

**CDC—A Brief Introduction**  
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### **What is the Centers for Disease Control and Prevention (CDC)?**

CDC's mission is to promote health and quality of life, to protect health and safety, provide credible information to enhance health decisions, and promote health through strong partnerships. CDC is the national focus for developing and applying disease prevention and control strategies, environmental health, and health promotion and education activities. CDC's goal is to improve the health of the people of the United States; however, CDC does not make regulations, recommend legislation, or impose programs on our state partners.

CDC is an agency within the U.S. Department of Health and Human Services and comprises 12 offices and centers, including the National Center for Environmental Health (NCEH) (see Figure 1). The mission of NCEH is to provide national leadership in preventing and controlling disease and death resulting from the interactions between people and their environment. The NCEH mission is implemented by working with state and local health agencies to identify, investigate, and prevent environmentally induced illnesses in people. With the exception of the National Institute for Occupational Safety and Health (NIOSH), CDC has a presence in a state or local health agency only at the specific request and consent of the agency.

CDC funding sources include congressional budget funds and other programs targeted to address a specific public health issue, such as the presence of *Pfiesteria piscicida* in eastern U.S. estuaries. CDC passes much of the funding it receives directly to states in the form of grants, cooperative agreements, and other assistance to health agencies.

### **CDC and Harmful Algal Blooms**

CDC has been directly involved in HABs research since the summer of 1997, when the discovery of *Pfiesteria piscicida* in the tributaries of the Chesapeake Bay created concerns about human exposure to the organism. CDC participated in the public health response to complaints of human illness associated with exposure to waters containing the organism by sending investigators to assist the state of Maryland in conducting their investigation. CDC held a number of workshops and program meetings to encourage federal and state health agencies to collaboratively develop a plan to assess the public health impact of environmental exposure to this organism. With the states, CDC defined a number of public health research needs: 1) Investigate *Pfiesteria piscicida*, including

the ecology and behavior of the organism, 2) Characterize the toxins elaborated by *P. piscicida* and other estuarine dinoflagellates, 3) Characterize the routes of human exposure, such as dermal contact or inhalation of aerosolized toxins, and 4) Conduct clinical and epidemiologic studies to determine the human health effects from exposure to the organism, similar organisms, and any toxins they produce.

CDC received direct congressional funding to support a number of harmful algal bloom (HAB) research activities, including cooperative agreements with six states (Delaware, Florida, North Carolina, Maryland, South Carolina, and Virginia) to assess the public health impact associated with the presence of *Pfiesteria piscicida* in estuarine waters used for recreational activities and commercial fishing. The states developed programs to: 1) Conduct epidemiologic studies, 2) Create public education materials, and 3) Develop biological markers of exposure to *P. piscicida* toxins and their biological effects. Detailed descriptions of CDC and state health agency activities associated with *P. piscicida* can be found in the Environmental Health Perspectives Supplement (2001) comprising papers presented at the CDC conference (*Pfiesteria: From Biology to Public Health*, Stone Mountain, Georgia, October 18-20, 2000).

CDC has conducted annual site visits with each of the states involved in the cooperative agreements. These site visits have provided opportunities to expand the research initially focused on a single organism to the more general issue of the impact of HABs on public health. For example, the cooperative agreements have supported a number of additional activities, including the investigation of exposure to aerosolized brevetoxins during red tide events, a study of the efficacy of home water filters to remove cyanobacterial toxins from water, this workshop, and the human health impact of exposure to waste products from confined animal feeding operations (CAFOs).

### **Future Challenges for CDC**

CDC has defined a number of future challenges in public health. They are: 1) Improve people's health, 2) Prevent violence and unintentional injury, 3) Meet health and safety needs of the workforce, 4) Provide credible health information, 5) Protect individuals from emerging diseases, 6) Eliminate racial/ethnic disparities, 7) Foster safe, healthy environments, and 10) Work with partners to improve global health. Collaborative efforts with public health partners will enable CDC to address these future challenges.

### **References**

Lorraine C. Backer, Lora E. Fleming, Alan Rowan, Yung-Sung Cheng, Janet Benson, Richard H. Pierce, Julia Zaias, Judy Bean, Gregory D. Bossart, David Johnson, Raul Quimbo, and Daniel G. Baden. Recreational Exposure to Aerosolized Brevetoxins During Florida Red Tide Events. *Harmful Algae*, 2, 2003:19-28

*Pfiesteria: From Biology to Public Health*. Environmental Health Perspectives, 109 (Suppl 5), 2001. 808 pp.

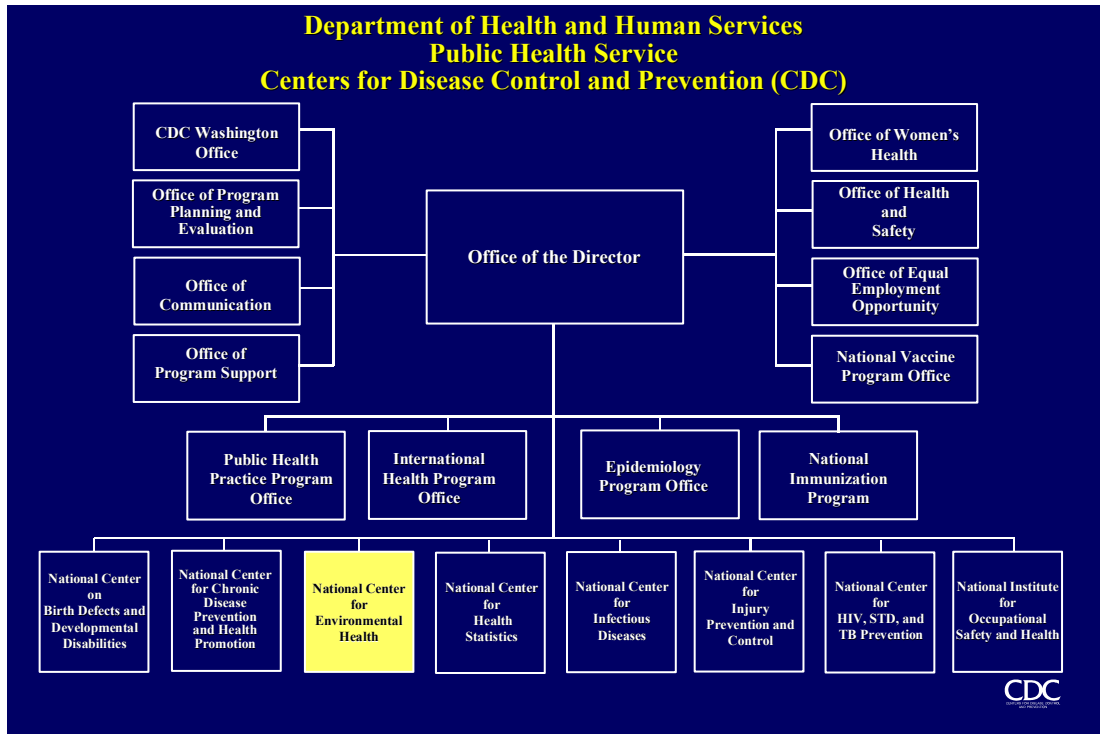


Figure 1. Organization chart for Centers for Disease Control and Prevention

## Australia's Cyanobacteria Experience Ian Falconer, D.Sc.

The most recent research into toxic cyanobacteria in Australia has had three main emphases. These are the ecology of cyanobacteria and its implications for management of water bodies; the toxicity and mechanism of action of cylindrospermopsin; and the bioaccumulation of toxins by plants and animals used for human consumption.

Blooms of toxic cyanobacteria in Australia are largely seasonal in occurrence. Some water storages have successions of blooms of different species, related to water temperature and residence time of water in lakes or weir pools. Often these are of toxic species, and hence a hazard to domestic animals or human consumers.

The species *Anabaena circinalis* is common in lakes, reservoirs and rivers in Australia and is toxic as a result of synthesizing a series of saxitoxin derivatives. These are stable to boiling and are not removed by conventional chlorination, flocculation, or rapid sand filtration water treatment. They can be removed by activated carbon and other more sophisticated water treatments.

The organism blooms most successfully in temperature-stratified rivers or lakes. Under these conditions anoxia at the sediment surface releases phosphate, which is the limiting nutrient for this nitrogen-fixing species. The implications for management to minimize cyanobacterial blooms of this species include flow control in rivers, setting water discharge rates to assist mixing, and varying discharge heights (Webster *et al.*, 2000).

Other ecological research has explored the toxic *Cylindrospermopsis raciborskii* that commonly occurs in the tropical lakes and rivers of Northern Australia. Monitoring 47 storage reservoirs and weir pools showed *C. raciborskii* in 70%, with highest bloom density in late summer/autumn. In 14 reservoirs for drinking water storage, the average cylindrospermopsin concentration was 3.4 µg/L. Toxin concentrations of 1.0 µg/L were reached at a cell concentration of 20,000 cells/ml, which was taken as the health trigger for monitoring drinking water for toxicity. High *C. raciborskii* concentrations were associated with long water residence time, high pH, high temperature, high incident radiation and a thermally stratified water column (McGregor and Fabbro, 2000).

Recent studies of cylindrospermopsin have investigated the toxin distribution in mice, and the pathway of excretion. Most appears in the urine, with some in the feces. The liver and kidney concentrate the toxin, but it occurs widely in the tissues (Norris *et al.*, 2001). Studies of possible carcinogenicity demonstrated 10% of cylindrospermopsin-dosed mice developed tumors over 210 days whereas no controls did so (Falconer and Humpage, 2001). Parallel studies using a cultured human white blood cell line demonstrated that micronucleus formation increased in the presence of cylindrospermopsin, indicating chromosome breakage and loss of whole chromosomes (Humpage *et al.*, 2000a). A sub chronic oral toxicity trial with cylindrospermopsin has just been completed (Humpage and Falconer, 2003).

The potential of microcystin, also an important toxic cyanobacterial contaminant of drinking water, as a tumor promoter in the lower gut was demonstrated by an increase in pre-neoplastic colon crypts in mice when microcystin was supplied in drinking water (Humpage *et al.*, 2000b).

As a result of major water blooms of *Nodularia* in a fish and shellfish harvesting area, analyses were done on tissues from commercial species. Sufficient toxin was found in tissues for shellfish and crustacean harvesting to be banned, and use of finfish restricted to 'cleaned' fish only (Van Bounder *et al.*, 2001).

## References

Falconer, I. R. and Humpage, A. R. (2001) Preliminary evidence for In-Vivo tumor initiation by oral administration of extracts of the Blue-Green Alga *Cylindrospermopsis raciborskii* containing the toxin cylindrospermopsin. *Environmental Toxicology* **16**, 192-195.

Humpage, A. R., Fenech, M., Thomas, P. and Falconer, I. R. (2000a) Micronucleus induction and chromosome loss in WIL2-NS cells exposed to the cyanobacterial toxin, cylindrospermopsin. *Mutation Research* **472**, 155-161.

Humpage, A. R., Hardy, S. J., Moore, E. J., Froschio, S. M. and Falconer, I. R. (2000b) Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon. *Journal of Toxicology and Environmental Health, Part A* **61**, 155-165.

Humpage, A.R. and Falconer, I.R. (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. *Environmental Toxicology* **18**, 94-103.

McGregor, G. B. and Fabbro, L. D. (2000) Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. *Lakes and Reservoirs: Research and Management* **5**, 195-205.

Norris, R. G. L., Seawright, A. A., Shaw, G. R., Smith, M. J., Chriswell, R. K. and Moore, M. R. (2001) Distribution of <sup>14</sup>C cylindrospermopsin *in vivo* in the mouse. *Environmental Toxicology* **16**, 498.

Van Buynder, P. G., Oughtred, T., Kirby, B., Phillips, S., Eaglesham, G., Thomas, K. and Burch, M. (2001) Nodularin uptake by seafood during a cyanobacterial bloom. *Environmental Toxicology* **16**, 468-471.

Webster, I. T., Sherman, B. S., Bormans, M. and Jones, G. (2000) Management strategies for cyanobacterial blooms in an impounded lowland river. *Regulated Rivers: Research and Management* **16**, 513-525.

# **CYANOBACTERIAL TOXINS: THE DEVELOPMENT AND EVALUATION OF METHODS TO DETERMINE MICROCYSTIN LEVELS IN CANADIAN WATER SUPPLIES**

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## **INTRODUCTION**

In Canada, the provision of safe drinking water demands the cooperation of all governments, particularly at the federal and provincial/territorial levels. Since the late 1960s, these governments have worked together to develop guidelines to maintain or improve the quality of drinking water across the country. Federal-provincial collaboration in this area ensures citizens have access to the highest quality water possible.

The Federal-Provincial-Territorial Committee on Drinking Water (formerly Subcommittee) is made up of representatives from Health Canada's Water Quality and Health Bureau, Environment Canada, and provincial and territorial government departments responsible for water quality. The group meets twice annually and via conference calls at other times to discuss issues related to drinking water quality, including:

- \* new and on-going research related to the microbiological quality of water,
- \* new and on-going research related to risks from exposure to particular chemical contaminants which may be present in Canadian drinking water supplies,
- \* the development of new, and revision of current, guidelines for contaminants found in Canadian drinking water supplies, and
- \* the publication of educational tools dealing with specific topics for the public, health practitioners, and drinking water authorities.

One of the substances currently under the Committee's evaluation is Microcystin-LR, a potent toxin that affects the liver (hepatotoxin). This hepatotoxin is naturally produced by many species of blue-green algae (cyanobacteria), which grow in shallow, warm, slow-moving or still bodies of water common throughout Canada. Many rural farm-type water supplies used for domestic purposes and for livestock watering are subject to repeated blue-green algal blooms, particularly during the hot summer months. While most species of blue-green algae are capable of producing toxins, not all blue-green algal blooms do. When present, the concentration of toxins varies dramatically within the body of water and over time. Microcystins are extremely stable and will persist for long periods once they are released into a water supply.

This presentation focuses on the development of a Canadian drinking water guideline for Microcystin-LR. In order to develop this guideline, federal and provincial governments have worked together to conduct surveys to assess risk and to improve available detection and testing methods. In particular, an analytical method was developed and a prototype field test kit created. The applicability of the analytical method and field test kit for routine monitoring will be discussed. The success and implementation of these two methods will provide a more accurate and cost-effective approach to the management of risk associated with blue-green algal toxins to humans and livestock.

## **GUIDELINE DEVELOPMENT**

### **History**

The first drinking water standards in Canada were developed in 1923. These standards dealt with the bacteriological quality of drinking water on ships in the Great Lakes and Inland Waters. In 1930 and 1937, they were extended to cover all common carriers and coastal shipping, and were adopted in 1954 as regulations under the National Health and Welfare Act. Until 1968, federal and provincial drinking water authorities generally used the United States Public Health Service Standards as the basis for water quality objectives in Canada. In 1968, the first comprehensive Canadian drinking water quality guidelines were published. These guidelines, referred to as the Canadian Drinking Water Standards and Objectives (Health and Welfare Canada, 1969), were developed by a joint committee made up of the Canadian Public Health Association Drinking Water Standards Committee and an Advisory Committee convened by the Department of National Health and Welfare (now Health Canada). Although the development of the 1968 drinking water standards was conceived as a federal initiative, it involved the active participation of representatives from provincial agencies.

Ten years later, the standards were updated, and the 1978 Guidelines for Canadian Drinking Water Quality (Health and Welfare Canada, 1979) were published. In addition to including more pesticides and organic chemicals, the 1978 Guidelines updated the earlier recommendations for radioactive substances. With the exception of pesticides and phenols, the presence of organic chemicals in drinking water had largely been ignored before the 1970s. Then, in 1974, scientists discovered that trihalomethanes (THMs), a group of chlorinated disinfection by-products (CDBPs), could be formed during the drinking water treatment process. This discovery awakened interest in the presence of organic chemicals in drinking water. Interest focused first on the health significance of the THMs themselves, and then on the presence and possible health significance of other organic chemicals in drinking water.

As with earlier efforts, a working group developed the 1978 Guidelines as a joint federal-provincial project with members drawn from each province and territory, as well as from the federal government. Health Canada provided the technical secretariat to the working group, which then reported through the Federal-Provincial Advisory Committee on Environmental and Occupational Health, now called the Committee on Environmental

and Occupational Health (CEOH), to the Conference of Deputy Ministers of Health. Once its task was completed in 1978, this working group was disbanded.

However, interest in drinking water quality remained, and in 1983 another working group under the same Federal-Provincial Committee began updating the 1978 edition of the guidelines. In 1986, this working group was changed to a standing subcommittee, and in 2002 it was changed to the Federal-Provincial-Territorial Committee on Drinking Water (Committee), which exists to this day.

Since the publication of the 1978 guidelines, further advances in analytical methods, particularly in the field of gas chromatography/mass spectrometry, have resulted in the detection of a large number of organic chemicals found in drinking water supplies that had previously gone unnoticed. Several hundred such chemicals have now been reported in Canadian drinking water supplies, and many hundreds more have been found in other parts of the world. The concentrations of most of these chemicals are very low, and little or no data exists to assess their toxicity. Government and international agencies have responded by assessing the need for standards or guidelines for a wide variety of substances.

The sixth, and most recent, edition of the Guidelines for Canadian Drinking Water Quality (Health Canada, 1996), published in September 1996, lists guidelines for more than 80 physical and chemical parameters and for 78 natural or artificial radionuclides. As in the 1978 edition, explanatory paragraphs concerning the guideline derivation process are an integral part of the booklet. The documents used to support the adoption of a particular guideline value are also made available on request, and can be found on Health Canada's water quality website (<http://www.hc-sc.gc.ca/waterquality> or <http://www.hc-sc.gc.ca/eauqualite>). In order to keep stakeholders apprised of new or revised guidelines between publications of the Guidelines booklet, a Summary Table is prepared and posted to the website once each year.

It should be emphasized that the guidelines are not enforceable standards unless they are embodied in provincial or territorial legislation. All provinces and territories use the guidelines as the basis for assessing the quality of their drinking water supplies. Most provinces have control over the operation of treatment facilities, including the issuance of operating permits.

The Committee continues to be made up of representatives from all of the provinces and territories, as well as from Health Canada and Environment Canada. Health Canada's Water Quality and Health Bureau also continues to provide the scientific expertise to the Committee, as its technical secretariat. The Committee meets biannually, alternating between Ottawa and a city in one of the provinces or territories; the latest meeting was held in Winnipeg, Manitoba in April, 2003. At each meeting, members review and revise the priority list of substances scheduled for assessment. In recent years, the Committee has worked to define the specific roles each level of government plays in the guideline development process, and to work together to identify national priorities which take into

account the needs of each jurisdiction. A detailed summary of the Committee's activities can be found on the water quality website.

### **Current Practices**

Because provincial and territorial governments are responsible for implementing the drinking water guidelines and for providing safe drinking water to Canadians, members of the Committee are accountable for the evaluation and approval steps of the drinking water guidelines development process. Each recommended guideline value, and its accompanying health risk assessment, is evaluated for its practicality and impact. Consultations carried out by the secretariat include posting documents for public comment on the water quality website. Regional consultations may be carried out by the provinces and territories. Through this consensus-based development process, a guideline is established, and the associated health risk assessment is modified to create a criteria summary that reflects the risk management decisions involved in the guideline's development.

The process of developing drinking water guidelines for microbiological, chemical/physical and radiological substances is based on risk management concepts and involves several steps: identification, assessment, evaluation, approval, announcement and publication (Federal-Provincial Subcommittee on Drinking Water, 1998; Health Canada, 1995). This process is flexible by nature, in that it must accommodate the diverse needs of various jurisdictions (i.e., provincial, territorial and federal) in order to be deemed successful. Certain steps within the development process may be modified in order to satisfy the needs of the jurisdictions involved. The process used in developing a guideline, from the selection of the parameters to be evaluated, through to approval and publication, takes several years to complete.

The guideline development process is broken down into a number of specific actions, completed by the various members of the Committee. In general terms, the secretariat (the Water Quality and Health Bureau, Safe Environments Programme, Healthy Environments and Consumer Safety Branch), representing the federal government, is responsible for providing scientific expertise and advice to the provinces and territories; compiling national data on substances under review; assessing health risks; and writing, revising and publishing the criteria summary documents and the guidelines booklet as needed. The secretariat also maintains the water quality website, which houses many of the documents related to the guideline development process.

The provincial and territorial representatives, on the other hand, are responsible for the information for and about their own specific jurisdictions. This includes regulating drinking water quality through the adoption of enforceable regulations, guidelines and objectives; conducting monitoring programs, and setting-up and maintaining distribution systems and treatment plants for drinking water. The Drinking Water Committee, as a group, evaluates all available scientific and economic data to establish or revise drinking water guidelines through a risk management process. In this way, the work conducted by

the various levels of government complement each other and duplication is kept to a minimum.

Drinking water guidelines are developed for substances that appear on the Committee's "drinking water substances priority list." In order to be placed on this list, and therefore qualify for evaluation, a substance must meet at least one of the following criteria: it must be frequently detected in many Canadian drinking water supplies, detected in frequently elevated concentrations, or there must be evidence (pre-assessment review) that the substance may cause adverse health effects. If the substance meets the criteria, the Committee and CEOH place it on a list, which is then approved. This final substance priority list can be found on Health Canada's water quality website. The evaluation of the substance begins once an evaluator becomes available. Due to limited resources and data gaps, many of the substances currently on the priority list are awaiting evaluation. Work is also coordinated with other standards-setting organizations, such as the U.S. Environmental Protection Agency and the World Health Organization, to minimize duplication of research or assessment.

The evaluator begins the risk assessment by researching the substance through an extensive literature and data search, including gathering information from other jurisdictions and partners. The provinces and territories become involved at this point by identifying whether exposure (monitoring) data on this particular substance is available from their own jurisdictions, whether from existing, current or future sampling programs. They also indicate when a summary of their monitoring data will be available. The results of both these searches are then used to determine whether a need really exists for a guideline on this particular substance.

If a need does exist, jurisdictions concerned about the substance initiate monitoring programs to satisfy their own informational needs (e.g., exposure estimates). They collect and summarize their monitoring data for the substance and submit their summaries to the secretariat for consideration in assessing national exposure to the contaminant. This step ensures the process is consistent with the population health directions and priorities of provincial, territorial and federal governments.

The evaluator obtains data on the effects of exposure to chemical agents from toxicological studies of animal species and in epidemiological studies of human populations, where such studies exist. Effects vary, depending upon the dosage, route of exposure (e.g., ingestion, inhalation or dermal), frequency or duration of exposure and the species, and the sex and age of the exposed population. The effects of exposure to chemicals are generally classified in the following broad categories: organ-specific, neurological/behavioral, reproductive, teratological and oncogenic/carcinogenic/mutagenic. Effects may be brief or prolonged, reversible or irreversible, immediate or delayed, single or multiple. In general, the nature, number, severity, incidence and/or prevalence of specific effects in a population generally increase with increasing dose; this is commonly referred to as the dose-response relationship.

For some types of toxic effects resulting from exposure to chemicals, it is believed that a dose (threshold) exists below which adverse effects will not occur. For other types of toxic effects, it is assumed, but not proven, that some probability of harm exists at any level of exposure (i.e., no threshold). At present, the latter assumption is generally considered to be appropriate only for carcinogenesis. For some types of carcinogens, specifically those that induce tumors by particular mechanisms, such as promotion, it is believed that a threshold dose may exist below which tumors will not occur.

Different approaches are adopted for the derivation of guidelines for compounds considered to be carcinogenic and probably carcinogenic, compounds considered to be possibly carcinogenic and those considered being probably not carcinogenic or for which data are inadequate for evaluation. Chemicals are therefore classified with respect to their potential carcinogenicity into various groups on the basis of rigorous examination of the quantity, quality and nature of the results of available toxicological and epidemiological studies. Chemicals classified as carcinogenic often also induce toxic effects other than malignant tumors; for these substances, the guideline is derived on the basis of the approach that leads to the most stringent value.

If the substance undergoing evaluation is found to be not carcinogenic to humans, its recommended drinking water guideline value is derived based on the application of an uncertainty factor to account for inter- and intraspecies variation to a no-observed-adverse-effect level observed in toxicological studies in which rats ingest the substance in drinking water daily for periods ranging from 90 days to two years.

Once the evaluator has compiled the above data, he or she drafts a criteria summary on the substance, incorporating the health risk assessment information, the overall environmental exposure to the substance, the fraction of its exposure attributed to drinking water, existing analytical/treatment techniques and capabilities, as well as a recommended guideline value.

The first review of the draft criteria summary is completed within the Water Quality and Health Bureau; the evaluator defends the classification of the substance and its proposed guideline value to a senior evaluator. The criteria summary is then revised to reflect the experience of the senior evaluator, who must be completely satisfied with the criteria summary before it is forwarded for an external review.

The evaluator then sends the draft criteria summary to three external or third party reviewers who have expert knowledge of the substance. These third-party reviewers are from Canadian or American universities, the U.S. Environmental Protection Agency Drinking Water Program or a Member State of the World Health Organization. Also included among the reviewers is a drinking water treatment specialist, often recommended by the Canadian Water and Wastewater Association. These experts critically review the criteria summary in accordance with the Canadian published approach policy and respond to questions set out in a guide for peer reviewers. Their review focuses on the scientific component of the summary.

Once the third-party reviewers written comments are received, the evaluator assesses the comments and considers any additional information identified by the reviewers. The evaluator then revises the criteria summary and submits it for a final internal review.

The criteria summary is then distributed to the Committee members for their review of the substance's health risk assessment and the proposed guideline value. Provincial/territorial review and assessment of the criteria summary varies from a brief departmental (internal) review to detailed evaluations by an external agency or non-governmental organization. Written comments from all Committee members are forwarded to the secretariat for consideration by the evaluator.

Once the risk assessment phase winds down, the process of risk management becomes more prominent. Each jurisdiction evaluates the feasibility of implementing the recommended guideline for the substance in drinking water by taking treatment costs and socio-economic factors into consideration. A revised criteria summary is then redrafted for public comment. This document contains as much provincial exposure and economic information as is available at the time of printing. It is then made available to the public for comment through the provincial representatives and the secretariat. The documents are also available through the water quality website for public comment during a specified period (usually 4 months).

Following the public review, all comments are again reviewed and summarized by the evaluator and presented to the Committee. Jurisdictions concerned that people living in their area may be exposed to drinking water containing the substance at concentrations that exceed the recommended guideline value will estimate the costs for treating drinking water to reduce the concentration of the substance. The costs of controlling exposure to the substance from sources other than drinking water may also be estimated in order to confirm that modifying water treatment is in fact the most cost-effective way of reducing intake of the substance. Control of other routes of exposure may be identified as a more effective means for reducing the particular health risk. The development of these cost estimates is the responsibility of Committee members; the level of detail of the cost estimates is left to their discretion.

Weighed against these costs are the benefits of reducing exposure to the substance via drinking water if such data are available. For example, there may be direct savings in health care costs that would otherwise be incurred from a specific health problem associated with the substance, or indirect savings in the form of socio-economic benefits such as savings in sick leave and subsequent increases in production. Any side benefits that are an outcome of improved drinking water treatment to control the substance (e.g., the removal of other contaminants or the extension of the life of the water distribution system) may be considered in a cost-benefit analysis.

When the new or revised guideline for a particular substance has been approved by the Committee and then CEOH, the secretariat evaluator makes all the required revisions to the criteria summary in preparation for its publication in the Guidelines for Canadian

Drinking Water Quality Supporting Documentation. The final criteria summary is published in both official languages, and is made available on the water quality website.

The guideline value for the substance is included in the summary table of drinking water guidelines found in the “Guidelines for Canadian Drinking Water Quality” supporting documentation and is posted on the website. This table is updated annually, following each spring meeting of the Committee. Notice is also sent out to subscribers to the Water Quality and Health Bureau’s List serve/ mailing list. The guideline is also included in the “Guidelines for Canadian Drinking Water Quality” booklet when updated.

Re-evaluation of existing guidelines is a continuous process. Although the secretariat is charged with the responsibility of identifying outdated guidelines each year when the Committee list of substances is established, any Committee member or interested party can identify an outdated guideline. The availability of new research, monitoring data, analytical methodology or treatment process may prompt a re-evaluation of an existing guideline.

Although its primary task was initially to develop drinking water guidelines, the role of the Committee has evolved to deal with a variety of issues concerning treatment technologies and the quality of drinking water. A partial list of the Committee's current priorities in the area of risk management and guideline development focuses both on the microbiological quality of drinking water and on chemical substances or groups of substances from the drinking water disinfection process: protozoa, chlorinated disinfection by-products (CDBPs), uranium, and cyanobacterial toxins (Microcystin-LR). Furthermore, Health Canada provides timely advice to other government agencies and departments, the provinces, the territories, and the public in response to emergency situations, such as spills.

## **CYANOBACTERIAL BLOOMS AND MICROCYSTIN-LR**

Cyanobacteria, uni- or multi-cellular prokaryotes that contain chlorophyll, form in shallow, warm, slow moving or still water. A mass of cyanobacteria in a body of water (commonly referred to as pond scum) is called a bloom. Far from being a new phenomenon, cyanobacterial blooms have been around for centuries. Cyanobacteria sometimes produce toxins that either affect the nervous system (neurotoxins) or the liver (hepatotoxins). Cyanobacterial neurotoxins are extremely unstable, which makes them difficult to isolate and study; cyanobacterial hepatotoxins, on the other hand, are extremely stable.

Most hepatotoxins produced and released by cyanobacteria are called microcystins because they were first isolated from a cyanobacterium called *Microcystis aeruginosa*. Microcystins are the most common of the cyanobacterial toxins found in water, and probably the ones most often responsible for poisoning animals and humans who come into contact with toxic blooms. Microcystins are extremely stable in water because of their chemical structure, which means they can survive in both warm and cold water and

can tolerate radical changes in water chemistry, including pH. So far, more than 50 different kinds of microcystins have been isolated and identified.

Regardless of the type of toxin, determining which cyanobacterial blooms are toxic and which are harmless is a complicated task. Researchers generally agree that 30 to 50 per cent of cyanobacterial blooms are harmless because they contain only non-toxic species of freshwater cyanobacteria. Currently, toxicity can only be determined through laboratory testing. However, because cyanobacterial blooms are becoming more and more common in surface waters across Canada, Health Canada and Committee members from affected jurisdictions have initiated a project that will help ascertain the toxicity of blooms in a simple, accurate, and efficient manner. One of the project's outcomes is a simple field test and analytical method that will allow regular and more cost-effective monitoring of this substance.

### **Canadian Drinking Water Guideline for Microcystin-LR**

Health Canada, as the technical secretariat to the Committee, has adopted a guideline value of 1.5 µg/L for Microcystin-LR. It is expected that this value will be protective of effects from other microcystins since M-LR is the most common of the toxins found in Canadian drinking water sources. The final criteria summary document for Microcystin-LR is posted on the water quality website. The value is believed to be conservative as it is based on lifetime oral exposure. For climatic reasons, toxins are probably not present in Canadian water supplies more than four or five months per year.

Some municipal treatment plants are now required to monitor for the presence of Microcystin-LR in their water supplies, especially if the source is prone to cyanobacterial blooms. Because maintaining drinking water quality in Canada is a provincial responsibility, monitoring and treatment strategies will vary between provinces. To help provincial authorities in dealing with elevated levels of toxins found in a given water supply, the Committee has also developed a risk management strategy (flow chart) as part of the guideline document.

### **Cyanobacterial Blooms and Toxins in Canada**

While cyanobacterial blooms occur in all provinces, they are most commonly found in the small, shallow, slow-moving bodies of water common to the prairies (Alberta, Saskatchewan, and Manitoba). Rural prairie towns are the most affected as they generally rely on surface water from shallow, nutrient-rich lakes for drinking water, and dugouts for domestic purposes and for watering livestock. Cyanobacterial blooms and their toxins do not appear in groundwater supplies.

In the summer of 1990, microcystin-LR was detected in cyanobacterial blooms on three shallow Alberta lakes used as sources for municipal drinking water (Kenefick et al., 1992). Microcystin-LR was present in concentrations up to 500 µg/g of algal biomass. More than 70% of more than 380 bloom biomass samples taken from 19 lakes in Alberta between 1990 and 1992 showed detectable levels of the toxin (>1 µg microcystin-LR/g of

dry biomass). Levels of microcystin-LR in Alberta lakes and dugout ponds, measured using high-performance liquid chromatography with ultraviolet detection, ranged from 4 to 605 µg/g dry weight of biomass or up to 1500 µg/g.

In late August 1993, a large algal bloom developed in Deacon Reservoir, Winnipeg's main storage facility for water from Shoal Lake. The water from this lake is generally considered to be of high quality and requires only disinfection with chlorine prior to distribution and consumption. In an attempt to control algal density and taste and odor problems, municipal officials isolated the reservoir and treated it with copper sulphate. However, this action raised the concern that if the algal bloom contained toxin-producing algae, significant quantities of the toxins may have been released into the reservoir.

Sampling determined that toxin-producing blue-green algae were not present in the Deacon Reservoir, but they were present in Shoal Lake. Analysis of water samples (using the protein phosphatase bioassay) indicated that microcystin-LR was present in samples collected from Shoal Lake and from within the distribution system, but was not present at detectable levels ( $>0.05$  µg/L) in samples from Deacon Reservoir. Maximum microcystin-LR concentrations measured in the raw water of Shoal Lake and in treated tap water were approximately 0.45 µg/L and 0.55 µg/L, respectively. Subsequent monitoring during the fall period showed a steep decline in concentrations, suggesting that higher microcystin-LR levels may have been present earlier in August 1993.

Because the weather during the summer of 1993 was characterized by below-normal temperatures and above-normal precipitation, conditions not usually supportive of algal bloom formation, a concern was raised that higher levels of microcystin-LR could develop in Shoal Lake during the more usual, relatively hot, dry summers. As a result, Manitoba Environment, in cooperation with the City of Winnipeg, continued to monitor for microcystin-LR in Winnipeg's water supply. Microcystin-LR was detected at concentrations ranging from 0.1 to 0.5 µg/L on six occasions between 1994 and the end of 1996.

Because Shoal Lake, a relatively nutrient-poor water supply, supported a toxic blue-green algal bloom, Manitoba Environment became concerned that toxic blooms may also be occurring in more nutrient-rich rural surface water supplies throughout southern Manitoba. To address these concerns, a comprehensive two-year study (1995 and 1996) was conducted on water quality in rural southwestern Manitoba surface water supplies. In the first year of the study, microcystin-LR was found to be widely distributed in all water supply categories. Rural municipal water supplies had a higher detection frequency (93%) than on-farm domestic/livestock dugouts (57%), showing that conventional treatment methods may be only partially successful in removing the toxin. Mean concentrations ranged from 0.23 µg/L in recreational sites to 0.35 µg/L in dugouts used exclusively for livestock. In the second year of the study, seven rural surface water supplies were intensively sampled for microcystin-LR. The hepatotoxin was found throughout the entire sampling period (June through December 1996), sometimes at levels exceeding 0.5 µg/L, the "Emergency Health Advisory Guideline" formulated by Health Canada in response to the 1993 incident.

Several incidents of suspected animal poisonings have been associated with toxic algal blooms in Manitoba lakes. In June 1995, hepatotoxin poisoning was suspected in the deaths of two calves watering directly from Pelican Lake, where a massive bloom was associated with microcystin-LR concentrations ranging from 0.8 to 1.2 µg/L. In mid-June 1996, 16 cattle and a dog died after drinking water from a creek impoundment near Balder. Although microcystin-LR concentrations were relatively low (0.4 and 0.23 µg/L), the neurotoxin-producing blue-green algal genus *Anabaena* dominated the algal composition. In July 1996, three dogs died after drinking water from recreational beaches along Dauphin Lake. Again, water samples contained low levels of microcystin-LR (<0.1 to 0.1 µg/L), but the dominant algal species were neurotoxin-producing *Anabaena* species. "Toxic Algae Advisory" signs were posted along Dauphin Lake beaches to warn residents about the risks associated with using the lake for drinking water, pet watering, or swimming.

### **Treatment Technology and Management**

Good control technology must reflect:

- \* proper management of the watershed and reservoir to prevent algal growth,
- \* an appropriate monitoring program, and
- \* correct treatment technology for both the cyanobacteria and their toxins.

Management options are similar to common techniques used to control algal populations in reservoirs, but with a few exceptions. For example, the use of chemicals that would lead to the disintegration (lysis) of cyanobacterial cells must be avoided to prevent the release of their toxins. In the past, chemical treatment with copper sulphate had been the most common technique used to control algal blooms in drinking water reservoirs. However, Kenefick et al. (1993) found that algal bloom material from a Canadian prairie lake treated with copper sulphate released most of its toxins during the first three days following treatment. Their results indicated that a 99% reduction in microcystin levels would take approximately three weeks, suggesting that copper sulphate should not be used to treat potentially toxic cyanobacterial blooms in waters to be consumed by humans within several weeks following treatment.

An appropriate monitoring program is essential to the overall control of cyanobacteria and their toxins. At present, most municipal water treatment plants in Canada do not regularly monitor their water supplies for cyanobacterial toxins. However, because cyanobacteria have strong smells and tastes and interfere with certain water treatment processes, most municipalities with a history of blooms monitor their surface water supplies for cyanobacteria.

Monitoring programs must take spatial and temporal variability in microcystin levels into consideration, as there may be large fluctuations in the levels of cyanobacteria and their

toxins resulting from the interplay of a variety of physical, chemical and biological factors. Kotak et al. (1995) studied the patterns of occurrence of microcystin-LR (measured as  $\mu\text{g/g}$  biomass of *Microcystis aeruginosa*) in three hypereutrophic hard water lakes in central Alberta over three seasons. *Microcystis aeruginosa* was highly variable both temporally and spatially, with differences up to three orders of magnitude within each lake over one year, between years in an individual lake and between lakes in a year. Seasonal changes in microcystin-LR concentration were positively correlated to the abundance and biomass of the *M. aeruginosa*, total and total dissolved phosphorus concentration, pH, and chlorophyll. There was a negative correlation between microcystin-LR concentration and nitrate concentration and no correlation with water temperature. Over a 24-hour period, the concentration of microcystin-LR was six times lower at night than during the day.

The final step in controlling cyanobacteria and their toxins is the drinking water treatment process. Although conventional surface water treatment plants using coagulation, clarification, and filtration are effective in removing cyanobacterial cells, the Manitoba study noted above (Jones, 1996) illustrated that conventional water treatment methods may be only partially successful in removing cyanobacterial toxins. This has been confirmed by Lambert et al. (1996), who examined the removal of microcystins from drinking water at two full-scale treatment plants in Alberta that employed coagulation-sedimentation, dual-media filtration, and chlorination combined with either granular activated carbon or powdered activated carbon filtration. The two processes generally removed more than 80% of the microcystin from raw water, particularly when the raw water concentrations were high; however, a residual concentration of 0.05-0.2  $\mu\text{g}$  microcystin-LR equivalents/L was observed at both treatment facilities. The data thus suggests that drinking water consumers could be chronically exposed to low levels of microcystin-LR for the duration of cyanobacterial blooms. As previously mentioned, Lambert et al. (1996) also found that chlorination was unsuccessful in achieving any reduction in microcystins.

### **Development of an Analytical Method**

In 1997/98, Health Canada was approached by Saskatchewan Health to help develop an analytical method to detect and assess the toxicity of cyanobacterial blooms in an affordable, efficient, reliable, and routine way. After discussion with the Saskatchewan Health Provincial Laboratory staff, it was concluded that the most suitable method would use High Performance Liquid Chromatography with UV detection (HPLC-UV).

Among the several analytical methods using HPLC-UV for microcystin analysis, a procedure published by HMSO (London, UK) in 1998 was chosen for modification. This method is part of the series Methods for the Examination of Waters and Associated Materials, and was developed to detect and quantify dissolved microcystin-LR, microcystin-RR, microcystin-YR and nodularin in raw and treated waters (extra-cellular toxins).

The procedure consists of multiple steps. First, samples are filtered to remove algal cells. The filtrate is then extracted using a preconditioned C18 phase extraction cartridge. The cartridge is washed with aqueous methanol before elution with methanolic trifluoroacetic acid (0.1%v/v). The acidified methanol is reduced to dryness; the residue is redissolved in methanol and again evaporated to dryness. The residue is taken up in 70 percent aqueous methanol and centrifuged (if necessary) to remove solid material. A portion of the supernatant solution is analyzed by reversed-phase high performance liquid chromatography with UV detection at 238 nm, using a photodiode array detector.

During the initial method evaluation conducted by Health Canada, two major difficulties arose. The first problem was the high background and interference peaks in the HPLC chromatograms. The second was the obtained detection limit, comparatively higher than reported in the published method.

Multiple attempts were carried out by changing several of the procedure parameters. The high background and interference peaks disappeared after the cartridge conditioning procedure was modified by passing 5 mL of methanolic trifluoroacetic acid (0.1%v/v) through it at the very beginning of the conditioning. The detection limit was slightly improved by increasing the cartridge elution volume (4.0 mL instead of 3.0 mL of methanolic trifluoroacetic acid (0.1%v/v)). During the development of the analytical method, duplicate samples were sent to a Health Canada lab in Ottawa in addition to the University of Alberta Protein Phosphatase Bioassay Service, the only laboratory in Canada that had the capacity to routinely quantify blue-green algae toxins in water. The per sample cost for testing via this method is high, therefore making this method unsuitable for routine monitoring practices.

Using the modified analytical method for a 500 mL water sample, microcystin-LR can be detected at levels as low as 0.3 µg/L. The minimum quantifiable limit (MQL) is 0.8 µg/L. The MQL for the three other microcystins (-RR, -LA and -YR) is similar (0.5-0.8 µg/L). Work is continuing on refining of the method.

### **Evaluation and Testing**

During the evaluation and testing phase, sampling was carried out at various sites in several provinces in Canada. During 1998, 279 raw water samples were collected from different surface water sources (lakes, rivers, dugouts, etc.). These samples were tested using the protein phosphatase method; 28 samples tested positive (0.3 µg/L to 14 µg/L). Thirty-two duplicate samples (randomly selected from the original 279 samples) were sent to the Health Canada lab for testing using the HPLC method. Unfortunately, none of these samples tested positive. (Because the samples were selected randomly, there was no guarantee of hitting a positive sample).

During the 1999 surveys, 92 samples were collected and analyzed using the protein phosphatase method, and 49 randomly selected duplicate samples were sent to the Health Canada lab for testing using the HPLC method. This time, 27 of the original 92 samples tested positive (0.3 µg/L to 9.4 µg/L), as did seven of the 49 random samples.

In both surveys, treated water samples were analyzed only if microcystins were detected in the raw water supply. The flow charts in Appendix A show the process used during the 1999 survey to determine which bodies of raw water to sample, when to test treated water, and when to notify the community and potentially switch to an alternative water supply or adjust treatment. Appendix A shows the process for water destined for human consumption.

## **Survey Results**

As mentioned above, no microcystins were detected in water samples tested using the HPLC method during the 1998 survey. In 1999, Microcystins -LR, -LA and -RR were detected in seven raw water samples (mainly recreational sites) at concentrations ranging from 0.3 to 3.1 µg/L. One sample contained both Microcystin-LR and -RR. None of the four screened microcystins were detected in treated water samples.

Other unknown compounds that could be part of the microcystin family were detected in several samples. Attempts to identify such compounds by liquid chromatography-mass spectrometry will be carried out during the summer of 2000.

## **Development of a Field Test Kit**

As mentioned earlier, the tools available to governments and the public to address concerns about the presence of toxins in cyanobacterial blooms are extremely limited. The field test kit, based on Dr. Holmes' protein phosphatase method, is being developed to complement the analytical method as a quick, easy to use, readily available tool to determine whether cyanobacterial toxins are present in an affected water supply. This tool will be useful for:

- \* municipal water utilities and agencies responsible for drinking water quality to monitor supplies for toxins,
- \* agencies responsible for recreational water quality to monitor surface water bodies used for recreational contact purposes for toxins, and
- \* livestock owners and agricultural agencies in determining the safety of water for livestock consumption.

The prototype field test kit is a non-radioactive assay which exploits the ability of microcystins to specifically inhibit the type-1 protein phosphatase (PP-1). This colorimetric assay is reproducible, fast, and capable of handling a large number of samples with little training or equipment. The kit allows untrained individuals to visually confirm the presence or absence of toxin in a water sample by comparing the color reaction against a control sample. The absence of toxin results in a clear yellow color whereas in the presence of the toxin, the solution becomes colorless. This comparison will also enable the individual performing the test to get an approximate idea of the

amount of toxin present in the sample (positive control is 1.5 µg/L M-LR). The prototype kit was validated during field trials in the summer of 2000. The summer trials will allow validation testing of variables such as the effects of turbidity, the stability of the PP-1, and the shelf life and storage time for the PP-1.

Test kit results will only be used as an initial indicator of the presence/absence of toxins. Final determination of water safety will be based on toxin level results derived from a laboratory analytical methodology. Currently, authorities must issue warnings regarding the use of water by humans, pets, or livestock where cyanobacterial blooms have developed. Where doubt exists about the safety of the water, the public must avoid the use of a particular water supply for drinking or recreation. This type of blanket approach is unsatisfactory

### **Future Applications**

In addition to its significance for drinking and recreational water authorities, the development of the field test kit is also important to livestock producers throughout the country, as the loss of any livestock can have serious financial implications. This tool will assist livestock owners to manage their resources by providing them with a rapid method to determine the safety of water used for their herds. Early detection of the toxins will not only result in decreasing the chances of financial loss due to livestock deaths, but will also help eliminate any concerns that may be held by livestock owners and buyers that meat from cattle which have consumed water contaminated by blue-green algae is tainted.

### **CONCLUSION**

In conclusion, the quality of drinking water in Canada, indeed the envy of most of the world, can be directly linked to a successful system of federal-provincial cooperation in assessing and managing health risks associated with its drinking water supplies. Over the past twenty years, the federal, provincial and territorial governments have worked together to develop and revise guidelines for substances found in Canadian water supplies to maintain and improve water quality. This relationship has evolved to include more than just guideline development, as can be seen by the current project to develop improved tools to monitor the presence of cyanobacterial toxins (microcystin-LR) in blue-green algae blooms. The success and implementation of the analytical method and the field test kit will provide a more accurate and cost-effective approach to the management of risk associated with blue-green algal toxins to humans and livestock.

### **References**

Health and Welfare Canada (1969). Canadian Drinking Water Standards and Objectives, 1968. Prepared by the Joint Committee on Drinking Water Standards of the Advisory Committee on Public Health Engineering, Department of National Health and Welfare and The Canadian Public Health Association. Ottawa.

- Health and Welfare Canada (1979), Guidelines for Canadian Drinking Water Quality, 1978. Supply and Services Canada, H48-10-1978.
- Health Canada (1996). Guidelines for Canadian Drinking Water Quality - Sixth Edition. Supply and Services Canada, H48-10/1996E.
- Federal-Provincial Subcommittee on Drinking Water (1998). Canadian Drinking Water Guidelines. Development Process. July 1998
- Health Canada (1995). Guidelines for Canadian Drinking Water Quality. Supporting Documentation. Part I. Approach to the Derivation of Drinking Water Guidelines. Ottawa. Environmental Health Directorate.
- Health Canada (1993) Guidelines for Canadian Drinking Water Quality. Supporting Documentation. Part II. Trihalomethanes. Ottawa. Environmental Health Directorate.
- Health and Welfare Canada (1978). Guidelines for Canadian Drinking Water Quality. Supporting Documentation. Uranium. Ottawa. Environmental Health Directorate.
- Health Canada (1987). Guidelines for Canadian Drinking Water Quality. Supporting Documentation. Part II. Uranium. Ottawa. Environmental Health Directorate.
- Health Canada (in press). Uranium in Drinking Water. Document for Public Comment. Ottawa. Environmental Health Directorate.
- Health Canada (1998). Guidelines for Canadian Drinking Water Quality. Supporting Documentation. Part II Aluminum. Ottawa. Environmental Health Directorate.
- Health Canada (1998). Cyanobacterial Toxins - Microcystins in Drinking Water. Document for Public Comment. Ottawa. Environmental Health Directorate.
- HMSO. (1998). The determination of microcystin algal toxins in raw and treated waters by high performance liquid chromatography. Methods for the Examination of Waters and Associated Materials. London, UK, 1998.
- Hrudey, S.E., Lambert, T.W. and Kenefick, S.L. (1994). Health risk assessment of microcystins in drinking water supplies. In: Toxic cyanobacteria a global perspective. March 28, 1994, Adelaide, South Australia. Australian Centre for Water Quality Research, Salisbury, Australia. pp.12.
- Gurney, S. and Jones, G. (1997) Toxic algae occurrence and distribution in Manitoba surface waters. Proceedings of the Rural Water Quality Symposium, March 25-26, 1997, Winnipeg, Manitoba.
- Jones, G., Gurney, S. and Rocan, D. (1996) Water Quality/Toxic Algae Study Interim Report: Summary of the 1995 Field Season Results. Manitoba Environment, June 4, 1996.

- Jones, G. (1996). Toxic algae study summary. Manitoba Department of Environment, February.
- Kenefick, S.L., Hrudey, S.E., Peterson, H.G. and Prepas, E.E. (1993). Toxin release from *Microcystis aeruginosa* after chemical treatment. *Water Sci. Technol.*, 27: 433-440.
- Kenefick, S.L., Hrudey, S.E., Prepas, E.E., Motkosky, N. and Peterson, H.G. (1992). Odorous substances and cyanobacterial toxins in prairie drinking water sources. *Water Sci. Technol.*, 25: 147-154.
- Kotak, B.G., Kenefick, S.L., Fritz, D.L., Rousseaux, C.G., Prepas, E.E. and Hrudey, S.E. (1993). Occurrence and toxicological evaluation of cyanobacterial toxins in Alberta lakes and farm dugouts. *Water Res.*, 27(3): 495-506.
- Kotak, B.G., Lam, A. J-Y., Prepas, E.E., Kenefick, S.L. and Hrudey, S.E. (1995). Variability of the hepatotoxin microcystin-LR in hypereutrophic drinking water lakes. *J. Phycol.*, 31: 248-263.
- Lambert, T.W., Holmes, C.F.B. and Hrudey, S.E. (1996). Adsorption of microcystin-LR by activated carbon and removal in full-scale water treatment. *Water Res.*, 30 (6): 1411-1422.

## **Annex A: Stepwise Protocol for Microcystin-LR in Water Supplies Used for Human Consumption**

### **Preamble**

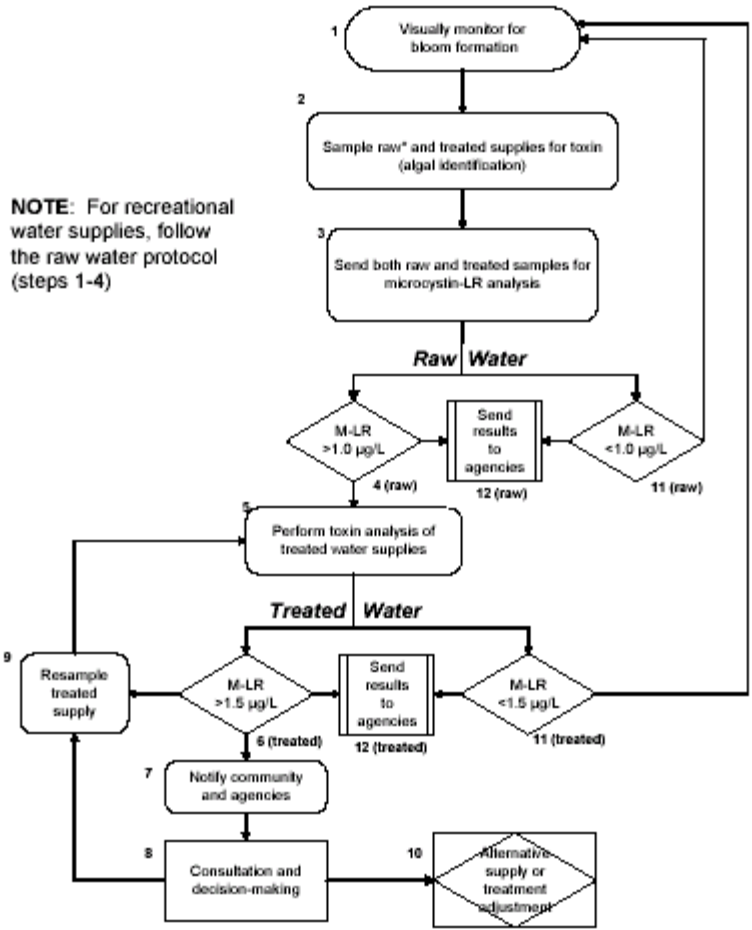
Summer conditions can lead to the growth of blue-green algae in bodies of water (generally smaller or shallow lakes, reservoirs, sloughs or dugouts) throughout Canada. In many cases, blooms tend to recur within the same bodies of water year after year. While most species of blue-green algae are capable of producing nerve and liver toxins, not all blue-green algal blooms produce toxins, and, when present, the amount of toxins varies dramatically within the body of water and over time. Analytical studies over the past few years in dugouts and other water supplies in Manitoba, Saskatchewan and the Peace River region of Alberta indicate that blue-green algal toxins are much more common in rural water supplies than originally thought. Although there are few quantitative data available, there are indications that these toxins may also be occurring in various water supplies in other provinces (e.g., Ontario, British Columbia, Quebec and Prince Edward Island).

The factors inducing the production of toxins by cyanobacteria are not well known. Laboratory studies demonstrate that some environmental factors, such as temperature, light, nitrogen concentrations, carbon availability (in the form of bicarbonate, carbonate

and carbon dioxide), phosphate concentrations and pH, could be important. As toxin production varies greatly among different strains of the same species, genetic differences and metabolic processes may also be important in the production of these secondary metabolites. Studies have shown that the ability to produce toxins can vary temporally and spatially at a particular site. Cyanobacterial toxins tend to be associated with cyanobacterial cells and may be membrane bound or occur free within the cells. In laboratory studies, most of the toxin release occurs as cells age and die and passively leak their cellular contents; some active release of toxins can also occur from young, growing cells. Toxin levels do not necessarily coincide with maximum algal biomass; there can be significant variation in the amount of toxin per unit biomass of cyanobacteria over time, which is independent of changes in the blue-green algal population. Concentrations of microcystins were higher in blooms taken during the day than at night in one study, for example, and no significant difference in toxin concentrations from cyanobacteria incubated for 24 hours at different depths in a reservoir was observed in another study. Microcystins are relatively persistent in the aquatic environment. Studies in Australia have shown that microcystin-LR was present up to 21 days following treatment of a *Microcystin aeruginosa* bloom with an algicide.

Recent studies have led to increasing concern by government agencies and the public regarding the safety of water supplies that may be potential sources of these toxins. The purpose of this annex and the accompanying flow chart (see next page) is to provide water purveyors and health and environment authorities with a summary of the important factors that should be considered during bloom events and recommendations on actions that may be taken to address the issue.

**ANNEX A**  
**Cyanobacterial Toxins -- Microcystin-LR**  
**Flow Chart**  
**- Water Supplies for Human Consumption -**



**NOTE:** For recreational water supplies, follow the raw water protocol (steps 1-4)

\* A field kit could be used for screening. A validation sample should be sent to a laboratory for confirmation of actual levels following a positive field test

April, 2002

**Step 1: Visually monitor for bloom formation**

As blooms tend to recur in the same water supplies, water bodies that have historically exhibited algal blooms should be visually monitored for bloom formation and hence toxin production during the peak season (usually late May to early October). Authorities should visually monitor supplies for algal growth and conduct sampling during and after collapse of the bloom. As well, bodies of water that, due to variables such as size, water depth and nutrient content, may be susceptible to algal blooms should be considered for monitoring programs. Toxins may persist following the collapse of blooms, particularly in the late summer and early fall, when the onset of colder temperatures and decrease in light intensity result in decreased rates of toxin degradation. A visual bloom is identified by

the appearance of “soupy” water. Colours can range from grey or tan to blue-green or bright blue or reddish. The appearance of blooms may also be described as fine grass clippings or small clumps.

### **Step 2: Sample raw and treated supplies for toxin (algal identification)**

Samples of raw and treated supplies (if applicable) should be taken at the same time in order to save time and money. For laboratory analysis, raw water samples should be collected prior to any treatment, including filtration; samples from the raw water tap at the treatment plant are acceptable if no pretreatment is applied. Sampling from a reservoir should be done as close to the inlet/shore and/or the bloom formation as possible. However, when possible, it is suggested that samples from several sites be pooled for the determination of toxicity, as cyanobacterial species/cell abundance and biomass vary spatially within a water body (e.g., cells may be transported by wind currents). Treated samples should be taken at the treated water tap of the treatment plant or within the distribution system.

Samples, in amounts required by the analytical laboratory, should be collected in glass containers, as studies indicate that the toxin, if present, can be adsorbed to the plastic. Agencies may also wish to sample for algal identification. Species identification, especially from sites positive for toxin, can provide additional information regarding the source, conditions and type of other toxins that might be present.

† A field test kit could be used as a screening tool to determine the presence or absence of toxin in a water supply. If the presence of toxin is detected in a sample using the field test kit, the sampling agency will need to submit a sample to a recognized laboratory for confirmatory analytical analysis (see Step 3).

### **Step 3: Send both raw and treated samples for microcystin-LR analysis**

Both raw and treated samples should be sent (in coolers) to the laboratory for analysis as soon as possible (preferably within 24 hours). Sampling agencies should contact their local or regional departments of health or environment for information on laboratories capable of conducting toxin analyses. Upon receipt of samples from sampling agencies and in order to avoid unnecessary costs, the laboratory should store the treated samples until the results of the microcystin-LR analysis of the raw samples are available. A microcystin-LR result of  $>1.0 \mu\text{g/L}$  in the raw sample will cause further action, as outlined in Steps 4–10.

### **Step 4: Microcystin-LR $>1.0 \mu\text{g/L}$ (raw)**

Results will be reported to the sampling agency, as per Step 12 (raw). A result of  $>1.0 \mu\text{g/L}$  should cause further action, as outlined in Steps 5–10.

### **Step 5: Perform toxin analysis of treated water samples**

Testing will be performed on treated samples when a level of  $>1.0 \mu\text{g}$  microcystin-LR/L is found in a raw sample from the same site.

### **Step 6: Microcystin-LR $>1.5 \mu\text{g/L}$ (treated)**

A result of >1.5 µg microcystin-LR/L for the treated sample will cause the sampling agency to take appropriate action, as outlined in Steps 7–10. Microcystin-LR results of >1.5 µg/L in a treated supply will result in a resampling of the treated supply as soon as possible (Step 9) and notification of the community and appropriate agencies (Step 7).

#### **Step 7: Notify community and agencies**

Upon receiving the results, the sampling agency will follow standard protocol for notifying communities and appropriate agencies. Consideration will be given as to which agency should be taking the lead role in this regard. Additionally, the investigation should take into account the history of the source/supply and the type of treatment at the plant. Dialysis treatment units in the community should also be notified, especially if it is a first-time occurrence for blooms on this supply. A Health Canada fact sheet on microcystin-LR (*It's Your Health: Blue-Green Algae (Cyanobacteria) and their Toxins*) is available to help convey information to communities.

#### **Step 8: Consultation and decision-making**

In the case of community (municipal) supplies, risk assessment discussions should take place regarding additional action to be taken. Health agencies, municipal councils and the lead regulatory agency responsible for municipal systems should be included in these discussions. The risk assessment discussion process should include, for example, such items as the history of the site, the size and location of the bloom, available treatment technology (if a treated site), uses of the source water (recreational versus domestic uses) and monitoring of the environmental conditions that might affect the bloom (e.g., wind). Where possible, lysing of the bloom by the addition of copper sulphate or Blue Stone should be avoided, as this action will immediately release toxin from the cells into the water supply. If the bloom is a common occurrence, nutrient monitoring may be considered. Weekly monitoring should be continued. In the case of non-community (non-municipal) supplies, the sampling agency will consult with health agencies and agencies having treatment expertise.

**Note:** See Step 10.

#### **Step 9: Resample treated supply**

Following receipt of results indicating that the treated water contains >1.5 µg microcystin-LR/L, the treated supply will be resampled as soon as possible or as determined by the sampling agency.

#### **Step 10: Alternative supply or treatment adjustment**

During the Step 8 decision-making process, discussions regarding treatment adjustments or alternative supplies should be reviewed (Step 10). The lead agency should advise the owner of the supply of any treatment options, such as additional granular activated carbon filtration or ozonation. It should be pointed out that boiling is not effective in reducing or removing these toxins, although some point-of-use devices may be effective. **Note:** As blooms may be of short duration (ranging from days to weeks), health and environment agencies may recommend, after consultations with the community (see Step 8), that consumers seek alternative supplies of safe drinking water until there is no longer a visible bloom and the toxin level has dropped below 1.5 µg/L.

**Step 11 (raw): Microcystin-LR <1.0 µg/L**

A result of <1.0 µg microcystin-LR/L in the raw water will cause the sampling agency to continue to visually monitor the raw water for the recurrence of blooms and resample if necessary.

**Step 11 (treated): Microcystin-LR <1.5 µg/L**

A result of <1.5 µg microcystin-LR/L in the treated water will cause the sampling agency to continue to monitor the raw water as per Step 11 (raw).

**Step 12 (raw): Send results to agencies**

Microcystin-LR results from laboratories (and field test kit results when available) will be reported to the sampling agency as soon as possible from time of receipt of the sample. Sampling agencies are responsible for reporting results to the water supply owner.

**Step 12 (treated): Send results to agencies**

Treated water microcystin-LR results will be reported to the sampling agency as soon as possible from the time of the positive raw water results for the same sampling site. Sampling agencies are responsible for reporting results to the water supply owner and/or responsible agencies.

## **Blue-green algae and their toxins – Great Britain’s perspective**

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### **ABSTRACT**

Details of research described in this paper can be found under Foundation for Water Research publications at [www.fwr.org](http://www.fwr.org)

This paper reviews outputs from the United Kingdom’s collaborative research program on algal toxins and their significance in drinking water treatment. Information on the DWI policy towards control of algal toxins in water supplies and current water industry control measures is also given.

### **INTRODUCTION**

Blue-green algae in some raw water sources had caused sporadic operational problems, such as blockage of raw water filters or the production of tastes and odors in finished waters, for water suppliers in the UK for decades. However, in 1989 following well publicized blue-green algal blooms, some of which occurred in raw water storage reservoirs, it was considered possible that mammalian toxins produced by some of these algae could pose a threat to the health of drinking water consumers. The most commonly encountered species in the 1989 incidents were *Microcystis aeruginosa*, *Aphanizomenon* sp., *Anabaena* sp. and *Oscillatoria* sp. Animal toxicity tests on samples from 78 water bodies indicated that 68 percent were toxin producing.

At this time, there was no reliable information regarding the presence of algal toxins in raw or treated waters in the UK, no toxicological information relating to the potential route of exposure existed, and it was not known whether water treatment processes then in use in the UK were effective in removing toxins (if present) from raw waters. To address these deficiencies, a national collaborative research program was initiated in 1990 by the then Department of the Environment and the National Rivers Authority (now the Environment Agency). In 1991 and 1992, contributions to funding were made by UK Water Industry Research Ltd. The research program was managed by the Foundation for Water Research.

### **R&D PROGRAMME (1990-1996)**

This research program had several objectives, as follows:

- development of sensitive analytical methods to allow toxins to be quantified in raw water sources and drinking waters;
- design of a toxicity testing program to establish levels of concern for the toxins;
- investigation of the effectiveness of various water treatment processes used in the production of drinking waters for toxin removal.

At the time this research began there were several factors that influenced the approach that could be adopted. Knowledge concerning the toxins produced by blue-green algae was limited, although it was considered that the toxin of greatest relevance to the UK was the cyclic heptapeptide hepatotoxin microcystin-LR, so research efforts were initially focused on this compound. The fact that it was one of the first hepatotoxins to be commercially available was also influential, as pure standards are required to develop quantitative analytical methods, to use for toxicity testing and for use in water treatment studies. Similar work was also undertaken for the neurotoxin anatoxin-a, which was also considered to be of relevance with respect to UK water supplies.

## **DEVELOPMENT OF ANALYTICAL METHODS**

It was necessary to develop quantitative analytical methods for toxins in water to establish whether they were present in raw waters and drinking waters, to provide a check of the dosing levels used in the toxicity testing program and to determine the efficiency of removal when various water treatment processes were applied to toxin-containing waters. It was also a requirement that the methods developed should be usable on a routine basis by water company laboratory staff; this constraint ruled out the use of advanced techniques e.g. liquid chromatography-mass spectrometry (LCMS).

### **Microcystin-LR**

Work on method development commenced in early 1991 and as noted earlier, this was one of the then known microcystin congeners that was commercially available. A literature survey revealed that although there were several reports of its determination in algal cells, little information was available on its analysis in water samples. The optimized method developed involved solid-phase extraction followed by analysis using high performance liquid chromatography (HPLC) with ultra-violet (UV) detection. Nodularin, a cyclic pentapeptide toxin produced by blue-green algae (*Nodularia* sp.) normally found in brackish waters, was used as an internal standard. A limited evaluation of the method for drinking waters and reservoir waters in the autumn of 1992 showed that at the 1 µg/l level, the relative standard deviation was less than 10%. The method was then performance tested by five laboratories in 1993. This involved the analysis of over one hundred samples by each laboratory, and demonstrated that the limit of detection was 0.5 µg/l.

It was also shown that electrospray ionization LCMS could be used to provide confirmatory data.

In 1996 another analytical method, which determined some additional microcystin congeners (-RR, -YR) and nodularin, was performance tested. It was shown to be satisfactory and could be applied to determine other microcystins, but the lack of commercially available pure standards only allowed it to be tested for the aforementioned toxins.

### **Anatoxin-a**

An analytical method for anatoxin-a in water, which involved solid-phase extraction and analysis of the extracts using HPLC with UV detection, was developed in 1993. The limit of detection of this method was 1 µg/l. In 1994, it was shown that a custom-synthesized internal standard (d<sub>3</sub>-homoanatoxin-a) improved the performance of the method.

## **TOXICITY TESTING**

### **Microcystin-LR**

The only published information relating to the toxicity of microcystin-LR that was available prior to these studies was that its LD<sub>50</sub> via intra-peritoneal (i.p.) injection in mice was about 50 µg/kg. The toxicity-testing program, devised in consultation with the contract toxicology laboratory that undertook the work, consisted of an acute toxicity study to compare the toxicity via the oral and i.p. routes, an investigation of the effects on the fetus in the mouse, and a repeat dose study over thirteen weeks by the oral route, again in the mouse. The main reason for using the mouse rather than the rat in the latter two studies was the availability and cost of pure microcystin-LR. Even when using the mouse, the cost of the pure toxin used was over \$30,000.

The mouse oral LD<sub>50</sub> in the acute study was found to be about 5000 µg/kg, while the rat oral LD<sub>50</sub> was greater than 5000 µg/kg. The mouse i.p. LD<sub>50</sub> was between 50 and 158 µg/kg. This meant that the difference in acute toxicity between i.p. injection and oral dosing was between 30 and 100-fold, with the mouse being slightly more sensitive than the rat.

The No Observed Adverse Effect Level (NOAEL) for developmental toxicity (daily oral administration during organogenesis) was 600 µg/kg bodyweight per day. In the 13 week repeat dose study, the clear NOAEL for pathological changes in the liver of the mouse when administered orally by gavage once daily was 40 µg/kg bodyweight per day. The effects observed at 200 µg/kg bodyweight per day were relatively minor and few animals were affected.

In terms of risk assessment the 40 µg/kg bodyweight per day dose would be 400 times greater than that received by a 10 kg child drinking 1 liter of water per day, and the margin of safety would be much greater than this.

## **Anatoxin-a**

Toxicity data relating to the acute effects of this neurotoxin were already available, but in order to undertake a better-informed risk assessment it was considered necessary to carry out some longer-term studies, so a 28-day oral *in vivo* toxicity study and a developmental toxicity screening study were carried out in the mouse. A series of *in vitro* and *in vivo* screening studies were also done in order to properly characterize the pharmacological activity of anatoxin-a relative to nicotine (anatoxin-a was known to be a stereospecific nicotinic agonist).

In the pharmacological screening studies anatoxin-a elicited similar responses to those of standard nicotine solutions, but it was a more potent agonist than nicotine. In a twenty-eight day oral repeat dose study the NOAEL found was 0.098 mg/kg bodyweight per day. The NOAEL for teratogenicity over the period of organogenesis was 2.46 mg/kg bodyweight per day. The conclusion of this latter study (that anatoxin-a was not a specific developmental toxicant in the mouse) is in agreement with an earlier study carried out in the hamster.

Based on the most conservative NOAEL from these studies, a guideline value of 1 µg/l for drinking water would provide a significant margin of safety of about three orders of magnitude for a 10 kg child consuming 1 liter of water per day.

## **TREATMENT STUDIES**

Because work already published had shown that conventional water treatment processes (chemical coagulation, sand filtration, chlorination) were not particularly effective in removing microcystin-LR, advanced water treatment processes were investigated.

### **Microcystin-LR**

It was shown that activated carbon adsorption, oxidation and nanofiltration could all be used to remove this toxin from lowland river-derived reservoir water. Powdered activated carbon (PAC) dosing was effective, but the degree of removal was strongly dependent on the dose applied and wood-based PAC was more effective than those derived from coal or coconut source materials. Coal, peat and wood based granular activated carbon (GAC) were also effective in removing microcystin-LR although rapid breakthrough of the toxin occurred.

Ozonation and treatment with potassium permanganate under practicable water treatment dosing conditions were the most effective oxidative processes, with higher removal occurring when dosed into clarified or sand filtered water. Chlorination (applied chlorine dose 1.7 mg/l) was effective, but dependent on pH e.g. at pH5, removal was >90% within 30 minutes, but at pH7 90% removal was only obtained after 22 hours and at pH9 removal was even slower.

When using a polymeric nanofiltration membrane with a nominal molecular weight cut-off of 200, and a permeate to retentate ratio of 0.25, complete rejection of microcystin occurred and no toxin accumulated on the membrane itself.

### **Anatoxin-a**

Oxidative processes also removed anatoxin-a, although as for microcystin-LR, they were more effective when applied to treated waters. For both ozone and potassium permanganate, a dose of 2 mg/l gave a reduction of >90 percent in the dissolved toxin concentration. However, no significant removal was observed with chlorination.

PAC and GAC were effective in removing anatoxin-a, although relatively high doses of PAC were required to achieve >85 percent toxin removal. Results obtained with GAC suggested that for anatoxin-a biodegradation occurs on the GAC.

## **OTHER STUDIES**

### **Monitoring**

Since the analytical methods described above were developed, several surveys of raw and treated waters have been carried out in England and Wales, and in Northern Ireland. The most comprehensive of these surveys was conducted in Northern Ireland to allay concerns regarding drinking water produced by a new drinking water treatment works taking raw water from a water body where there have been blue-green algal blooms (usually *Oscillatoria* sp.) in the past. A total of two hundred and seventy three samples were analyzed during the period June 1998 – July 1999 for microcystin-LR and anatoxin-a. In one of these samples, anatoxin-a was detected at about 1 µg/l and its presence confirmed using GCMS following derivatisation of the extract. This concentration found was not considered to be of significance to human health. No microcystin-LR was detected in any of the samples.

### **Toxin persistence**

The analytical methods have also been applied to study the persistence of microcystin-LR and anatoxin-a in raw water reservoirs. For microcystin-LR the half-life in reservoir water was found to be about six days, and with addition of bed sediment this was reduced to about 3 days. The half-life of anatoxin-a was found to be 4-5 days in reservoir water at pH8 in the presence of bed sediment. At pH4, anatoxin-a is persistent but as it is unlikely that natural waters at this pH would support populations of anatoxin-a producing blue-green algae, this finding was not considered significant.

## **GUIDELINES AND STANDARDS FOR ALGAL TOXINS**

The water industry in England and Wales was privatized in 1989 and the Government's technical regulator for the industry is the Drinking Water Inspectorate (DWI). The Water Supply (Water Quality) Regulations (1989), which DWI enforces, cover some 55 specific

parameters that do not include algal toxins. However, the regulations require that no substance may be present in drinking waters at concentrations that would cause a risk to health. In this respect water utilities would be required to monitor for algal toxins, if a risk situation existed. In the UK, water utilities currently base that risk assessment on the potential for algal loadings to overwhelm treatment processes and contaminate supplies.

In respect of regulatory standards, there are insufficient data to determine health-based guidelines or standards for even a representative selection of the toxins. The best studied is microcystin LR, although uncertainties exist, particularly with regard to its tumor promoting capability. Guideline values have only been set for microcystin LR/microcystins. WHO proposed a provisional guideline value in 1998 for microcystin LR and Health Canada set a guideline value for microcystins at about the same time. Both of these were based on the data generated by the UK national research program.

Some data have been generated for anatoxin-a that provides sufficient information to give a reasonable level of reassurance regarding the concentrations encountered in UK waters. Data on cylindrospermopsin have been recently produced in Australia but these data have a number of problems with regard to reporting and interpretation. It is understood that WHO will consider the data in the rolling revision of its Guidelines for Drinking Water Quality. There is pressure from some of the groups working on toxins to produce “standards” for toxins but reports from WHO indicate that this is not seen as an appropriate approach.

At the Global Meeting on the WHO Guidelines in Tokyo in May 2002, there was considerable discussion of blue-green algae and their toxins. The general view was that toxic blooms need to be taken seriously. There was particular concern in parts of Asia, where there can be significant growths throughout the year. The main issue is clearly one for developing countries where there are many small, under-resourced, supplies with only basic treatment. It was generally agreed that a preventative approach is preferred and that any attempt to control the risk by routine monitoring for toxins was not practicable. Analytical methods are complicated and not sufficiently robust for routine monitoring purposes. Standards are very expensive and are available for only a few toxins. In addition, there are almost certainly a number of as yet unidentified toxins.

Currently, setting a standard based on the few toxins for which there were adequate data could be construed as potentially misleading because the absence of a particular toxin does not indicate the absence of a problem. In addition, the potential for changes in the presence and absence of toxins means that sampling to give an appropriate level of reassurance could be problematical. Prevention of blooms forming is the best way forward, although this may present some difficulties. Control of eutrophication is an important issue for the Environment Agency in the UK and at the European level; it will be an important consideration in the Implementation of the Water Framework Directive.

It is understood that the WHO approach will be supported by guidelines for some toxins, developed when there are adequate data but the production of guideline values for an

increasing list of toxins is seen as potentially counter-productive. The WHO paragraph to be included in volume 1 of the revised guidelines currently reads as follows:

*Cyanobacteria occur widely in lakes, reservoirs, ponds and slow flowing rivers. Many species are known to produce toxins, a number of which are of concern for health. There are many cyanotoxins, which vary in structure and may be found within cells or released into water. There is wide variation in the toxicity of recognized toxins (including amongst different varieties of a single toxin, e.g., Microcystins) and it is likely that further toxins remain unrecognized.*

*The health hazard is primarily associated with overgrowth (bloom) events. Such blooms may develop rapidly and they may be of short duration. In most circumstances, but not all, they are seasonal.*

*Analysis of these substances is also difficult although rapid methods are becoming available for a small number, e.g, microcystins, in addition analytical standards are frequently not available. The preferred approach is, therefore, monitoring of source water for evidence of blooms, or bloom forming potential, and increased vigilance where such events occur.*

*A variety of actions are available to decrease the probability of bloom occurrence and some effective treatments are available for removal of cyanobacteria or cyanotoxins. For these reasons, monitoring of cyanotoxins is not the preferred focus of routine monitoring and is primarily used in response to bloom events. Whilst guideline values are derived where sufficient data exist, they are intended to inform the interpretation of data from the above monitoring and not to indicate that there is a requirement for routine monitoring by chemical analysis.*

## **OPERATIONAL ISSUES IN THE UK**

Because of the comprehensive treatment processes that are already in place at water treatment works, monitoring for algal toxins is normally only undertaken when there is a risk that algal cell concentrations could adversely affect treatment processes. Algal blooms occur seasonally in many impounding reservoirs where water is abstracted from lowland rivers. These rivers drain agricultural catchments and there are usually significant inputs of treated sewage effluent. Although phosphate removal is practiced at some locations, this treatment rarely achieves the overall reduction in phosphorus input needed to limit development of algal blooms.

Operational measures focus on forced mixing/aeration systems. These are aimed primarily at avoiding stratification but also limit algal growth by reducing light penetration. Multiple draw-off levels are used to facilitate selection of best quality water and where alternative sources are available, continuous fluorimetry is used to enable switching of sources to reduce algal load in raw water. Monitoring of reservoirs is normally carried out from boats, with samples taken throughout the water column. Measurements include: a-chlorophyll, particulate organic carbon, algae identification and

estimation of abundance and inorganic algal nutrients. These data are assessed in the light of potential to cause blocking of filters and no water utilities carry out routine analysis for algal toxins.

Public access is permitted on many lowland reservoirs and contact water sports are commonly practiced. Livestock grazing to the water's edge is rarely allowed but dogs and horses may drink from reservoirs, especially where footpaths and bridleways border the reservoir. Where access to reservoirs is permitted, warning signs alert the public to the risk of contact with water, or allowing animals access to the water. These signs are usually permanently installed, rather than being deployed only when an algal bloom is underway.

## **CONCLUSIONS**

The comprehensive R&D program that was funded by the UK government and the water industry addressed the potential threat to drinking water supplies from blue-green algal toxins. The research outputs have enabled a better understanding of the problems posed by these toxins and the ways in which the potential risks can be effectively addressed. The main part of this program was conducted over the period from 1991-1996 and the cost to the funding bodies was in excess of 1.5 million dollars.

In the UK, there have been relatively few problems relating to blue-green algal toxins and drinking waters since 1989, and there was only a limited demand for monitoring for microcystins and anatoxin-a during the period 1992-1996. The only significant monitoring that has been undertaken since was for water samples from Northern Ireland between June 1998-July 1999. A further indication of the lack of demand for monitoring is illustrated by the fact that with one exception (WRc-NSF), no contract laboratories currently offer the analysis of blue-green algal toxins.

Most lowland waters in the UK receive multiple barrier treatment, often including ozone and/or carbon treatment. The national research program provided reassurance that, as long as algal loadings are not allowed to overwhelm treatment, exposure to algal toxins via drinking water would not constitute an identifiable risk to health. This situation could of course change if water temperatures and nutrient inputs continue to increase.

The main potential problem for the UK water industry at present concerns disposal of filter backwash water from those water treatment works which experience significant algal blooms in their raw water storage reservoirs. The Environment Agency is actively seeking to place discharge consents on the disposal of such waters (particularly where there is little dilution by the receiving waters), although no consents have yet been applied.

**Summary of: WHO Guidelines  
Presented by: Ian Falconer, D.Sc.**

Dr. Ian R. Falconer, from the Cooperative Research Center for Water Quality and Treatment and the University of Adelaide Medical School, gave an additional presentation on the World Health Organization (WHO) and their approach to cyanobacterial toxin guidelines. WHO drinking water guidelines, based on potential hazards to human health, were first released in 1985, and have undergone numerous revisions. Cyanobacterial toxins were considered as part of the WHO drinking water guidelines in April 1997, following the production of a WHO monograph on the subject. The group that produced the monograph recommended that a Guideline Value of 1 microgram per liter be made for microcystin, based largely on animal and human data from Australia.

Dr. Falconer discussed research, much of which was his own, on microcystin. The tolerable daily intake (TDI) was determined from 10-13 week oral dosing tests in mice and pigs. The NOAEL in mice was 40 µg/kg/day, and the LOAEL in pigs was 100 µg/kg/day. The TDI was created by dividing the NOAEL in mice, 40 µg/kg/day by three ten-fold safety factors, yielding 0.04 µg/kg/day. The WHO then generated a guideline value by multiplying the TDI by a 60 kg body mass and a 0.8 proportion of dose due to drinking water and dividing by 2.0 L/day water consumption. This yields 0.96 µg/L, or roughly 1.0 µg/L.

As Dr. Falconer mentioned, the Chemical Substances Working Group reviewed the matter of cyanobacterial toxins in May of 2002. The Toxic Cyanobacteria in Water monograph, originally written in 1997, is to be revised and reissued. The WHO will reconsider cylindrospermopsin, for which an oral toxicity study was completed, and other cyanobacterial toxins in 2003/4. In light of actions by the WHO, Australia, Canada, New Zealand, Poland and Brazil have established a guideline for microcystin, and have provisional standards for other cyanobacterial toxins. Lastly, monitoring and water treatment techniques are being improved, and attentiveness to cyanobacterial toxins is rising.