University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

U.S. Environmental Protection Agency Papers | U.S. Environmental Protection Agency

2008

Cyanobacterial Harmful Algal Blooms: Chapter 26: Human Health Effects Workgroup Report

Sandra Azevedo
Federal University of Rio de Janeiro

Neil Chernoff
USEPA

Ian Falconer
University of Adelaide

Michael Gage

Elizabeth Hilborn
USEPA

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/usepapapers

 Part of the Civil and Environmental Engineering Commons

Azevedo, Sandra; Chernoff, Neil; Falconer, Ian; Gage, Michael; Hilborn, Elizabeth; Hooth, Michelle; Jensen, Karl; MacPhail, Robert; Rogers, Ellen; Shaw, Glen; and Stewart, Ian, "Cyanobacterial Harmful Algal Blooms: Chapter 26: Human Health Effects Workgroup Report" (2008). *U.S. Environmental Protection Agency Papers*. 43.
https://digitalcommons.unl.edu/usepapapers/43

This Article is brought to you for free and open access by the U.S. Environmental Protection Agency at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in U.S. Environmental Protection Agency Papers by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Sandra Azevedo, Neil Chernoff, Ian Falconer, Michael Gage, Elizabeth Hilborn, Michelle Hooth, Karl Jensen, Robert MacPhail, Ellen Rogers, Glen Shaw, and Ian Stewart

This article is available at DigitalCommons@University of Nebraska - Lincoln: https://digitalcommons.unl.edu/usepapapers/43

Published in Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, ed. H. Kenneth Hudnell (Springer, 2008)

Chapter 26: Human Health Effects Workgroup Report

Workgroup Co–Chairs: Elizabeth D Hilborn, John W Fournie

Workgroup Members[1]**:** Sandra MFO Azevedo, Neil Chernoff, Ian R Falconer, Michelle J Hooth, Karl Jensen, Robert MacPhail, Ian Stewart

Authors: Sandra MFO Azevedo, Neil Chernoff, Ian R Falconer, Michael Gage, Elizabeth D Hilborn, Michelle J Hooth, Karl Jensen, Robert MacPhail, Ellen Rogers, Glen R Shaw, Ian Stewart

Introduction

Two types of approaches may be used to evaluate the toxicity of environmental agents:

- Observational population studies may be used to investigate humans or animals in contact with the agent of interest, either prospectively or retrospectively.
- Experimental studies may be performed under controlled conditions on animals, living tissues or cells. These results may be extrapolated to human and animal populations.

Observational epidemiological studies of human and animal populations have the advantage of investigating the effects of environmentally relevant exposures to naturally–occurring mixtures of toxins. Health effects may be identified in the target species of concern. These studies are difficult to implement however, as monitoring of cyanobacteria toxin occurrence in ambient water is essential to document exposure status, and specific associations between exposure and effect are difficult to establish in free–living populations.

[1] See workgroup member affiliations in Invited Participants section.

For author affiliations, see following page.

Human Health Effects Workgroup

*Workgroup Members**

John W Fournie, Co-chair
USEPA
fournie.john@epa.gov

Elizabeth D Hilborn, Co-chair
USEPA
hilborn.e@epa.gov

Sandra Azevedo
Federal University of Rio de Janeiro
sazevedo@biof.ufrj.br

Neil Chernoff
USEPA
chernoff.neil@epa.gov

Ian Falconer
Pharmacology Department
University of Adelaide
+62 2 6251 1345

Michelle Hooth
National Institute of Environmental Health Science
hooth@niehs.nih.gov

Karl Jensen
USEPA
Jensen.karl@epa.gov

Robert MacPhail
Neurotoxicology
USEPA
macphail.robert.epa.gov

Ian Stewart
Research Fellow
School of Public Health
Griffith University
and
Research Officer
Organic Chemistry
Queensland Health Scientific Services
39 Kessels Road
COOPERS PLAINS
QLD 4108, Australia

*Michael Gage, Ellen Rogers, and Glen Shaw also contributed to the Workgroup Report.

Invited Speakers on Human Health Effects

Ian Falconer
(see above)

Ian Stewart
(see above)

Louis Pilotto
Faculty of Medicine
University of New South Wales
+61 (2) 69335111
l.pilotto@unsw.edu.au

Health studies of standard test species in controlled environments address some of the difficulties described above, but they often involve the use of species other than those of primary interest. Laboratory studies may not closely approximate the toxin, dose or duration of exposure relevant to environmental conditions. The effects of mixtures of natural materials and toxins may be difficult to reproduce during repeated experiments. The advantages and disadvantages of these two strategies reveal that each may complement and enhance the other. Neither approach is complete in itself. Both are essential to the understanding of the potential effects of cyanobacterial toxins on human and animal populations.

The use of laboratory animals in toxicology is based on the premise that the data obtained during controlled exposures may be extrapolated with some degree of confidence to other species including our own. Cyanobacterial toxins are a heterogeneous group of compounds with unrelated structures, toxic endpoints, and mechanisms of action. Cyanobacterial toxins include hepatotoxins, renal toxins, immunotoxins, neurotoxins, skin irritants and sensitizers, although mechanisms of pathogenesis are not fully understood. Future cyanobacterial toxicity studies must consider the following components:

1. Selection of test species. The science of toxicology is replete with examples of highly significant inter–species variability. Differences in susceptibility to acute toxicity (e.g. TCDD), carcinogenicity (e.g. aflatoxin), and teratogenicity (e.g. thalidomide) are well documented. The response of different test species to a given dose of cyanobacterial toxin will depend onspecies–related metabolic/ toxicokinetic characteristics and differences in sensitivity to the agent studied (toxicodynamics). The majority of studies have used the mouse because it is the smallest rodent test species and therefore requires the least amount of toxin. Rodents are commonly used test animals but other animals must be studied in order to address the occurrence of specific toxicity endpoints across species. Determination of the "appropriate" test species will therefore depend on the information known about the site(s) of action and metabolism of the study agent. Although these factors are critical to understanding the effects of cyanobacterial toxins, there are major gaps in our knowledge of these factors for most of the toxins. Given these data gaps, the generally accepted testing strategy is to utilize multiple test species and to compare the results obtained in each species with those known to occur in the target species of concern.
2. Duration of exposure. Studies need to be conducted that approximate environmental exposures to cyanobacterial toxins. This may involve

multiple study designs as free–living populations may be exposed to cyanobacterial toxins chronically at low doses, and/or may experience episodic short–term exposures to high doses. These different exposure scenarios may, in turn, result in different spectrums of toxicity. For example, the data from cylindrospermopsin studies (Hawkins et al. 1997) have shown that the acute LD50 is 2000 ug/kg, contrasted with the 5–day LD50 of only 200 ug/kg. This suggests that the mortality seen in the two exposure groups of animals may be the result of different types of injury, one type being acute, and the other cumulative. Adequate characterization of cyanobacterial–induced toxicity should include a range of exposure scenarios.

3. Route of exposure. Oral, inhalation, and cutaneous exposures to cyanobacterial toxins may be the most likely routes of exposure among human and animal populations. Two episodes of human microcystin exposure by the intravenous route have been documented among patients undergoing dialysis (Jochimsen et al. 1998, Soares et al. 2006). In contrast, most animal toxicology studies have reported health effects associated with intraperitoneal (i.p.) exposure to cyanobacterial toxins. Expense and lack of sufficient test material are the primary reasons for most cyanobacterial toxin studies having been performed using the i.p. exposure route. The oral route of administration has been prohibitively expensive for extended studies since the toxins are far less toxic orally than by the i.p. route (generally by factors of 10 or more). Future laboratory animal studies should include oral, inhalation, and cutaneous exposures to evaluate effects that may be associated with these environmentally relevant exposure routes.

4. Characterization of cyanobacterial toxins and effects of mixtures. Multiple cyanobacteria genera produce the same toxin, and some produce multiple toxins. Many cyanobacterial toxins have yet to be identified and/or characterized. The production of cyanobacterial toxins can be highly variable, and factors associated with toxin gene expression are poorly understood. *Cylindrospermopsis raciborskii*, for example, produces widely varying amounts of cylindrospermopsin depending upon its geographical location and environmental conditions. A recently described cyanobacterial toxin is β–N–methylamino–L–alanine (BMAA). This compound may be associated with adverse neurological effects in humans. It is possible that BMAA occurs in multiple species of cyanobacteria including some that occur in fresh water (Cox et al. 2005). Microcystins may vary temporally, spatially and chemically within a water body, and

microcystin variants differ significantly in their potential to induce mammalian toxicity (Sivonen and Jones 1999). Experimental evidence also indicates that any given bloom may produce more than one cyanobacterial toxin and the resultant toxicity may be the result of additive and/or synergistic effects among these agents. Studies on cyanobacterial cellular extracts containing either anatoxin–a or cylindrospermopsin have shown that the resulting toxicity was not directly proportional to the amount of toxin in the test material itself (Stevens and Krieger 1991; Falconer et al. 1999). In both reports, the authors hypothesized the existence of other, as yet unidentified toxins that were contributing to the observed adverse effects.

Studies designed to assess the effects of purified cyanobacterial toxins such as microcystin–LR, anatoxin–a, and cylindrospermopsin, are needed to: characterize toxicokinetics and toxicodynamics, develop biomarkers of exposure and effect, and study specific health effects. However, this approach is hindered by a lack of toxin standards, and the fact that the study of individual toxins does not approximate environmentally relevant exposures. The use of cellular extracts from cyanobacterial clonal cultures addresses these limitations. Cultures can be characterized, batched and used for multiple studies among laboratories. The use of these cultures would allow effects research to proceed in the absence of toxin standards, and may be more representative of the spectrum and mix of toxins to which human and animal populations are exposed.

Cyanobacteria occurrence is generally increasing as a result of eutrophication and warming of surface waters. When human and domestic animal populations congregate around surface water sources their waste, and nutrients such as nitrogen and phosphorous, enters the water. Local populations may depend on surface waters to provide drinking water, irrigation, food fish, and recreation. However, as population densities increase, the occurrence of blooms increases, thus heightening the risk of human and animal exposure to cyanobacteria toxins. Given the potency, heterogeneity, and documented occurrence of cyanobacterial toxins in US surface waters, they will increasingly be recognized as a serious public and environmental health concern.

Charge 1

What materials do we need to perform health effects research?

Ideally, investigators need well characterized, pure cyanobacterial toxins to perform toxicity and carcinogenicity studies. The results of studies us-

ing pure toxins are easier to interpret, and have fewer confounding variables. However, extended toxicity studies require significant quantities of toxin to dose sufficient numbers of animals by relevant routes of exposure (dermal, oral, and inhalation). These studies are necessary for risk assessment and the development of guidance values.

Currently the supply of pure toxin is limited. Only two oral dosing toxicity studies using pure microcystin (Fawell et al. 1994) and cylindrospermopsin (Humpage and Falconer 2003) have been implemented to date. However, the impact of these types of studies is significant. The Fawell study was used by the World Health Organization (WHO) as the basis for a Guideline Value determination for microcystins. The cylindrospermopsin study is currently under review by WHO. Despite the importance of these types of studies, few are conducted because sufficient quantities of purified toxin are not currently available. There are published chemical synthesis methods for microcystins, including production of the 3–amino–9–methoxy–2,6,8–trimethyl–10–phenyl–4,6–decadienoic acid (ADDA) residue which has been successfully synthesized (Humphrey et al. 1996; Candy et al.1999). However, no commercial production of pure cylindrospermopsin in bulk has been reported. Although research groups have reported chemical methods to synthesize cylindrospermopsin (Xie et al. 2000), this method is prohibitively expensive due to the 20 steps required for synthesis.

Few toxicology studies using pure toxins have been conducted in species other than mice. Because of the limited supply of pure toxin, mice are preferred as an animal model because of their size. However, they may not be the best model for every target species or system of interest. Alternatively, studies with thoroughly characterized toxic extracts of cyanobacteria have yielded valuable data for domestic animals, including a study with growing pigs which was also used by WHO in the derivation of the human drinking water guideline (Falconer et al. 1994).

Pure toxin isolated from live cultures of cyanobacteria is available privately and commercially in limited amounts allowing the conduct of mechanistic studies which require less material. Some investigators have used *C. raciborskii* cultures (Hawkins et al. 1997; Chiswell et al. 1999; Shaw et al. 2000). Cylindrospermopsin has been purified from *C. raciborskii* cultures using high performance liquid chromatography (HPLC) (Chiswell et al. 1999). Other investigators have conducted studies with highly purified cylindrospermopsin isolated from a bloom of *Umezakia natans* collected from Lake Mikata, Fukui, Japan (Harada et al. 1994; Terao et al. 1994). Likewise, studies with approximately 95% pure microcystin–LR have been conducted with toxin produced from *Microcystis aeruginosa*, laboratory strain 7820, and purified by HPLC (Hooser et al.

1990). Limited quantities of characterized microcystin variants LA, LR, RR, and YR, cylindrospermopsin and nodularin are available commercially from Sigma–Aldrich (St. Louis, MO, US) and microcystin variants LF, LR, LW, and RR from EMD Biosciences (San Diego, CA, US). Although the production method is not disclosed, these materials are suspected to have been isolated from live cultures. Regardless of source, it is always important to verify the identity and purity of these toxins, as investigators have reported inconsistencies in research results among different lots of toxins.

Freeze–dried natural bloom material is more widely available in larger quantities for research studies. Dr. Ian Falconer and his colleagues have conducted long–term microcystin toxicity and cancer promotion studies in mice with extracts from dried natural bloom material that was from a batch collected at Lake Mokoan in Victoria, Australia and from Malpas Dam in New South Wales, Australia (Falconer and Humpage 1996). Both sources of material have been characterized and used in previous research (Falconer et al. 1988, 1994). Ten peaks exhibiting the characteristic absorbance spectrum of microcystins were detected using HPLC analysis. However, none of these peaks corresponded to microcystins for which standards were available (microcystin–LR, –YR, and –RR). Bloom materials offer the advantage of reflecting real–world exposure scenarios. However, these materials have to be carefully evaluated for use in studies that may be included in risk assessments. Bloom materials may not be completely characterized and the toxicity of the mixture may be greater than that of the identified individual components indicating that: 1) the mixture may contain additional unidentified toxins; 2) that there may be a synergistic effect among bioactive components of the bloom material.

Since the lack of availability of pure cyanobacterial toxins is currently limiting the ability to perform health effects research, this workgroup strongly recommends the development and maintenance of bulk clonal cyanobacteria cultures shared among laboratories to facilitate screening, bioassay development and long–term exposure studies. Extensive chemical and bioassay characterization of the test materials is needed to ensure appropriate interpretation of study results. Due to the hazardous nature of these materials, clarification and standardization of the import/export regulations is needed to facilitate the use of shared cyanobacterial test materials among laboratories (Metcalf et al. 2006).

Charge 1: What materials do we need to perform health effects research?

Near-term Research Priorities

- Develop and maintain bulk clonal cyanobacteria cultures that can be shared among laboratories to facilitate screening, bioassay development and long–term exposure studies.

Long-term Research Priorities

- Develop lower cost methods of chemical synthesis to produce sufficient quantities of standard cyanobacterial toxins.

Charge 2

What are the health effects associated with chronic and episodic exposures?

Risk assessments of the effects of cyanobacterial toxins on human and animal health require information produced during studies of chronic and episodic exposures, and are insufficient if based solely on studies of acute exposures and lethal endpoints. However, much of our knowledge of toxicity rests upon acute exposures: poisoning of animals in the natural environment, and laboratory animal studies of lethal endpoints. Considerable advances have been made, however, in our understanding of the sub-lethal effects of cyanobacterial toxins, and this must become the focus of research in order to insure continued progress in the understanding of the risks associated with cyanobacterial toxin exposures.

There are strong experimental studies that demonstrate the tumor promotion activity of microcystins and nodularin, and well–designed genotoxicity studies that show cylindrospermopsin to be a potential carcinogen (Falconer 2005). To perform effective risk assessment it is essential to carry out carcinogenicity studies on both groups of cyanobacterial toxins. The present limitation to both National Toxicology Program (NTP) protocol studies is the lack of available purified toxin. It is preferable to use highly characterized toxic extracts of cultures rather than to indefinitely defer these essential carcinogenicity trials, as humans may be currently exposed to cylindrospermopsin via drinking and recreational water exposure.

The highest priority work is to implement carcinogenicity trials with cylindrospermopsin because of the structure of the toxin, preliminary evidence of carcinogenicity, and the current strength of evidence of genotoxicity in a human lymphoblastoid cell line (Humpage et al. 2000a). An *in vivo* study conducted by Falconer and Humpage provided preliminary evidence of the carcinogenicity of cylindrospermopsin in mice (Falconer and Humpage 2001).

Neurodevelopmental studies are required for all groups of cyanobacterial toxins. Currently little is known about neurodevelopmental toxicity. Early work with chronic *Microcystis* extract exposure to male and female mice throughout pregnancy showed cytotoxicity in the hippocampus of neonatal offspring (Falconer et al. 1988). These studies are especially relevant as there is the potential for neonates to be exposed via drinking water in formula during a period of their rapid neurologic development.

Immunomodulation and immunosuppression after exposure to microcystins has been documented (Shen et al. 2003; Shi et al. 2004; Chen et al. 2005). Adverse effects on human leukocytes *in vitro* have been reported at very low doses (Hernandez et al. 2000). The potential immunotoxic effects of microcystin in human and animal populations are uncharacterized. To our knowledge, no studies of the potential immunotoxic effects of cylindrospermopsin have been reported.

There is no strong evidence to date for the teratogenicity of microcystin and anatoxin–a (Chernoff et al. 2002, Rogers et al. 2005, MacPhail et al. 2005). Teratogenicity studies of cylindrospermopsin are underway. Multigenerational studies are needed for most toxins although the implementation of these will need to be deferred until sufficient toxin or characterized clonal bloom material is available.

Charge 2: What are the health effects associated with chronic and episodic exposures?

Near-term Research Priorities

- Conduct carcinogenicity studies with cylindrospermopsin.
- Conduct neurodevelopmental studies for all groups of cyanobacterial toxins.
- Conduct immunotoxicology studies for all groups of cyanobacterial toxins.

- Conduct studies of the effects of acute, episodic and chronic exposures at sublethal concentrations.
- Implement toxicological studies using oral, inhalation, and cutaneous exposure routes.

Long-term Research Priorities

- Implement multigenerational studies for all groups of cyanobacterial toxins.

Charge 3

What are the health effects associated with environmental mixtures of toxins?

In naturally occurring cyanobacterial blooms, a mixture of toxins is present. Most commonly, these are mixtures of microcystins when the bloom is *Microcystis* or *Planktothrix* or of cylindrospermopsins when the bloom is *Cylindrospermopsis, Aphanizomenon, Raphidiopsis* or *Umezakia*. Anatoxin–a and anatoxin–a(s) are uncommonly found occurring with microcystins or cylindrospermopsins in the environment, but when this mixture does occur, synergistic effects are possible. Bloom toxicity therefore represents the combined toxicities of the constituent toxin variants present, and can best be expressed as toxicity equivalents (Falconer 2005).

Under natural circumstances eutrophic water bodies form a sequence of toxic blooms that appear to be determined primarily by water temperature. An observed sequence in Australia is neurotoxic *Anabaena*, followed by hepatotoxic *Microcystis*, followed by cytotoxic *Cylindrospermopsis* in late summer (Bowling 1994). Populations can then potentially be exposed to saxitoxins, followed by successive hepatotoxins with different mechanisms of action.

Effective risk assessment of natural toxic bloom events requires a systematic assessment of mixtures of toxins for which there is currently no data. We have little information about the health effects of exposure to concurrent or sequential mixtures. Monitoring of cyanobacteria toxin occurrence in environmental samples is essential to detecting and understanding potentially associated health effects of exposure to mixtures of toxins.

Risk assessment of populations exposed to natural blooms needs to take into consideration a range of pathological effects generated by different cyanobacterial toxins, in sequence and in combination. These may include measures of hepatic, immune system, and kidney impairment (Falconer 2005). In human populations, neurological impairment can be measured by the study of the neurodevelopment of young children and with a variety of standard tests among adults. Cancer registries may be used to assess potential carcinogenetic effects of cyanobacterial toxins if large populations are found to be exposed.

Charge 3: What are the health effects associated with environmental mixtures of toxins?

Near-term Research Priorities

- Implement toxicology studies of animal models exposed to mixtures of cyanobacterial toxins to evaluate toxin synergism.
- Implement epidemiologic studies of exposed populations to assess a variety of cyanobacterial toxin–associated health effects.
- Perform risk assessments of populations exposed to natural blooms and multiple cyanobacterial toxins, in sequence and in combination.

Charge 4

What research is needed to better understand the public health effects of exposures to cyanobacteria?

A well–designed study of the health effects associated with cyanobacteria exposure in human populations requires comprehensive exposure assessment. Currently, the United States does not routinely monitor drinking or recreational waters for the presence of cyanobacterial toxins despite the reported presence in these waters of potentially toxic blooms. Therefore, the magnitude of the risk of cyanobacteria toxin exposure and associated effects to the US population is unknown. Human exposures to cyanobacterial toxins may be broadly categorized for the purpose of public health investigation into acute or chronic (episodic) exposures. Study methods

and approaches will vary depending upon the size of the population and the characteristics of exposure.

Acute exposures to cyanobacteria and their toxins may occur via the oral, dermal, inhalational or intravenous exposure routes. The most common exposures are believed to occur during recreational and occupational contact with cyanobacteria in lakes, rivers and marine waters (Osborne et al. 2001; Stewart et al. 2006). Acute, chronic, and episodic exposures may arise from drinking water (Annadotter et al. 2001; Ressom et al. 1994; Kuiper–Goodman et al. 1999; Duy et al. 2000; Falconer 2005), dietary intake via consumption of cyanobacterial toxin–contaminated foods (Negri and Jones 1995; Nagai et al. 1996; Codd et al. 1999; Saker and Eaglesham 1999; de Magalhães et al. 2001), and dietary supplements (Schaeffer et al. 1999; Gilroy et al. 2000; Lawrence et al. 2001; Saker et al. 2005). Acute human exposure has been documented via contamination of dialysate in hemodialysis clinics (Hindman et al. 1975; Carmichael et al. 2001; Azevedo et al. 2002; Soares et al. 2006). Outcomes may range from no apparent effect to serious morbidity or death associated with toxicosis. Non life–threatening allergic and allergic–like reactions (rhinitis, asthma, eczema, conjunctivitis) have been reported but are poorly quantified, as are acute illnesses (e.g. flu–like reactions, skin rashes) in (presumably) non–allergic individuals (Stewart et al. 2006).

Reports of acute illness have been received from workers sampling potentially toxic cyanobacteria blooms (Reich 2005). These ranged from anaphylactic–like reactions, to dermatitis, gastroenteritis, and respiratory irritation. However, published reports and descriptions of exposed and ill individuals (cases) and case series are needed as the literature is sparse (Turner et al. 1990). This information is needed to develop more specific case definitions, to describe the spectrum of cyanobacteria–associated health effects, and to identify potential susceptibility factors.

Chronic or episodic exposure to cyanobacterial toxins may occur when people are exposed occupationally or by their drinking water. The principal concern is that some cyanobacterial toxins, such as the hepatotoxins, may have carcinogenic potential. Some epidemiological investigations have suggested that hepatocellular carcinoma (HCC) and colorectal cancer rates were significantly higher in regions of China where consumption of untreated pond or ditch water was common when compared to rates in populations drinking deep well water (Ressom et al. 1994; Falconer 2005). However, the epidemiological evidence is contradictory; a recent retrospective study failed to identify a relationship between HCC and consumption of ditch water (Yu et al. 2002). China is a high–risk area for HCC, accounting for some 45% of worldwide mortality and HCC is associated with other risk factors such as chronic viral hepatitis and exposure to afla-

toxins (Ming et al. 2002; Yu and Yuan 2004; Shi et al. 2005). Therefore, cyanobacterial toxin exposure assessment is essential to the successful design and implementation of studies to investigate the risk of cancer associated with repeated exposure to these toxins.

Because of the lack of currently identified cyanobacteria toxin–specific health effects, we urgently need to develop specific biomarkers of exposure and effect to use in health studies. Biomarkers of exposure include the toxin itself, metabolites of the parent compound, and DNA or protein adducts specific to the toxin. Recently, the use of a simple colorimetric assay was proposed to screen human serum samples for the presence of microcystins (Hilborn et al. 2005). Work is in progress to identify cylindrospermopsin metabolites and possible adducts that can be used as human biomarkers of exposure (Humpage and Falconer 2005). Biomarkers of effect include: biochemical markers such as serum concentrations of liver transaminases, and quantification of micronuclei in leukocytes (Falconer et al. 1983; Humpage et al. 2000a). The use of biomarkers will strengthen the specificity of association between exposure to cyanobacterial toxins and health endpoints. The carcinogenicity of aflatoxin is now much better understood through the ability to measure aflatoxin B_1, its metabolites and DNA–adducts in urine (Ross et al. 1992; Qian et al. 1994); the epidemiology of cyanobacterial toxins awaits similar advances. Potential future areas for biomarker development include the use of DNA mutations and adducts as markers of genotoxicity. Targeted studies using genomic and proteomic techniques may be useful, however are frequently difficult to interpret. Currently, these approaches are best guided by and aligned with the results of traditional toxicologic studies.

Epidemiologic studies are needed to evaluate the effects of exposures to cyanobacteria and their toxins on human and animal health. Population–based observational study designs that assess cyanobacteria exposure retrospectively are probably the most cost–effective approaches to investigate cyanobacteria bloom–associated health effects at this time. Of particular importance are recent event–triggered investigations. Both acute and chronic health effects may be investigated. However, assigning exposure histories to individuals may be problematic, particularly to those individuals or populations with chronic or episodic exposures. Retrospective studies may lack the information to examine the temporal relationship between exposure and effect, and one design, the case–control study, requires specific case definitions which are not well defined at present.

Prospective population–based observational studies have a greater ability to associate exposure and effect due to improved exposure assessment, which is done before health effects occur. However this approach requires large numbers of exposed persons, is expensive, and time consuming. The

dynamic nature of cyanobacteria blooms and toxin production presents significant challenges for exposure characterization, study planning and implementation.

An experimental study design can provide a higher degree of the certainty of associations between exposure and effect. The principal advantage of a large, randomized, controlled exposure study is that random assignment to a study group minimizes the differences between exposure groups that may affect the interpretation of the study results. However, the specific details of any proposed randomized exposure trial would need to be closely reviewed for its scientific merits and for the protection of human subjects. Within human ethical guidelines, an experimental design could, in theory, be applied to the study of certain acute, low risk exposures to cyanobacteria. However, randomized, controlled exposure studies are expensive and labor intensive, and therefore are not recommended as a research priority at this time. Resources should instead be directed to the implementation of well–designed observational studies to investigate the effects of human exposures to ambient concentrations of cyanobacteria and their toxins.

Charge 4: What research is needed to better understand the public health effects of exposures to cyanobacteria?

Near-term Research Priorities

- Develop specific biomarkers of exposure and effect.
- Implement retrospective population–based observational studies.
- Systematically collect descriptions of exposed and ill individuals (cases) and case series.

Long-term Research Priorities

- Implement prospective population–based observational studies in those communities with recurrent exposure events.

Charge 5

What are the determinants of host susceptibility?

Human beings are a diverse lot, varying in age, sex, genetics, nutrition, exposure history and health status. Accurate prediction of the human health risks of cyanobacterial toxins must be based on recognition that each one of these variables, either alone or in combination, could affect an individual's susceptibility to the effects of these toxins. However, little information is available at this time regarding the importance of these (and other) factors in determining host susceptibility. This lack of knowledge is due primarily to the sporadic nature of poisoning episodes in humans and in other animals (i.e. wildlife, livestock, domestic animals), and the absence of a centralized mechanism for collecting and summarizing data from these types of events. Laboratory studies have focused largely on toxicity produced by acute exposures in relatively homogenous laboratory test species, such as mice. Some useful data exists on the effects of cyanobacterial toxins in some domestic animals (Falconer 2005). However, there is far less data on the effects of toxins following sublethal acute, episodic or chronic exposures. As a result, attempts to predict the human health risks of cyanobacterial toxins are riddled with uncertainty.

Despite the paucity of data, it seems likely that several factors could influence the severity of outcome in humans following exposure to cyanobacterial toxins. For example, liver failure would likely be more common following exposure to hepatotoxins in people with prior hepatic disease or insufficiency, and irritation caused by exposure to air–borne toxins may be greater in people with underlying respiratory disease.

Studies on laboratory animals are potentially more informative in identifying host susceptibility factors. The most common practice, referred to as the mouse bioassay, acutely exposes mice by the i.p. route to samples suspected to contain cyanobacterial toxins (Sullivan 1993). This approach has been widely used to determine the safety of seafood samples, as well as to gauge potency during the extraction and purification of toxins from environmental samples. A premium has therefore been placed on standardized conditions of testing among samples (Fernandez and Cembella 1995). As a consequence, the potential importance of most susceptibility factors that may be prevalent in the human population is unknown.

Two episodes of human poisonings have focused attention on age as a potential risk factor for adverse health effects. For example, a large scale outbreak of toxicoses among renal dialysis patients in Brazil found that

mortality was significantly higher among older (47 vs. 35 year old) patients (Jochimsen et al. 1998). However, these patients were ill, and exposed to cyanobacterial toxins by the intravenous route; this finding may not be applicable to people in general. Another large–scale poisoning episode involved exposure to *Cylindrospermopsis* via drinking water in Australia (Hawkins et. al 1985; Falconer 2005). This outbreak of hepato–enteritis affected considerably more children that adults. However, in contrast to children, very few adults in the community drank from the local water supply. Therefore, the higher rate of intoxication observed among children in the population may have been due to exposure rather than to age–related susceptibility. The role of human genetic susceptibility to effects associated with cyanobacterial toxins is unknown.

Some laboratory animal data indicate that age at exposure may influence susceptibility to cyanobacterial toxins. For example, the acute LD_{50} in rats of an extract prepared from Alaskan butter clams containing paralytic shellfish poisoning (PSP) toxins increased with age; it is has been surmised that the principal toxin in this extract was saxitoxin (WHO 1984). Compared to newborn rats, adult rats were about 10 times less sensitive following oral exposure, and about 2 times less sensitive following i.p. exposure to the extract (Watts et al. 1966). Regardless of age, however, LD_{50} values were considerably lower for the i.p. than the oral route of dosing (Wiberg and Stephenson 1960). On the other hand, in mice receiving a reported i.p. LD_{50} of purified microcystin LR, the time to death *decreased* substantially with age (from 6 to 36 weeks). A similar age–related decrease in time to death was also reported following an oral LD_{50} exposure of mice to a microcystin–containing extract (Rao et al. 2005). Recent studies found no developmental or long–term effects in mice following repeated prenatal exposure to sublethal doses of microcystin LR, or anatoxin–a (Chernoff et al. 2002; Rogers et al. 2005; MacPhail and Jarema 2005). These latter studies highlight the importance of systematic investigations into the effects of sublethal exposures involving acute, episodic and chronic exposures, in order to make accurate estimations of the health hazards associated with cyanobacterial toxins.

Charge 5: What are the determinants of host susceptibility?

Near-term Research Priorities

- Systematically examine and report susceptibility among multiple test species of animals exposed to cyanobacterial toxins in toxicology studies.
- Collect and report information about susceptibility during case reports and observational studies of humans and animals exposed to cyanobacterial toxins.
- Implement studies of the effects of sublethal acute, episodic and chronic exposures in healthy animals and in animal models of susceptibility.

Charge 6

What are the research needs after exposure/intoxication has occurred?

Little is known about the potential for human health effects that may persist or develop after intoxication with cyanobacterial toxins, although long term effects of microcystin poisoning in sheep have been reported (Carbis et al. 1995). A better understanding of post intoxication effects will depend upon longer–term toxicological studies and upon comprehensive epidemiological evidence, including longitudinal studies initiated after recognition of human and animal exposures.

Timely recognition of exposure events is needed. In global terms, human exposures to cyanobacterial toxins in drinking water supplies may occur sporadically as a consequence of treatment failures, deficiencies in drinking water systems operations, or from the addition of copper sulfate to reservoirs to terminate blooms of cyanobacteria. Epidemiological evidence is useful to describe the relationship between cyanobacterial toxin exposure and human health outcomes. However, comprehensive information is difficult to collect due to the lack of knowledge of the risk of human exposure to cyanobacterial toxins by water quality managers. When potentially toxic cyanobacterial blooms occur, they may be unrecognized as a health threat and public health authorities may not be involved early in the process. Therefore, the majority of outbreaks of human cyanobacterial

toxicoses have been studied retrospectively and complete epidemiological and environmental data has rarely been available.

A similar lack of knowledge may preclude identification of potential adverse health effects associated with other potential routes of cyanobacterial toxin exposure such as recreational water activities, consumption of contaminated fish, or dietary supplements contaminated by toxic cyanobacteria. Ideally, for health effects research, these risks would be recognized during the period of exposure to toxic cyanobacteria, and measures taken to protect human health. This timely identification of exposure would allow better collection of epidemiological evidence and consequently improve the analysis of associated health effects and recovery.

Although there are no known antidotes to treat poisonings associated with cyanobacterial toxins, people and animals may be treated by various supportive measures such as intravenous fluid and electrolyte replacement, corticosteroid therapy, assisted ventilation, and maintenance of acid–base balance. Methods to reduce absorption such as gastric lavage, activated charcoal or cholestyramine may also be used.

Potentially, if hepatotoxin poisoning is recognized soon after exposure, survival may be improved using recent advances in acute liver support therapy. Therapeutic use of the bioartificial liver, the molecular adsorbents recirculating system, portal vein arterialisation and human hepatocyte transplantation may supplement the standard treatment for fulminant liver failure – liver transplantation (Park and Lee 2005; Hay 2004; Nguyen et al. 2005; van de Kerkhove et al. 2005; Nardo et al. 2005; Baccarani et al. 2005; Tissières et al. 2005).

Maintenance of tissue oxygenation by intermittent positive pressure ventilation may benefit victims of neurotoxin poisoning. Some have investigated this technique for anatoxin–a poisoning, with mixed results (Carmichael et al. 1975; Carmichael et al. 1977; Beasley et al. 1989; Valentine et al. 1991). Artificial ventilation in conjunction with the potassium channel blocker 4–aminopyridine has shown promise in reversing experimental saxitoxin poisoning (Chang et al. 1996).

However, emergency interventions on human and animal victims of cyanobacterial toxicosis can only make a limited contribution to the understanding of post–intoxication intervention, as typically little is known about the total absorbed dose. There is a need for experimental work designed specifically to investigate the efficacy of various therapeutic approaches.

Although a large number of animal poisonings and animal toxicity tests have been reported, few have measured the effects of chronic or sub–chronic exposure (Falconer et al. 1988; Guzman and Solter 1992; Falconer et al. 1994; Fawell et al. 1994; Ito et al. 1997; Humpage et al. 2000b; Fal-

coner and Humpage 2001; Humpage and Falconer 2003). Therefore, the long–term risk associated with chronic, low–level exposure is less well understood. However, this type of exposure scenario may be the most common among human populations. The relative lack of animal data makes epidemiological studies difficult due to the lack of case definitions and biologic methods to confirm evidence of chronic or subchronic exposure to low levels of cyanobacterial toxins (Kuiper–Goodman et al. 1999).

There is a great disparity of information among cyanobacterial toxins. Microcystins are the group of toxins most frequently studied, and there is more information to support the understanding of the intoxication and detoxification process. However, even in the case of microcystins, research into long–term exposures is needed to investigate reproductive toxicity, teratogenicity and carcinogenicity effects in mammals. For other cyanobacterial toxins, such as cylindrospermopsin and the neurotoxins, anatoxins and saxitoxins, there are large knowledge gaps related to chronic toxicity and to the intoxication and detoxification process.

Charge 6: What are the research needs after exposure/ intoxication has occurred?

Near Term Research Priorities

- Initiate regular monitoring of water bodies at risk for toxic cyanobacteria blooms to enable remediation and timely health effects studies.
- Implement toxicologic studies that encompass multiple health endpoints at frequent time intervals to examine the relationship between initial intoxication, health effects and recovery at multiple life stages.
- Conduct studies to investigate the effectiveness of therapeutic approaches.
- Monitor food and supplements at risk for contamination with cyanobacterial toxins to enable public health intervention and timely health effects studies.

Charge 7

Where are we in the development of predictive models?

Predicting human health outcomes associated with cyanobacterial toxins requires linking exposure estimates with dose–response models. Estimates of toxin occurrence may be potentially derived from the models of bloom dynamics, toxin production, and the fate and transport of the toxins. Mechanistic models have been developed that can simulate a variety of bloom characteristics and they have been useful in determining the significance of a variety of environmental variables that influence bloom dynamics (Bonnet and Poulin 2002; Thébault and Rabouille 2003; Håkanson et al. 2003; Robson and Hamilton 2004; Arhonditsis and Brett 2005; Prokopkin et al. 2006) but their predictive capacity remains limited. In contrast, inductive models, such as those employing artificial neural networks, appear to have the capacity to predict significant aspects of bloom dynamics (Recknagel et al. 1997; Maier et al. 1998; Jeong et al. 2003).

A key step in using predictive models of bloom dynamics to predict exposures will be consideration of toxin fate and transport within aquatic ecosystems. This will help to determine the relative importance of different routes of human exposure to cyanobacterial toxins. For example, there is a potential for oral, inhalational and dermal exposures during recreational water use. Microcosm studies may provide an efficient means to develop and evaluate the models that integrate bloom dynamics, toxin production, fate and transport to estimate such multi–route exposures. Reliable models predicting exposure via drinking water may be more difficult to link to bloom dynamics given the uncertainties regarding the fate of toxins in water processing and the potential for by–products resulting from the interaction of disinfection agents with cyanobacterial toxins.

Mechanistic models of human health effects based on stepwise links from exposure to effect require toxicokinetic and toxicodynamic information that is largely unavailable for the majority of cyanobacterial toxins. Furthermore, dose–response information is only currently available for a small number of toxins for limited types of effects, suitable for use in more basic modeling efforts. Alternatively, more 'intelligent' inductive models may be used to predict human health outcomes from mixtures of cyanobacterial toxins based on data derived from experimental studies that employ characterized bloom extracts rather than pure toxin. While such extracts more accurately represent individual real–world blooms, their complex and unique characteristics may limit the inferences that can be

made until large numbers of blooms are characterized and their effects assessed.

The development of predictive models may also benefit from a more extensive characterization of the influence of temporal aspects of exposure on various types of health outcomes. Different health outcomes are likely to predominate as a result of acute, subchronic, chronic and episodic exposures. Only minimal data are currently available that are suitable for discriminating the impact of these temporal aspects of exposure on the variety of potential human health outcomes. Predictive models are needed that may be used by risk managers during decisions related to the costs and benefits of surface water use and exposure.

To the extent that future experimental studies implicate a wider variety of health outcomes as being influenced by cyanobacterial toxin exposure, models will need to be developed to address more comprehensive predictions that include a variety of health outcomes in the human population. Evaluation of such predictions will require corresponding biomarkers of effect suitable for use in human populations to evaluate the validity of model predictions.

While the primary linkage between bloom occurrence and human health effects is presumed to be exposure to cyanobacterial toxins, the effects of cyanobacterial blooms on ecosystem services may also influence human health. While several international efforts are modeling the impact of cyanobacterial blooms on ecosystems, assessing the impact of ecosystem services on human health is emerging as an important area of research. At this time it is difficult to assess the significance of ecosystem services for modeling the effect of cyanobacteria blooms on human health.

Charge 7: Where are we in the development of predictive models?

Near-term Research Priorities

- Implement toxicology studies to determine dose–response relationships using oral, inhalational and dermal exposure routes.
- Implement studies of cyanobacterial toxin fate and transport in environmental media.
- Characterize the toxicokinetics/toxicodynamics of parent toxin and metabolites.

Long-term Research Priorities

- Develop linkages between predictive models of bloom dynamics to dose–response models of human health and ecologic effects outcomes.
- Investigate how cyanobacterial blooms reduce ecosystem services, and the resulting effects on human and animal health.

Conclusion

In summary, we have identified multiple health effects research needs associated with exposure to cyanobacteria and cyanobacterial toxins.

- Affordable toxin standards are needed. However, in the short term, research may be conducted with characterized clonal cyanobacteria cultures that can be shared among laboratories.
- Studies of the health effects of chronic, episodic, and low–dose exposures by environmentally—relevant routes are needed. Carcinogenicity, neurodevelopmental, neurotoxicology, and immunotoxicology studies are of immediate importance.
- Experimental studies of laboratory animals and observational studies of populations exposed to mixtures of cyanobacterial toxins are needed. A goal is to develop risk assessments of populations exposed to natural blooms and mixtures of cyanobacterial toxins.
- The public health consequences of exposure to cyanobacteria and cyanobacterial toxins are poorly characterized. Biomarkers of exposure and effect are needed to use in human studies. Case reports and observational epidemiologic studies are needed.
- Host susceptibility is poorly defined and should be systematically examined among individuals involved in outbreaks of toxicoses, and during experimental and observational studies.
- Timely and accurate exposure assessment is critical to detecting and understanding cyanobacterial toxin—associated health effects. Systematic toxin occurrence monitoring is needed in water and food at risk for contamination with potentially toxic cyanobacteria.
- Critical data gaps remain before predictive modeling of cyanobacteria–associated health effects in populations is possible. Information about the environmental fate and transport of toxins, the effects of cyanobacteria blooms on ecosystem services and population dynamics, the

toxicokinetics/toxicodynamics of parent compounds and metabolites, and dose–response data are needed.

References

Annadotter H, Cronberg G, Lawton L, Hansson HB, Göthe U, Skulberg O (2001) An extensive outbreak of gastroenteritis associated with the toxic cyanobacterium Planktothrix agardhii (Oscillatoriales, Cyanophyceae) in Scania, south Sweden. In: Cyanotoxins – occurrence, causes, consequences. Edited by Chorus I. Berlin: Springer–Verlag; 200–208

Arhonditsis GB, Brett MT (2005) Eutrophication model for Lake Washington (USA) Part I. Model description and sensitivity analysis. Ecol Modell 187(2–3):140–178

Azevedo SM, Carmichael WW, Jochimsen EM, Rinehart KL, Lau S, Shaw GR, Eaglesham GK (2002) Human intoxication by microcystins during renal dialysis treatment in Caruaru–Brazil. Toxicology 181–182:441–446

Baccarani U, Adani GL, Sainz M, Donini A, Risaliti A, Bresadola F (2005) Human hepatocyte transplantation for acute liver failure: state of the art and analysis of cell sources. Transplant Proc 37(6):2702–2704

Beasley VR, Dahlem AM, Cook WO, Valentine WM, Lovell RA, Hooser SB, Harada K, Suzuki M, Carmichael WW (1989) Diagnostic and clinically important aspects of cyanobacterial (blue–green algae) toxicoses. J Vet Diagn Invest 1(4):359–365

Bonnet MP, Poulin M (2002) Numerical modelling of the planktonic succession in a nutrient–rich reservoir: environmental and physiological factors leading to Microcystis aeruginosa dominance. Ecol Modell 156(2–3):93–112

Bowling L (1994) Occurrence and possible causes of a severe cyanobacterial bloom in Lake Cargelligo, New South Wales. Aust J Mar Freshwater Res 45(5):737–745

Candy DJ, Donohue AC, McCarthy TD (1999) An asymmetric synthesis of ADDA and ADDA–glycine dipeptide using the beta–lactam synthon method. J Chem Soc Perkin Trans I 5:559–567

Carbis CR, Waldron DL, Mitchell GF, Anderson JW, McCauley I (1995) Recovery of hepatic function and latent mortalities in sheep exposed to the blue–green alga Microcystis aeruginosa Vet Rec 137(1):12–15

Carmichael WW, Azevedo SM, An JS, Molica RJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK (2001) Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ Health Perspect 109(7):663–668

Carmichael WW, Biggs DF, Gorham PR (1975) Toxicology and pharmacological action of Anabaena flos–aquae toxin. Science 187(4176):542–544

Carmichael WW, Gorham PR, Biggs DF (1977) Two laboratory case studies on the oral toxicity to calves of the freshwater cyanophyte (blue–green algae) Anabaena flos–aquae NRC–44–1. Can Vet J 18(3):71–75

Chang FCT, Bauer RM, Benton BJ, Keller SA, Capacio BR (1996) 4–aminopyridine antagonizes saxitoxin– and tetrodotoxin–induced cardiorespiratory depression. Toxicon 34(6):671–690

Chen T, Shen P, Zhang J, Hua Z (2005) Effects of microcystin–LR on patterns of iNOS and cytokine mRNA expression in macrophages in vitro. Environ Toxicol 20(1):85–91

Chernoff N, Hunter ES 3rd, Hall LL, Rosen MB, Brownie CF, Malarkey D, Marr M, Herkovits J (2002) Lack of teratogenicity of microcystin–LR in the mouse and toad. J Appl Toxicol 22(1):13–17

Chiswell RK, Shaw GR, Eaglesham G, Smith MJ, Norris RL, Seawright AA, Moore MR (1999) Stability of cylindrospermopsin, the toxin from the cyanobacterium, Cylindrospermopsis raciborskii: Effect of pH, temperature, and sunlight on decomposition. Environ Toxicol 14(1): 155–161

Codd GA, Metcalf JS, Beattie KA (1999) Retention of Microcystis aeruginosa and microcystin by salad lettuce (Lactuca sativa) after spray irrigation with water containing cyanobacteria. Toxicon 37(8):1181–1185

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RB, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce β–N–methylamino–L–alanine, a neurotoxic amino acid. Proc Natl Acad Sci U S A 102(14):5074–5078

de Magalhães VF, Soares RM, Azevedo SMFO (2001) Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39(7):1077–1085

Duy TN, Lam PKS, Shaw GR, Connell DW (2000) Toxicology and risk assessment of freshwater cyanobacterial (blue–green algal) toxins in water. Rev Environ Contam Toxicol 163:113–185

Falconer IR (2005) Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. Boca Raton: CRC Press. 279pp.

Falconer IR, Beresford AM, Runnegar MT (1983) Evidence of liver damage by toxin from a bloom of the blue–green alga Microcystis aeruginosa. Med J Aust 1(11):511–514

Falconer IR, Burch MD, Steffensen DA, Choice M, and Coverdale OR (1994) Toxicity of the blue–green alga (cyanobacterium) Microcystis aeruginosa in drinking water to growing pigs, as an animal model for human injury and risk assessment. Environ Toxicol Water Qual 9(2):131–139.

Falconer IR, Hardy SJ, Humpage AR, Froscio SM, Tozer GJ, Hawkins PR (1999) Hepatic and renal toxicity of the blue–green alga (cyanobacterium) Cylindrospermopsis raciborskii in male Swiss albino mice. Environ Toxicol 14(1): 143–150

Falconer IR, Humpage AR (1996) Tumour promotion by cyanobacterial toxins. Phycologia 35(6 Suppl):74–79

Falconer IR, Humpage AR (2001) Preliminary evidence for in vivo tumour initiation by oral administration of extracts of the blue–green alga Cylindrospermopsis raciborskii containing the toxin cylindrospermopsin. Environ Toxicol 16(2):192–195

Falconer IR, Smith JV, Jackson AR, Jones A, Runnegar, MT (1988) Oral toxicity of a bloom of the cyanobacterium Microcystis aeruginosa administered to mice over periods up to one year. J Toxicol Environ Health 24(3):291–305.

Fawell JK, James CP, James HA (1994) Toxins from blue–green algae: Toxicological assessment of microcystin–LR and a method for its determination in water. Water Research Centre, Medenham, England Report number FR 0359/2/DoE 3358/2

Fernandez M, Cembella AD (1995) Mammalian bioassays. In: Manual on harmful marine microalgae, IOC Manuals and Guides No. 33. Edited by Hallegraeff GM, Anderson DM, Cembella AD. Paris: UNESCO 213-224

Gilroy DJ, Kauffman KW, Hall RA, Huang X, Chu FS (2000) Assessing potential health risks from microcystin toxins in blue–green algae dietary supplements. Environ Health Perspect 108(5):435–439

Guzman RE, Solter PF (2002) Characterization of sublethal microcystin–LR exposure in mice Vet Pathol 39(1):17–26

Håkanson L, Malmaeus JM, Bodemer U, Gerhardt V (2003) Coefficients of variation for chlorophyll, green algae, diatoms, cryptophytes and blue–greens in rivers as a basis for predictive modelling and aquatic management. Ecol Modell 169(1):179–196

Harada KI, Ohtani I, Iwamoto K, Suzuki M, Watanbe MF, Watanabe M, Terao K (1994) Isolation of cylindrospermopsin from a cyanobacterium Umezakia natans and its screening method. Toxicon 32(1):73–84

Hawkins PR, Chandrasena NR, Jones GJ, Humpage AR, Falconer IR (1997) Isolation and toxicity of Cylindrospermopsis raciborskii from an ornamental lake. Toxicon 35(3):341–346

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue–green alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic supply reservoir. Appl Environ Microbiol 50(5):1292–1295

Hay JE (2004) Acute liver failure. Curr Treat Options Gastroenterol 7(6):459–468

Hernandez M, Macia M, Padilla C, Del Campo FF (2000) Modulation of human polymorphonuclear leukocyte adherence by cyanopeptide toxins. Environ Res 84(1):64–68.

Hilborn ED, Carmichael WW, Yuan M, Azevedo SMFO (2005) A simple colorimetric method to detect biological evidence of human exposure to microcystins. Toxicon 46(2):218–221

Hindman SH, Favero MS, Carson LA, Petersen NJ, Schonberger LB, Solano JT (1975) Pyrogenic reactions during haemodialysis caused by extramural endotoxin. Lancet 2(7938):732–734

Hooser SB, Beasley VR, Basgall EJ, Carmichael WW, Haschek WM (1990) Microcystin–LR–induced ultrastructural changes in rats. Vet Pathol 27(1):9–15

Humpage AR, Falconer IR (2003) Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ Toxicol 18(2):94–103

Humpage AR, Falconer IR (Nov. 2005) Personal communication

Humpage AR, Fenech M, Thomas P, Falconer IR (2000a) Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin. Mutat Res 472(1–2):155–161

Humpage AR, Hardy SJ, Moore EJ, Froscio SM, Falconer IR (2000b) Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon. J Toxicol Environ Health A 61(3):155–165

Humphrey JM, Aggen JB, Chamberlin AR (1996) Total synthesis of the serine–threonine phosphatase inhibitor microcystin–LA. J Am Chem Soc 118(47): 11759–11770.

Ito E, Kondo F, Terao K, Harada K (1997) Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin–LR Toxicon 35(9):1453–1457

Jeong KS, Kim DK, Whigham P, Joo GJ (2003) Modelling Microcystis aeruginosa bloom dynamics in the Nakdong River by means of evolutionary computation and statistical approach Ecol Modell 161(1–2):67–78

Jochimsen EM, Carmichael WW, An J, Cardo DM, Cookson ST, Holmes CEM, Antunes MB, de Melo Filho DA, Lyra TM, Barreto VST, Azevedo SMFO, Jarvis WR (1998) Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. N Engl J Med 338(13):873–878

Kuiper–Goodman T, Falconer I, Fitzgerald J (1999) Human health aspects. In: Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. Edited by Chorus I, Bartram J. London: E & FN Spon on behalf of the World Health Organization 113–153

Lawrence JF, Niedzwiadek B, Menard C, Lau BPY, Lewis D, Kuper–Goodman T, Carbone S, Holmes C (2001) Comparison of liquid chromatography/mass spectrometry, ELISA, and phosphatase assay for the determination of microcystins in blue–green algae products. J AOAC Int 84(4):1035–1044

MacPhail RC, Farmer JD, Jarema KA, Chernoff N (2005) Nicotine effects on the activity of mice exposed prenatally to the nicotinic agonist anatoxin–a. Neurotoxicol Teratol 27(4):593–598

MacPhail RC, Jarema, KA (2005) Prospects on behavioral studies of marine and freshwater toxins. Neurotoxicol Teratol 27(5):695–699

Maier HR, Dandy GC, Burch MD (1998) Use of artificial neural networks for modelling cyanobacteria Anabaena spp. in the River Murray, South Australia. Ecol Modell 105(2–3):257–272

Metcalf JS, Meriluoto JA, Codd GA (2006) Legal and security requirements for the air transportation of cyanotoxins and toxigenic cyanobacterial cells for legitimate research and analytical purposes. Toxicol Lett 163(2):85–90

Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, Shao Y, Wu Z, Liu G, Wang X, Sun Z (2002) Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 36(5):1214–1220

Nagai H, Yasumoto T, Hokama Y (1996) Aplysiatoxin and debromoaplysiatoxin as the causative agents of a red alga Gracilaria coronopifolia poisoning in Hawaii. Toxicon 34(7):753–761

Nardo B, Caraceni P, Montalti R, Puviani L, Bertelli R, Beltempo P, Pacilè V, Rossi C, Gaiani S, Grigioni W, Bernardi M, Martinelli G, Cavallari A (2005) Portal vein arterialization: a new surgical option against acute liver failure? Transplant Proc 37(6):2544–2546

Negri AP, Jones GJ (1995) Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium Anabaena circinalis by the freshwater mussel Alathyria condola. Toxicon 33(5):667–678

Nguyen TH, Mai G, Villiger P, Oberholzer J, Salmon P, Morel P, Bühler L, Trono D (2005) Treatment of acetaminophen–induced acute liver failure in the mouse with conditionally immortalized human hepatocytes. J Hepatol 43(6):1031–1037

Osborne NJT, Webb PM, Shaw GR (2001) The toxins of Lyngbya majuscula and their human and ecological effects. Environ Int 27(5):381–392

Park JK, Lee DH (2005) Bioartificial liver systems: current status and future perspective. J Biosci Bioeng 99(4):311–319

Prokopkin IG, Gubanov VG, Gladyshev MI (2006) Modelling the effect of planktivorous fish removal in a reservoir on the biomass of cyanobacteria. Ecol Modell 190(3–4):419–431

Qian GS, Ross RK, Yu MC, Gao YT, Henderson BE, Wogan GN, Groopman JD (1994) A follow–up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 3(1):3–10

Rao PVL, Gupta N, Jayaraj R, Bhaskar ASB, Jatav PC (2005) Age–dependent effects on biochemical variables and toxicity induced by cyclic peptide toxin microcystin–LR in mice. Comp Biochem Physiol C Toxicol Pharmacol 140(1):11–19

Recknagel F, French M, Harkonen P, Yabunaka KI (1997) Artificial neural network approach for modelling and prediction of algal blooms. Ecol Modell 96(1–3):11–28

Reich A (Nov. 2005) Personal communication

Ressom R, Soong FS, Fitzgerald J, Turczynowicz L, El Saadi O, Roder D, Maynard T, Falconer I (1994) Health effects of toxic cyanobacteria (blue–green algae). Canberra: National Health and Medical Research Council & Australian Government Publishing Service

Robson BJ, Hamilton DP (2004) Three–dimensional modelling of a Microcystis bloom event in the Swan River estuary, Western Australia. Ecol Modell 174(1–2):203–222

Rogers EH, Hunter III ES, Moser VC, Phillips PM, Herkovits J, Munoz L, Hall LL, Chernoff N (2005) Potential developmental toxicity of anatoxin–a, a cyanobacterial toxin. J Appl Toxicol 25(6):527–534

Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, Groopman JD, Gao YT, Henderson BE (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet 339(8799):943–946

Saker ML, Eaglesham GK (1999) The accumulation of cylindrospermopsin from the cyanobacterium Cylindrospermopsis raciborskii in tissues of the Redclaw crayfish Cherax quadricarinatus. Toxicon 37(7):1065–1077

Saker ML, Jungblut AD, Neilan BA, Rawn DFK, Vasconcelos VM (2005) Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium Aphanizomenon flos–aquae. Toxicon 46(5):555–562

Schaeffer DJ, Malpas PB, Barton LL (1999) Risk assessment of microcystin in dietary Aphanizomenon flos–aquae. Ecotoxicol Environ Saf 44(1):73–80

Shaw GR, Seawright AA, Moore MR, Lam PKS (2000) Cylindrospermopsin, a cyanobacterial alkaloid: evaluation of its toxicologic activity. Ther Drug Monit 22(1):89–92

Shen PP, Zhao SW, Zheng WJ, Hua ZC, Shi Q, Liu ZT (2003) Effects of cyanobacteria bloom extract on some parameters of immune function in mice. Toxicol Lett 143(1):27–36

Shi J, Zhu L, Liu S, Xie WF (2005) A meta–analysis of case–control studies on the combined effect of hepatitis B and C infections in causing hepatocellular carcinoma in China. Br J Cancer. 92(3):607–612

Shi Q, Cui J, Zhang J, Kong FX, Hua ZC, Shen PP (2004) Expression modulation of multiple cytokines in vivo by cyanobacteria blooms extract from Taihu Lake, China. Toxicon 44(8):871–879

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. Edited by Chorus I, Bartram J. London: E & FN Spon on behalf of the World Health Organization 41–111

Soares RM, Yuan M, Servaites JC, Delgado A, Magalhães VF, Hilborn ED, Carmichael WW, Azevedo SMFO (2006) Sublethal exposure from microcystins to renal insufficiency patients in Rio de Janeiro, Brazil. Environ Toxicol 21(2):95–103

Stevens DK, Krieger RI (1991) Effect of route of exposure and repeated doses on the acute toxicity in mice of cyanobacterial nicotinic alkaloid anatoxin–a. Toxicon 29(1):134–138

Stewart I, Webb PM, Schluter PJ, Shaw GR (2006) Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. Environ Health 5(1):6

Sullivan JJ (1993) Methods of analysis for cyanobacterial toxins: dinoflagellate and diatom toxins. In: Cyanobacterial toxins in seafood and drinking water. Edited by Falconer IR. London: Academic Press 29-48

Terao K, Ohmori S, Igarashi K., Ohtani I, Watanabe MF, Harada KI, Ito E, Watanabe M (1994) Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue–green alga Umezakia natans. Toxicon 32(7):833–843

Thébault, JM, Rabouille, S (2003) Comparison between two mathematical formulations of the phytoplankton specific growth rate as a function of light and

temperature, in two simulation models (ASTER & YOYO) Ecol Modell 163(1–2):145–151

Tissières P, Sasbón JS, Devictor D (2005) Liver support for fulminant hepatic failure: is it time to use the molecular adsorbents recycling system in children? Pediatr Crit Care Med 6(5):585–591

Turner PC, Gammie AJ, Hollinrake K, Codd GA (1990) Pneumonia associated with contact with cyanobacteria. BMJ 300(6737):1440–1441

Valentine WM, Schaeffer DJ, Beasley VR (1991) Electromyographic assessment of the neuromuscular blockade produced in vivo by anatoxin–a in the rat. Toxicon 29(3):347–357

Van de Kerkhove AP, Poyck PPC, Deurholt T, Hoekstra R, Chamuleau RAFM, van Gulik TM (2005) Liver support therapy: an overview of the AMC–bioartificial liver research. Dig Surg 22(4):254–264

Watts JS, Reilly J, DaCosta FM, Krop S (1966) Acute toxicity of paralytic shellfish poison in rats of different ages. Toxicol Appl Pharmacol 8(2):286–294

WHO, International Programme on Chemical Safety (1984) Aquatic (marine and freshwater) biotoxins. Environmental Health Criteria 37. Geneva: World Health Organization. 95pp.

Wiberg GS, Stephenson NR (1960) Toxicologic studies on paralytic shellfish poison. Toxicol Appl Pharmacol 2:607–615

Xie C, Runnegar MTC, Snider BB (2000) Total synthesis of (±)-cylindrospermopsin. J Am Chem Soc 122(21):5017–5024

Yu MC, Yuan JM (2004) Environmental factors and risk for hepatocellular carcinoma. Gastroenterol 127(Suppl 1):S72–78

Yu SZ, Huang XE, Koide T, Cheng G, Chen GC, Harada K, Ueno Y, Sueoka E, Oda H, Tashiro F, Mizokami M, Ohno T, Xiang J, Tokudome S (2002) Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China. Jpn J Cancer Res 93(12):1287–1292