Martha North	22	81.5%	11.36	2	30	4	15	9.32	1.99
	Pine	57	71.3%	10.33	1	45	3	10	11.84	1.57
	Serene	28	56.0%	7.89	1	20	3	10	6.21	1.17
	Steel	25	73.5%	11.44	2	35	6	15	8.25	1.65
	Wilderness	31	81.6%	9.84	1	60	3	10	13.15	2.36
	Total	**229**	**59.9%**	**10.30**	**1**	**60**	**3**	**12**	**10.72**	**0.71**
Fishing	Cochran	12	85.7%	6.83	1	30	3	8	7.76	2.24
	Crabapple	8	40.0%	3.50	1	10	1	5	3.12	1.10
	Howard	13	39.4%	9.38	1	35	2	20	10.77	2.99
	Ketchum	15	37.5%	11.80	2	20	4	20	7.44	1.92
	Loma	10	41.7%	9.70	3	30	4	12	8.90	2.81
	Lost	9	40.9%	8.33	1	30	1	4	12.33	4.11
	Martha North	18	66.7%	8.56	1	15	5	12	4.66	1.10
	Pine	21	26.3%	9.05	1	47	2	8	13.15	2.87
	Serene	11	22.0%	3.45	1	10	1	4	2.94	0.89
	Steel	12	35.3%	5.08	1	15	2	6	4.87	1.41
	Wilderness	13	34.2%	3.46	1	15	1	3	3.73	1.04
	Total	**142**	**37.2%**	**7.55**	**1**	**47**	**2**	**10**	**8.54**	**0.72**
Wading/ Playing	Cochran	11	78.6%	12.82	2	30	5	20	8.47	2.55
	Crabapple	13	65.0%	26.54	1	50	8	50	21.27	5.90
	Howard	15	45.5%	12.67	1	40	3	20	12.34	3.19
	Ketchum	5	12.5%	2.20	1	5	1	2	1.64	0.73
	Loma	7	29.2%	19.00	1	60	1	60	28.04	10.60
	Lost	3	13.6%	2.00	2	2	2	2	0.00	0.00
	Martha North	17	63.0%	27.12	1	120	10	30	36.43	8.84
	Pine	41	51.3%	11.88	1	45	5	10	12.83	2.00
	Serene	24	48.0%	7.42	2	25	3	10	6.57	1.34
	Steel	26	76.5%	11.85	1	60	5	15	12.33	2.42
	Wilderness	26	71.1%	7.81	1	60	2	10	11.93	2.30
	Total	**188**	**49.5%**	**13.14**	**1**	**120**	**3**	**15**	**17.56**	**1.28**
Boating	Cochran	14	100.0%	11.64	2	25	8	15	6.36	1.70
	Crabapple	11	55.0%	12.00	2	20	3	15	6.53	1.97
	Howard	23	69.7%	6.83	1	40	2	6	8.49	1.77
	Ketchum	10	25.0%	8.80	1	20	3	20	7.98	2.52
	Loma	17	70.8%	14.00	2	60	3	15	15.29	3.71
	Lost	15	68.2%	11.80	1	30	2	30	12.26	3.17
	Martha North	20	74.1%	11.75	1	40	5	20	11.91	2.66
	Pine	63	78.8%	15.03	1	75	4	20	16.16	2.04
	Serene	34	68.0%	6.82	1	40	2	8	7.38	1.26
	Steel	28	82.4%	7.82	2	22	3	10	5.95	1.12
	Wilderness	32	84.2%	15.59	3	90	5	14	20.15	3.56
	Total	**267**	**69.9%**	**11.56**	**1**	**90**	**3**	**15**	**13.19**	**0.81**

[a] N/total number of individuals reporting swim days per lake

Event frequency (days per June-September) by gender

	Gender	Individuals [a] Represented	Percent Reported	N	Mean	Min.	Max.	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	**Male**	182	59.34%	108	11.9	1	167	3	15	18.36	1.77
	Female	186	61.83%	115	10.6	1	60	3	12	11.09	1.03
Fishing	**Male**	182	48.35%	88	8.1	1	47	2	10	8.98	0.96
	Female	186	26.88%	50	6.6	1	43	2	8	7.93	1.12
Wading/Playing	**Male**	182	49.45%	90	11.9	1	60	3	15	13.97	1.47
	Female	186	51.61%	96	14.5	1	120	3	18	20.48	2.09
Boating	**Male**	182	70.88%	129	12.8	1	75	4	15	13.57	1.19
	Female	186	69.89%	130	10.8	1	90	3	15	13.04	1.14

[a] total number of individuals reporting their gender

Event frequency (days per June-September) by age

	Age	Individuals [a] Represented	Percent Reported	N	Mean	Min.	Max.	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	**< 18**	45	88.9%	40	16.83	1	60	5	30	13.99	2.21
	≥ 18	325	56.3%	183	10.01	1	167	3	10	15.01	1.11
Fishing	**< 18**	45	55.6%	25	5.08	1	15	2	5	4.79	0.96
	≥ 18	325	34.5%	112	8.04	1	47	2	10	9.19	0.87
Wading/Playing	**< 18**	45	77.8%	35	19.57	1	120	5	30	23.38	3.95
	≥ 18	325	47.1%	153	11.67	1	120	2	12	15.67	1.27
Boating	**< 18**	45	82.2%	37	9.54	2	50	3	10	11.81	1.94
	≥ 18	325	69.8%	227	11.98	1	90	4	15	13.46	0.89

[a] total number of individuals reporting their age

Appendix F: Event Duration

Event duration (hours per event) by lake

	Lake	N	Percent Reported [a]	Mean	Min	Max	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	Cochran	11	78.6%	1.18	0.50	3	1.00	1.00	0.72	0.22
	Crabapple	18	90.0%	0.87	0.25	2	0.30	1.00	0.68	0.16
	Howard	22	66.7%	0.93	0.10	3	0.50	1.00	0.66	0.14
	Ketchum	5	12.5%	0.70	0.25	1	0.25	1.00	0.41	0.18
	Loma	7	29.2%	0.96	0.25	2	0.50	1.00	0.55	0.21
	Lost	5	22.7%	0.70	0.50	1	0.50	1.00	0.27	0.12
	Martha North	22	81.5%	1.62	0.10	6	1.00	2.00	1.53	0.33
	Pine	57	71.3%	1.16	0.10	3	0.50	2.00	0.86	0.11
	Serene	24	48.0%	1.03	0.30	4	0.50	1.00	0.94	0.19
	Steel	24	70.6%	1.02	0.25	3	0.50	1.00	0.76	0.15
	Wilderness	27	71.1%	1.40	0.20	3	1.00	2.00	0.74	0.14
	Total	222	58.1%	1.05	0.10	6	0.60	1.27	0.74	0.18
Fishing	Cochran	12	85.7%	1.79	1.00	3	1.25	2.00	0.58	0.17
	Crabapple	12	60.0%	2.04	0.50	4	1.00	3.50	1.36	0.39
	Howard	14	42.4%	2.82	0.50	8	1.00	4.00	2.00	0.53
	Ketchum	13	32.5%	3.08	1.00	5	2.00	4.00	1.26	0.35
	Loma	12	50.0%	2.00	1.00	4	1.00	2.50	0.90	0.26
	Lost	5	22.7%	1.80	1.50	2	1.50	2.00	0.27	0.12
	Martha North	18	66.7%	2.53	1.00	5	2.00	3.00	1.10	0.26
	Pine	22	27.5%	2.27	0.50	6	1.00	3.00	1.41	0.30
	Serene	8	16.0%	1.88	1.00	3	1.00	2.50	0.83	0.30
	Steel	12	35.3%	1.63	1.00	3	1.00	2.00	0.71	0.21
	Wilderness	11	28.9%	2.05	1.00	5	1.00	2.50	1.19	0.36
	Total	139	36.4%	2.17	0.50	8	1.25	2.82	1.06	0.30
Wading/ Playing	Cochran	8	57.1%	0.94	0.50	3	0.50	1.00	0.86	0.31
	Crabapple	17	85.0%	0.79	0.25	3	0.25	1.00	0.80	0.19
	Howard	11	33.3%	1.05	0.50	3	0.50	1.00	0.69	0.21
	Ketchum	3	7.5%	0.75	0.25	1	0.25	1.00	0.43	0.25
	Loma	6	25.0%	0.96	0.25	2	0.50	2.00	0.81	0.33
	Lost	3	13.6%	0.67	0.50	1	0.50	1.00	0.29	0.17
	Martha North	17	63.0%	1.13	0.10	3	1.00	1.00	0.82	0.20
	Pine	39	48.8%	1.24	0.20	5	0.50	1.00	1.15	0.18
	Serene	21	42.0%	1.05	0.03	4	0.50	1.00	0.83	0.18
	Steel	24	70.6%	1.18	0.25	3	0.50	1.00	0.90	0.18
	Wilderness	21	55.3%	1.11	0.10	2	1.00	2.00	0.67	0.15
	Total	170	44.5%	0.99	0.03	5	0.55	1.18	0.75	0.21
Boating	Cochran	13	92.9%	1.31	1.00	2	1.00	2.00	0.48	0.13
	Crabapple	15	75.0%	0.87	0.50	2	0.50	1.00	0.40	0.10
	Howard	25	75.8%	1.37	0.50	4	1.00	2.00	0.85	0.17
	Ketchum	6	15.0%	2.67	2.00	3	2.00	3.00	0.52	0.21
	Loma	17	70.8%	1.65	0.30	4	1.00	2.50	1.01	0.25
	Lost	12	54.5%	1.29	0.50	2	0.75	2.00	0.62	0.18
	Martha North	20	74.1%	1.35	1.00	3	1.00	2.00	0.59	0.13
	Pine	67	83.8%	1.29	0.50	4	1.00	2.00	0.78	0.09
	Serene	30	60.0%	1.14	0.50	3	1.00	1.00	0.64	0.12
	Steel	23	67.6%	1.29	0.25	3	1.00	2.00	0.65	0.14
	Wilderness	29	76.3%	2.17	1.00	6	1.00	2.00	1.43	0.27
	Total	257	67.3%	1.49	0.25	6	1.02	1.95	0.72	0.16

[a] N/total number of individuals reporting swim days per lake

Event duration (hours per event) by gender

	Gender	Individuals [a] Represented	Percent Reported	N	Mean	Min.	Max.	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	**Male**	182	56.59%	103	1.04	0	4	1	1	0.78	0.08
	Female	186	58.60%	109	1.13	0	3	1	1	0.76	0.07
Fishing	**Male**	182	45.60%	83	2.18	1	8	1	3	1.20	0.13
	Female	186	27.42%	51	2.12	1	6	1	3	1.17	0.16
Wading/Playing	**Male**	182	44.51%	81	1.07	0	5	1	1	0.89	0.10
	Female	186	46.24%	86	1.09	0	5	1	1	0.88	0.09
Boating	**Male**	182	68.13%	124	1.46	0	6	1	2	0.92	0.08
	Female	186	66.13%	123	1.40	0	6	1	2	0.87	0.08

[a] total number of individuals reporting their gender

Event duration (hours per event) by age

	Age	Individuals [a] Represented	Percent Reported	N	Mean	Min.	Max.	Percentile 25	Percentile 75	Standard Deviation	Standard Error
Swimming	**< 18**	45	80.0%	36	1.35	0	4	1	2	1.02	0.17
	≥ 18	325	53.5%	174	1.00	0	3	1	1	0.69	0.05
Fishing	**< 18**	45	44.4%	20	1.60	1	4	1	2	0.79	0.18
	≥ 18	325	34.2%	111	2.21	1	8	1	3	1.19	0.11
Wading/Playing	**< 18**	45	64.4%	29	1.48	0	5	1	2	1.37	0.25
	≥ 18	325	43.1%	140	0.99	0	3	1	1	0.72	0.06
Boating	**< 18**	45	73.3%	33	1.27	1	4	1	2	0.73	0.13
	≥ 18	325	66.5%	216	1.41	0	6	1	2	0.88	0.06

[a] total number of individuals reporting their age