

WaBoLu-Hefte

WaBoLu

**02
05**

ISSN
0175-4211

**Current approaches to
cyanotoxin risk assessment,
risk management and
regulations in different
countries**

**Umwelt
Bundes
Amt** 
Für Mensch und Umwelt



**Current approaches to
cyanotoxin risk assessment,
risk management and
regulations in different
countries**

Compiled and edited by

Ingrid Chorus

Federal Environmental Agency

This publication is also available as download under
<http://www.umweltbundesamt.de>

Acknowledgements: Compilation and editing of this booklet was supported by the EU in the context of the project "Peptides in Cyanobacteria", Contract No. QLRT-2001-02634.
Special thanks are due to Bettina Schiers for format and lay-out of the text.

Publisher: Federal Environmental Agency (Umweltbundesamt)
P.O.B. 14 06
06844 Dessau
Tel.: +49-340-2103-0
Telefax: +49-340-2103 2285
Internet: <http://www.umweltbundesamt.de>

Edited by: Section II 3.3
Dr. Ingrid Chorus

Berlin, June 2005

Table of Contents

Editorial and Summary	1
Australia: Regulation and Management	9
Belgium and Luxembourg: Cyanobacterial Blooms	21
Brazil: Management and Regulatory Approaches for Cyanobacteria and Cyanotoxins	27
Canada: The Development of a Microcystin Drinking Water Guideline	31
Czech Republic: Management and Regulation of Cyanobacteria and Cyanotoxins	37
Denmark: Occurrence, Monitoring and Management of Toxic Cyanobacteria	41
Finland: The Network of Monitoring Cyanobacteria and their Toxins in (1998 – 2004).....	47
France: The Occurrence of Cyanobacteria in Management and Regulatory Approaches.....	55
Germany: Approaches to Assessing and Managing the Cyanotoxin Risk.....	59
Greece: Cyanotoxin Risk Assessment, Risk Management and Regulation	69
Hungary: Regulation on Drinking Water and Bathing Water Quality including Cyanobacteria.....	77
Italy: Cyanobacteria in Surface Water.....	81
Netherlands: Risks of Toxic Cyanobacterial Blooms in Recreational Waters: Guidelines.....	85
New Zealand: Risk assessment, Management and Regulatory Approach for Cyanobacteria and Cyanotoxins in Drinking-water.....	93
Poland: Regulation on Cyanotoxins in Legislation.....	99
South Africa: Regulatory Approaches to Cyanobacteria.....	103
Spain: Legislation regarding Cyanotoxins.....	107
United States of America: Cyanobacteria and the Status of Regulatory Approaches	111

EDITORIAL AND SUMMARY

INGRID CHORUS¹

¹ Federal Environmental Agency. E-mail: ingrid.chorus@uba.de

Background for this booklet

At the VIth International Conference on Toxic Cyanobacteria, held from 21 – 27th of June 2004 in Bergen, Norway, 190 participants from 36 countries presented results covering a wide range of aspects of research on toxic cyanobacteria. The conference included a special session in which scientists and regulators reported on the current status of awareness of the toxic cyanobacterial hazards in their country, on discussions towards implementing regulations, and on regulatory approaches already implemented. This session demonstrated substantial recent progress in the perception of cyanotoxins as risk to human health and in risk management. Furthermore, differences in the approaches taken to risk management in different countries proved worth sharing. Participants concluded that it would be useful to compile this emerging experience, including contributions from those who could not attend the conference, both for readers seeking to promote the development of regulatory approaches in their own country as well as for further improvement of approaches already implemented.

This booklet therefore compiles the responses to a post-conference call mailed to all delegates asking for contributions. Although chances are that a wide range of regulatory approaches to cyanotoxins is included, coverage is neither globally balanced nor comprehensive. Also, contributions do not represent authorised government positions, but rather the personal views of the authors, largely scientists. The experience reported in many of these contributions demonstrates that research results may have a significant impact on the development of awareness and subsequently, of governmental policy. It is therefore hoped that this compilation will trigger and support national discussions on assessing health hazards from cyanotoxins and on the best management approaches for protecting human health.

Contributions in this booklet are organised alphabetically by country. Readers not very familiar with cyanotoxins are referred to the contribution from Australia which provides an introductory overview of the groups of cyanotoxins, their specific effects, and monitoring methods. The following text provides a brief overview of the types of approaches taken towards protecting human health from cyanotoxin exposure. It further reports prospects discussed at the Bergen conference towards improving approaches, including the draft EU Bathing Water Directive.

Range of approaches taken in different countries

In many countries, a general picture of the extend of cyanobacterial and cyanotoxin occurrence is emerging from scientific research, initial surveys and ongoing monitoring. Often, some of this was funded in part by public authorities interested in a first overview or by water utilities, or it is a spin-off from other programmes, e.g. long-standing phytoplankton monitoring of drinking-water reservoirs. Examples of situations where sufficient information has now been compiled to demonstrate the need for addressing cyanotoxins in hazard assessment risk management are the reports from Greece, Belgium and Luxembourg.

In several countries, measures to protect public health from cyanotoxins have been implemented even where formal regulations are not – or perhaps not yet – in place. These were achieved on the basis of widespread awareness developed through different combinations of activities such as long-standing phytoplankton and/or cyanobacterial monitoring programmes, cyanotoxin research activities, public information, and also from programmes for reducing eutrophication in order to improve the ecological quality of aquatic habitats. This

experience shows that transitions from collecting information to taking action may be gradual and often begin with advisories to public health authorities. This may include informal information networks such as in Denmark, where administrative units and research institutions collaborate to collate information, and the *Danish National Environmental Research Institute* posts a national overview of toxic cyanobacterial occurrence on its website. In South Africa, cyanotoxin work spans a comprehensive set of activities including an assessment of trophic state, eutrophication control policies, identification of research needs, monitoring manuals, formulation of a strategic research protocol for cyanobacteria and cyanotoxins, and an Alert Levels Framework for drinking-water treatment. Although a standard was considered premature because of the cost implications this would impose on the smaller water providers, a "Target Water Quality Range" of 0 – 0.8 µg/L for microcystin-LR, supported by guidelines for chlorophyll-a and cell counts, is recommended. In the USA on a national level, cyanobacteria and cyanotoxins have been included in the US EPA "contaminant candidate list" for further research to clarify the need for their regulation. However, some states have implemented cyanotoxin regulations or taken other interventions, including declaration of a disaster area by the Governor of Kansas in response to a cyanotoxic bloom in a water supply reservoir.

Drinking-water standards or, in federally organised countries, national guideline values have been adopted for cyanotoxins in six countries covered in this booklet. For microcystin(s), all of these draw on the World Health Organisation's provisional TDI (Tolerable Daily Intake) value for microcystin-LR in drinking-water or directly use its provisional Guideline value, but details of how these are used vary between countries: Brazilian federal legislation bases monitoring on cyanobacterial occurrence in drinking-water supply systems and requires more intensive monitoring, including toxin analyses or toxicity testing if cyanobacteria in raw water exceed 10,000 cells/ml or 1 mm³ biovolume. This includes mandatory standard of 1 µg/L is applied for microcystins (variants not specified), and recommendations are given for saxitoxins (3 µg/L) and for cylindrospermopsin (15 µg/L). Canada, as a result of a federal-provincial-territorial cooperative effort, set a *maximum accepted concentration* (MAC) for microcystin-LR in drinking-water at 1.5 µg/L, and research by Health Canada is currently addressing the need to comprehensively include other congeners in surveys and in monitoring. Polish regulations require a limit of 1 µg/L to be met for microcystin-LR in drinking-water. Czech legislation requires monitoring of tap water for microcystin-LR with a limit of 1 µg/L, and an update of the ordinance is expected in 2005 which will include alternatives to microcystin analysis such as quantification of cyanobacterial biomass in raw water or bioassays in conjunction with cell counts, requiring toxin analyses only if thresholds for cyanobacterial biomass are exceeded. The French Drinking-water Decree includes a maximum limit of 1 µg/L microcystin-LR, with analyses being required in the event of cyanobacterial proliferation in the raw water. The Spanish decree establishing the water quality criteria for human consumption includes a limit for "microcystin" (variants not specified) of 1 µg/L, to be reviewed at 5-year intervals, with sampling regimes specified in relation to size of population served.

In two countries, the provisional WHO Guideline value for microcystin-LR is important for implementation of regulations which do not explicitly address microcystins: In Germany, and very similarly in Finland, the national Drinking-water Ordinances stipulates that drinking-water should contain no substances in concentrations that may be harmful to human health, and the provisional WHO value for microcystin-LR provides an important definition of such concentrations. A prerequisite for this approach was that drinking-water suppliers using surface water run long-established phytoplankton monitoring programmes as basis for adapting treatment to raw water quality, usually have effective treatment in place, and are aware of the cyanotoxin hazard. In Italy also, no limit value has been implemented, but the national drinking-water decree considers algae as an accessory parameter to be monitored in case local authorities suspect a risk to human health, with the provisional WHO Guideline of 1 µg/L microcystin-LR used as basis for this assessment.

In Hungary: the decree on drinking-water quality and the ordinance on monitoring include cyanobacteria among biological parameters to be monitored by microscopy, though no limit

is given for cyanotoxins, only for the number of cyanobacterial cells. In Finland, starting in the late 1980's the waterworks have also been advised to monitor cyanobacteria microscopically, and if cyanobacterial cells occur in raw or treated water, to analyse toxins.

Recreational exposure is addressed in advisories for protecting public health following quite diverse approaches, some of which more or less closely and explicitly follow that suggested by the World Health Organisation (Chorus & Bartram 1999; WHO 2003) using two levels of cyanobacterial cell density and scums to trigger different degrees of intervention, from warning to closure of the site. The Netherlands use the provisional WHO TDI for microcystin-LR as basis for a guideline of 10 µg/L for issuing warnings, 20 µg/L for closure of bathing sites, and scums to trigger at least warning and continued monitoring. In France, three levels of cyanobacterial cell density were defined by the *Public Hygiene Council* that trigger management responses up to prohibition of water contact sports. Information on cell numbers is published on the internet within not more than 5 days of sampling.

In Australia, monitoring cell densities is often preferred to toxin limits because cell counting is widely available, cost-effective and is performed rapidly. Draft *Guidelines for Managing Risks in Recreational Water* use three levels of thresholds prompting actions and include an assessment of the susceptibility for cyanobacterial growth based on the water-body's bloom history as well as on physico-chemical conditions that are risk factors for the development of cyanobacteria. This assessment leads to scoring as "good, fair, or poor". In Germany, recreational exposure is addressed through a 3-step guideline based on visual inspection and assessment of the nutrient capacity for blooms followed by assessing cyanobacterial biomass, with thresholds for warning or closure and an option for allowing sites to remain open if microcystin levels are low even in face of high cyanobacterial levels.

The Danish Bathing Water Instruction requires that when massive blooms occur, the material is investigated, the risk assessed and authority alarm groups trigger posting of warning signs at the waterfront as well as dissemination of information particularly to local water-body user groups. In Finland, health authorities were provided with guidelines already in the late 1980's, and a cost-effective monitoring network of nuisance algal occurrence is based on a long-term data on nuisance algal occurrence collected since 1967, now also including involvement of private citizens for visual monitoring.

The Italian decree on the quality of bathing water addresses cyanobacterial proliferation indirectly on the basis of dissolved oxygen: permission for derogations above the limit may be applied for, and they are typically granted if it is demonstrated that excessively high levels of oxygen are not caused by toxic algal proliferation. Hungary also addresses cyanobacterial blooms in natural bathing waters indirectly through a limit for chlorophyll-a, and the revision of the EU Bathing-water directive is awaited as basis for addressing toxic cyanobacteria more explicitly.

Risk-based approaches for drinking-water and recreational water use explicitly including cyanobacteria are reported from in two countries. Similarly to the regulatory approaches summarised above, these use defined values for cyanotoxin concentrations that should not be exceeded and are recommended on a national level in the context of determining the public health targets. However, compliance to such values is not the primary target of these approaches. Rather, they focus on improving the understanding of potentially occurring hazards in specific, individual supply systems and the system's efficacy in controlling them, including the development of management plans to ensure controls are working, emergency and contingency strategies exist, lines of communication are clear, and assessments as well as records of system performance are documented. Thus, the regulatory approach is to require a comprehensive management system, within which guideline values determine the target.

The *Australian Drinking Water Guidelines* (ADWG) provide guidance for developing such management plans. They include fact sheets for 4 cyanotoxins – microcystins, nodularin, saxitoxins and cylindrospermopsin. For total microcystins these recommend a guideline value of 1.3 µg/L, and alternatively they give a value for cell density of 6500 cells per mL,

based on a toxin cell quota of 0.2 pg total microcystins per cell (which would lead to 1,3 µg/L of microcystin). In contrast to cyanotoxin standards in other countries, the values in the ADWG are not mandatory legally enforceable standards. Rather, they provide a framework for analysing hazards and assessing risks for individual water supply systems, similar to the *Water Safety Plan* suggested by the 3rd Edition of the WHO Guidelines for Drinking-water Quality (WHO 2004). In many cases, they are now being adopted by water authorities as agreed quality targets or as contract conditions where water supply is outsourced. They are seen as useful targets and performance indicators for audits of process performance.

In New Zealand the management of cyanotoxin risks is an integral part of the Ministry of Health's *Drinking-Water Public Health Management Plans*. In this context, individual *Water Safety Plans* are developed for each drinking-water supply system. These follow a comprehensive multi-barrier approach, a central element of which is process control. Hazard assessment is performed in the overall context of priorities for public health by using four priority classes with Priority 1 assigned to pathogens and their indicators (i.e. *E. coli*, *Giardia* and *Cryptosporidium*). Cyanotoxins, when present at concentrations above 50 % of the *Maximum Acceptable Value* (MAV) are assigned Priority 2 by the *Medical Officer of Health* responsible for the supply. Provisional MAV's for both cyanobacteria and cyanotoxins as well as minimum frequencies and methods for monitoring are defined in the *Drinking-Water Standards for New Zealand 2005*. The development of such plans follows a five-stage process, the first of which is the identification of barriers to contamination. For cyanotoxins, considerations begin with barriers to contamination of raw water with eutrophicating nutrients in order to reduce the likelihood of cyanobacterial cells and/or toxins entering the supply in the first place, but they also include removal of cells and/or destruction of toxins by treatment. A '*Barriers to Contamination*' guide assists suppliers in assessing barriers and in estimating risk of cyanotoxin occurrence. An important stage of plan development is *Performance Assessment*, i.e. the safety of the system and its control measures. Finally, plans include the identification of reporting and communication pathways, i.e. who receives which information and how often, and documentation.

Further regulatory issues flagged in this booklet

A key question particularly for risk-based approaches is the availability of guidance on the health hazard caused by cyanotoxins. WHO Guideline values have proven highly valuable for this purpose, as they provide a scientifically substantiated target with a transparent explanation of the health-based considerations that lead to derivation of the value. Discussions in Bergen at the 6th International Conference on Toxic Cyanobacteria therefore highlighted a pronounced demand for a WHO Guideline value for cylindrospermopsin, and the contribution to this booklet from Brazil reports that this toxin was reported to occur in at least 5 states of the country.

Livestock is likely to be exposed to cyanotoxins in a number of settings worldwide. Accordingly, in Australia, regulatory approaches include *Livestock Drinking Water Guidelines* for Cyanobacteria. Furthermore, microcystins and nodularins may occur in fish and shellfish, and some countries have addressed this issue. For example, Australia has derived 'health alert' levels ranging from 250 µg/kg for fish to 1500 µg/kg for mussels. These are based on tolerable daily intake levels for adults, modified to be protective for short-term exposure. Denmark provides a service telephone line for information on closure of areas for mussel harvesting due to algal toxins (including cyanotoxins).

Problems related to security concerns for working with toxins are flagged in the contributions from Canada, where exchanging samples and cultures with laboratories the USA faces license and containment requirements. In the Czech Republic, registration of persons and institutions using cyanotoxins at the State Office for Nuclear Safety is required. This endeavours to limit the number of registered users, however, this target is seen to be in conflict with the target of establishing widespread cyanotoxin monitoring.

In some countries, legislation also addresses the application of algicides and treatment chemicals that may promote cell lysis and thus release cell-bound toxins: e.g. in Brazil their application is not allowed above a threshold of at > 20,000 cells/ml, and in France, algicide treatment is authorised only upon request and only for preventive use.

Prospects for effective approaches to reducing cyanotoxin risks

An encouraging development reported from many countries is the growing awareness of health risks from exposure to cyanotoxins, promoted through the increasing provision of educational materials such as manuals about cyanobacteria and cyanotoxins or training courses for professionals from e.g. state health authorities and water companies. Awareness is critical for educating water-body users towards recognising blooms and avoiding exposure. Examples reported from Finland and from Berlin, Germany, highlight how intensively web-based information and telephone hotlines may be used by the general public.

Awareness is also an important basis for recognising health impacts where they occur, and for developing acceptance of restrictions of water-body use. Symptoms after exposure to cyanobacteria are reported from the *Helsinki University Hospital's Poison Information Centre* which has frequently been contacted in relation to cyanobacterial exposure. Evaluation of these data indicate symptoms particularly after exposure to *Anabaena lemmermannii*. In the Netherlands, surveying activities include an inventory of health complaints related to recreation in surface waters. Although some of these made direct reference to cyanobacteria, their specific contribution to the symptoms remained unknown. In France, temporary closure of recreational sites occurred rather frequently from 2000 onwards, and their public acceptance appears to have increased after cases of dermatitis and 20 dog deaths were attributed to cyanobacteria.

Developments towards reducing cyanobacterial occurrence are emerging from some countries, largely as result of regulations targeting eutrophication, such as the North-Sea Convention. For example, in Denmark, where cyanobacterial occurrence is widespread, with about half of the phytoplankton in freshwaters estimated to consist of cyanobacteria (as average of the growing season), phosphorus loading has been reduced by more than half since 1989, but in most lakes water quality has not yet reached target levels. In Germany, a phosphorus levels in the major rivers have also been reduced by more than half, although many water-bodies require substantial further reduction for controlling cyanobacterial proliferation. The experience reported from France indicates that recreational site closure has accelerated the implementation of measures to control nutrient loading.

Comprehensive risk-based approaches emerging from some countries include the assessment of eutrophication risks leading to cyanobacterial growth and proliferation, as first step in the chain of assessing barriers to cyanotoxin exposure. Thus, they improve chances for addressing the immediate cause of the problem. In France, the management of cyanobacteria is perceived as falling within the scope of the EU Water Framework Directive, which calls for a '*good ecological status*' of the water resources by the year 2015. Indeed, where the natural reference state of water-bodies is a low trophic status, cyanobacteria may still occur occasionally, but usually not in potentially hazardous cell densities, and thus this piece of EU legislation – if implemented as intended – should help reverse the currently perceived general increase of cyanobacteria.

A further step towards comprehensive and locally adequate risk-based approaches is the draft EU Bathing-water Directive. Box 1 gives the key statements relevant for cyanobacterial assessment and management. The draft stipulates the determination of a '*bathing-water profile*' which describes the risk of pollution. It also explicitly includes an article on cyanobacterial risks (Article 8). This specifies neither monitoring approaches and time schedules nor management measures, because these will be more effective if adequately adapted to the specific situation. Article 15, however, allows supplementing the Directive with detailed rules

for the implementation of Article 8 if this should prove necessary. The context of Article 8 within the establishment of bathing water profiles points to assessing and mitigating the causes. While classification of bathing waters is based on occurrence of pathogen indicators, information of the public of cyanobacterial proliferation is required explicitly.

Box 1: Draft Directive of the European Parliament and of the Council concerning the management of bathing water quality* (Political agreement 16 July 2004)

— Statements related to cyanobacteria

- (11) Monitoring actions and frequency should be related to the bathing water's history and classification, and regional climatic conditions, putting emphasis on bathing waters where risks may occur. Conformity should be a matter of appropriate management measures and quality assurance, not merely of measuring and calculation. A system of bathing water profiles is therefore appropriate to provide a better understanding of risks as a basis for management measures. ...

This Directive shall apply to any element of surface water where the competent authority expects a large number of people to practice bathing and has not imposed a permanent prohibition on, or issued permanent advice against, bathing (hereafter "bathing water").

...

Article 2 Definitions

7. "Management measures" means the following measures undertaken with respect to bathing water:
- (a) establishing and maintaining a bathing water profile;
 - (b) establishing a monitoring calendar;
 - (c) monitoring bathing water;
 - (d) assessing bathing water quality;
 - (e) classifying bathing water;
 - (f) identifying and assessing causes of pollution that might affect bathing waters and impair bathers' health;
 - (g) giving information to the public
 - (h) taking action to prevent bathers' exposure to pollution
 - (i) taking action to reduce the risk of pollution

...

- 12: "Cyanobacterial proliferation" means an accumulation of cyanobacteria in the form of a bloom, mat or scum.

...

Article 3 Monitoring

- 3: The monitoring point shall be the location within the bathing water where:
- (a) most bathers are expected; or
 - (b) the greatest risk of pollution is expected, according to the bathing water profile.

Article 8 Cyanobacterial risks

- 1. When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks.
- 2. When cyanobacterial proliferation occurs and a health risk has been identified or presumed, adequate management measures shall be taken immediately to prevent exposure, including information to the public.

Article 9 Other parameters

- 1. When the bathing water profile indicates a tendency for proliferation of macro-algae and/or marine phytoplankton, investigations shall be undertaken to determine their acceptability and health risks and adequate management measures shall be taken, including information to the public.

...

Article 15 Technical adaptations and implementing measures

- 1. It may be decided:...

(b) to lay down detailed rules for the implementation of Articles 8 (1) and ...;

...

ANNEX III THE BATHING WATER PROFILE

1. The bathing water profile referred to in Article 6 is to consist of:

- (a) a description of the physical, geographical and hydrological characteristics of the bathing water, and of other surface waters in the catchment area of the bathing water concerned that could be a source of pollution, which are relevant to the purpose of this Directive and in accordance with Directive 2000/60/EC;
- (b) an identification and assessment of causes of pollution that might affect bathing waters and impair bathers' health;
- (c) an assessment of the potential for proliferation of cyanobacteria
- (d) an assessment of the potential for proliferation of macro-algae and/or phytoplankton;

...

*For the full text, please see: <http://register.consilium.eu.int/pdf/en/04/st12/st12884-re01.en04.pdf>

A common element of the discussion on developing and improving regulatory approaches to the cyanotoxin hazard is the perception of a pronounced need for a better understanding of the hazard, for further guideline values, as well as for certified analytical standards and reference materials. The VIth International Conference on Toxic Cyanobacteria identified these needs as quoted in Box 2.

Box 2: Recommendations of the 6th International Conference on Toxic Cyanobacteria

"At the 6th International Conference on Toxic Cyanobacteria in Bergen, 21-27 June 2004, attended by 190 expert participants from 36 countries, many of the results reported demonstrate the high frequency of cyanotoxin occurrence in concentrations that may be hazardous to human health. This particularly includes a variety of microcystins and cylindrospermopsin, but also toxic effects by as-yet unidentified cyanobacterial compounds. These observations emphasised the need for surveillance and for regulatory approaches in water management in order to reduce exposure risks. In a special session, delegates reported on recently implemented and currently emerging approaches to monitoring and regulating cyanotoxins in several countries. The following recommendations emerged:

1. Setting standards for cyanotoxins is necessary for enforcing both improved risk management and communication of the occurrence of elevated concentrations in drinking-water to the public.
2. For microcystins, the WHO Guideline Value specifies microcystin-LR. However, as microcystin variants in addition to MCYST-LR occur commonly, standards and guideline values for total microcystins are preferable.
3. Certified analytical standards for cyanotoxins and reference material for matrixes containing these toxins are required.
4. Technical workshops are needed to promote international skills transfer, competence-building and intercomparability.
5. Substantial gaps remain in the understanding and recognition of the hazards and risks presented by cyanobacterial cells and their toxins."

References

- Chorus, I. & J. Bartram (1999): **Toxic Cyanobacteria in Water: a Guide to Public Health Significance, Monitoring and Management**. Published on behalf of WHO by E & FN Spon /Chapman & Hall, London, 416 pp.
- World Health Organisation 2003: Guidelines for safe recreational waters, Volume 1 – Coastal and fresh waters, Chapter 8: Algae and cyanobacteria in fresh water. WHO Publishing, Geneva, pp. 136-158

AUSTRALIA:

Regulation and Management of Cyanobacteria

Michael BURCH and Andrew HUMPAGE

Cooperative Research Centre for Water Quality and Treatment
MB 3 Salisbury Post Office, Salisbury, SA, 5108, Australia.
Email: mike.burch@sawater.com.au

INTRODUCTION

Cyanobacteria are now recognised as a serious water quality problem with regard to both drinking water supply and recreational water use in Australia. The deterioration of our water resources through poor land and catchment management, and also water allocation practices, is now becoming better understood and acknowledged. Algal blooms are often a symptom of the resulting changes in water quality. The conditions which favour the growth of cyanobacteria and lead to blooms are nutrient enrichment (largely phosphorus but also nitrogen), warm temperatures, and calm stable water conditions such as those occurring in slow-flowing rivers and thermally stratified lakes. These conditions are often caused by human actions and activities, but can often be equally associated with natural climatic cycles which prevail over the wide geographic area of Australia. It has been argued that the growth of cyanobacteria may be favoured by simply impounding and storing water in dams and weirs in a hot dry climate such as ours. Similarly, highly variable river flows have always been a regular cyclical feature of the hydrology in a continent with regular droughts, however the regulation of large rivers, such as for example the Murray River, has led to an overall reduction in flow and flow duration characteristics (Walker and Thoms, 1993; Maheshwari *et al.*, 1995). The role of river regulation in the development of algal blooms and the effect on other aspects of riverine ecology needs to be better understood to allow for more informed management (MDBMC, 1994).

Notwithstanding these “thorny” water resource management issues, which require complex political and social solutions, the main water supply problems associated with cyanobacterial growth and proliferation in water supplies which include production of tastes, odours and toxins are now well recognised in the Australian water industry. There has been a large coordinated research effort over the last 15 years to address aspects of cyanotoxin occurrence, toxicity and toxicology, bloom occurrence and ecology, control, monitoring and detection, and molecular genetics.

The purpose of this paper is to discuss the regulation and management of cyanobacteria and cyanotoxins in Australia.

CYANOBACTERIAL TOXINS IN AUSTRALIA

Significance for Drinking Water Quality

Cyanobacteria produce a range of toxic compounds that have a deleterious effect upon drinking water quality. Table 1 presents a list of the compounds which have been found to occur in cyanobacteria in Australia, their public health and water quality significance, and provides comments on a range of guideline and management issues. The formulation of judgement of the water supply and public health significance of these toxins is guided by the information on their occurrence across Australia, which is now quite widespread, and also by the research knowledge on their toxicity. A short discussion on toxicity and properties of these toxins, with emphasis on Australian research follows.

Table 1 Cyanobacteria and Drinking Water Quality: Major classes of toxins produced by cyanobacteria in Australia, their significance to drinking water quality, and comments on their guideline status and the implications for management of water supply.

TYPE	COMPOUND	ORGANISMS	EFFECTS	DRINKING WATER QUALITY AND PUBLIC HEALTH SIGNIFICANCE	GUIDELINE STATUS	IMPLICATIONS FOR WATER SUPPLY MANAGEMENT
TOXINS						
Hepatotoxins	Microcystin	<i>Microcystis aeruginosa</i> <i>Anabaena</i> spp.	<ul style="list-style-type: none"> Liver Damage Tumour promotion in animal studies 	<ul style="list-style-type: none"> Acute toxicity in large water supply systems unlikely Chronic liver damage possible with chronic exposure The relationship between the animal tumour growth promotion properties of these toxins and human carcinogenicity needs to be determined 	<ul style="list-style-type: none"> WHO Provisional Guideline : 1 µg L-1 for microcystin-LR (only) released 1998 Australian Guideline: 1.3 µg L-1 expressed as microcystin-LR toxicity equivalents (TE), released in 2001 	<ul style="list-style-type: none"> The introduction of guidelines for microcystins obliges operators to monitor for the chemical compound, and this requires access to appropriate monitoring techniques These guidelines include a reference to cell numbers - guideline is equivalent to 6,500 cells mL-1 , for a highly toxic population of <i>M. aeruginosa</i>
	Nodularin	<i>Nodularia spumigena</i>	<ul style="list-style-type: none"> Liver Damage Tumour promotion in animal studies 	<ul style="list-style-type: none"> Acute toxicity in large water supply systems unlikely As for microcystin: chronic liver damage possible with chronic exposure 	<ul style="list-style-type: none"> No Australian Guideline However, hazard assessment is often guided by that for microcystin 	<ul style="list-style-type: none"> Only likely to be a significant drinking water quality issue in unusual situations where <i>Nodularia</i> blooms in freshwater (e.g. Lakes of Lower River Murray in South Australia)
	Cylindrospermopsin	<i>Cylindrospermopsis raciborskii</i> <i>Aphanizomenon ovalisporum</i> <i>Anabaena bergii</i>	<ul style="list-style-type: none"> Cytotoxic Liver, kidney and other organ damage genotoxic 	<ul style="list-style-type: none"> Risk of acute toxicity via drinking water supplies dependent upon circumstances Animal studies to investigate sub-chronic exposure have now been undertaken 	<ul style="list-style-type: none"> No Australian Guideline However the value proposed by Humpage & Falconer (2003) of 1 µg L-1 is used in some locations 	<ul style="list-style-type: none"> This toxin is a significant water quality issue for tropical Australia Cannot be disregarded as an issue in southern Australia
Neurotoxins	Saxitoxins (Paralytic Shellfish Poison – PSP's)	<i>Anabaena circinalis</i>	<ul style="list-style-type: none"> Sodium channel blocking agent – acute poisoning results in death by paralysis and respiratory failure 	<ul style="list-style-type: none"> Acute toxicity in large water supply systems unlikely Effects from chronic exposure not known Public health significance unclear No evidence of human illness from drinking water supply 	<ul style="list-style-type: none"> No Australian Guideline However a “Health Alert “of 3 µg L-1 for acute exposure is sometimes used for guidance. 	<ul style="list-style-type: none"> Not likely to be as significant a drinking water quality issue as microcystins
Endotoxins	Lipopolysaccharides (LPS)	Most cyanobacteria (outer cell wall component similar to LPS in cell walls of gram negative bacteria)	<ul style="list-style-type: none"> Implicated in Gastro-intestinal disorders, Skin, eye irritation, Respiratory symptoms 	<ul style="list-style-type: none"> Less toxic than hepato- or neurotoxins Recent studies with isolated cyanobacterial LPS indicate relatively low potency Effects from chronic exposure not known 	<ul style="list-style-type: none"> No Australian Guideline 	<ul style="list-style-type: none"> New research currently underway will assist to clarify potential health significance.

Microcystins

The microcystin toxins are a group of cyclic peptide hepato– (liver) toxins which are widely regarded as the most significant potential source of human injury from cyanobacteria on a world wide scale (Falconer, 2005). This is arguably not the case in many geographic regions of Australia where other toxins may be more prevalent. Nevertheless blooms of toxic *Microcystis aeruginosa*, which in Australia is the predominant cyanobacterium producing microcystin, are widespread throughout south-eastern Australia (Baker and Humpage, 1994). Microcystins can occasionally be produced by *Anabaena* spp, however this appears to be rare in Australia. Microcystins have been implicated in causing liver damage in a human population exposed via reticulated town water supply where the source water contained blooms of *Microcystis* (Falconer *et al.*, 1983). In addition microcystins promote the growth of tumours in, the liver (Falconer, 1991; Nishiwaki-Matsushima *et al.*, 1992; Humpage and Falconer, 1999) and colon (Humpage *et al.*, 2000b) of experimental animals, and the significance of this for humans who may be subject to chronic exposure via drinking water is unclear.

Cylindrospermopsin

Cylindrospermopsin is an alkaloid toxin that has been isolated from two species of cyanobacteria in Australia – *Cylindrospermopsis raciborskii* (Ohtani *et al.*, 1992) and recently from *Aphanizomenon ovalisporum* (Shaw *et al.*, 1999) and *Anabaena bergii* (Schembri *et al.*, 2001). *C. raciborskii* is a widespread bloom-forming organism in tropical and sub-tropical areas of Australia and this is a significant water quality issue due to the associated production of cylindrospermopsin. *C. raciborskii* also occurs in the phytoplankton in temperate regions but rarely forms blooms in the cooler climates (Baker, 1996). Cylindrospermopsin was implicated in a severe human poisoning episode associated with a bloom of the cyanobacterium *C. raciborskii* in a water supply reservoir on Palm Island, Queensland in 1979 (Hawkins *et al.*, 1985). Cylindrospermopsin potently inhibits protein synthesis (Terao *et al.* 1994; Froscio *et al.* 2003), a characteristic that has been used to develop a toxicity assay for the toxin (Froscio *et al.* 2001). The experimental dosing of mice with extracts of *C. raciborskii* leads to widespread tissue and organ damage, primarily in the liver (Hawkins, *et al.*, 1985; Seawright *et al.* 1999). Kidney damage is also observed on occasions (Falconer *et al.*, 1999) and it has been proposed that *C. raciborskii* may produce other unknown toxins in addition to the characterised hepatotoxin cylindrospermopsin (Falconer, 1998; Hawkins *et al.*, 1997). More recently, cylindrospermopsin has been shown to cause genetic damage *in vitro* (Humpage *et al.* 2000a; Humpage *et al.*, In Press) and *in vivo* (Falconer and Humpage, 2001; Shen *et al.* 2002). A sub-chronic oral dosing study found a NOAEL of 30 µg/kg/d, leading the authors to propose a Guideline Value of 1 µg/L (Humpage and Falconer, 2003).

Neurotoxins

One of the most common bloom-forming cyanobacteria in Australia is *Anabaena circinalis* and this has been shown relatively recently to often produce the saxitoxin (PSP – Paralytic Shellfish Poisons) class of neurotoxins (Humpage *et al.*, 1994; Negri *et al.*, 1997). These toxins disrupt the normal signalling between nerves and muscles and can cause death by respiratory paralysis when given in high enough doses to both humans and other animals. An assay has been developed based on these toxins' specific binding to saxiphillin (Llewellyn *et al.* 2001). The saxitoxins have been responsible for significant human illness and mortality in situations where they have been ingested with contaminated shellfish which accumulate the toxins in the marine environment (Kao, 1993). These toxins may not accumulate and appear not to produce chronic health effects in humans as they are cleared from the body relatively quickly (Kao, 1993). There is no evidence of human health effects caused directly from water that contains saxitoxin – producing cyanobacteria (Fitzgerald *et al.*, 1999). The neurotoxins produced by *A. circinalis* may reservedly be considered as less of a threat to public health via public water supply than hepatotoxins. The reservations are that these are highly toxic compounds, which have not yet

been the subject of medium– or long–term chronic animal studies, and there is little information on their occurrence in drinking water.

Endotoxins

Lipopolysaccharides (LPS) or endotoxins as they are commonly called, are major components of the cell wall in most gram-negative bacteria, marine pseudomonads and blue-green algae (cyanobacteria) (Buttke and Ingram 1975). LPS from both gram-negative and cyanobacteria usually consist of a polysaccharide backbone comprising of an O-specific oligosaccharide chain and a relatively small oligosaccharide (core region), which in turn, is covalently bonded to a lipid unit known as lipid A (Sykora *et al.* 1980). It is well established that LPS of gram-negative organisms (e.g., *Escherichia coli*) are responsible for the severe pathophysiological effects (fever, trauma, multi-organ failure and septic shock) observed following infection of a host (Galanos *et al.* 1985, Holst *et al.* 1996). Therefore, there has been a significant amount of work on the elucidation of the structure and biological significance (with respect to toxicity) of LPS from gram-negative bacteria.

In contrast, up until recent times, analogous studies involving LPS from cyanobacteria has been relatively limited. A comprehensive investigation is now underway within the CRC for Water Quality and Treatment (AwwaRF Project "Determination and Significance of Emerging Algal Toxins") into the potency of some physiological effects exhibited by LPS isolated from a range of cyanobacteria (*Microcystis flos aquae*, *Microcystis aeruginosa*, *Anabaena circinalis*, *Cylindrospermopsis raciborskii* and *Phormidium* spp.). Initial results from this study indicate that cyanobacterial LPS is significantly less potent than typical gram negative bacterial (e.g. *Escherichia coli*) LPS. Therefore it is envisaged that health effects on humans following exposure to cyanobacterial contaminated waters would be insignificant.

GUIDELINES – CURRENT STATUS IN AUSTRALIA

Drinking Water

The Australian Drinking Water Guidelines (ADWG) have been undergoing a process of rolling revision by National Health & Medical Research Council (NHMRC) since 1998. As part of that process the NHMRC/ARMCANZ Drinking Water Review Coordinating Group decided that guidelines for cyanobacteria and their toxins would be developed as part of the review for 1999/2000. A working party completed a review of information on cyanobacteria and their toxins in relation to drinking water and public health in 2000. This has resulted in the production of four "Fact Sheets" for individual classes of toxins: microcystins, nodularin, saxitoxins, and cylindrospermopsin (Fact Sheets 17a-17d). These are available at <http://www.nhmrc.gov.au/publications/pdf/awg5.pdf>

The guidelines are now published as part of the 2004 Australian Drinking Water Guidelines (NHMRC/NRMMC, 2004). The outcome of the review and subsequent consultation process was that a guideline value was recommended for total microcystins (Fact Sheet 17a), and that no guideline values could be set for concentrations of nodularin, saxitoxins or cylindrospermopsin due to the lack of adequate data.

The process for guideline derivation for microcystins taken directly from NHMRC/NRMMC (2004) was as follows:

Derivation of Guideline

$$1.3 \mu\text{g/L} = \frac{40 \mu\text{g / kg bodyweight per day} \times 70 \text{ kg} \times 0.9}{2 \text{ L / day} \times 1000}$$

where:

- 40 µg/kg body weight per day is the No Observed Adverse Effect Level (NOAEL) from a 13-week ingestion study with microcystin-LR in mice based on liver histopathology and serum enzyme level changes (Fawell *et al.*, 1994)
- 70kg is the average weight of an adult
- 0.9 is the proportion of total daily intake attributed to the consumption of water
- 2 L/day is the average amount of water consumed by an adult
- 1000 is the safety factor derived from extrapolation of an animal study to humans (10 for interspecies variability, 10 for intraspecies variability and 10 for limitations in the database, related particularly to the lack of data on chronic toxicity and carcinogenicity).

The guideline is derived for total microcystins and expressed as microcystin-LR toxicity equivalents (TE). This is because the total microcystin concentration should be considered in relation to potential health impacts.

The NHMRC/NRMMC (2004) provided a comparison to the WHO guideline as follows:

"The World Health Organization has recently undertaken an evaluation of the health-related information for cyanobacterial toxins (Gupta, 1998; WHO, 1998; Chorus and Bartram, 1999 Chap. 5). It was concluded that there are insufficient data to allow a guideline value to be derived for any cyanobacterial toxins other than microcystin-LR. The guideline recommended by the WHO for drinking water is 1 µg/L (rounded figure) for total microcystin-LR (free plus cell-bound), based on the Fawell *et al.* (1994) sub-chronic study. This guideline value for microcystin-LR is provisional, as the database is regarded as limited (WHO, 1998). The approach being taken for guideline derivation here is essentially similar to that used by WHO (Chorus and Bartram, 1999 Chap. 5). The same ingestion study in mice was used to calculate the NOAEL. The Australian guideline of 1.3 µg/L total microcystin (as microcystin-LR TE) differs from the WHO provisional guideline of 1 µg/L microcystin-LR due to the incorporation of a different average body weight for an adult (70 kg versus 60 kg), and to a difference with regard to the proportion of the daily intake of microcystin being attributed to the consumption of drinking water. The proportion for the Australian situation is regarded to be 0.9, which is higher than 0.8 selected by WHO. This is due to lower potential exposure in Australia from other environmental sources, such as contaminated bathing water, and via dietary supplements potentially containing microcystins."

The NHMRC/NRMMC (2004) also provided some guidance to assist with assessment of potential toxin contamination in the initial absence of toxin monitoring data as follows:

"In situations where *M. aeruginosa* occurs in drinking water supplies, and toxin monitoring data are unavailable, cell numbers can be used to provide a preliminary orientation to the potential hazard to public health. As an indication, for a highly toxic population of *M. aeruginosa* (toxin cell quota of 0.2 pg total microcystins/cell), a cell density of approximately 6,500 cells/mL is equivalent to the guideline of 1.3 µg/L microcystin-LR (TE), if the toxin were fully released into the water. It is important to note that this number is preliminary and for indicative purposes only, and for health risk assessment toxin determination is required."

A significant difference between the provisional WHO guideline and the NHMRC/NRMMC guideline is that the Australian guideline gives advice in relation to the concentration of total microcystins whereas WHO restricts its advice to the single compound microcystin-LR. Microcystin-LR was the first variant to be well characterised and used widely in toxicological studies from this class of hepatotoxins, of which there are in excess of 75 structural types.

The Fact Sheet for microcystins (17a) in the ADWG states that the Australian guideline for Total Microcystins is 1.3 µg/L expressed as toxicity equivalents of microcystin-LR.

The rationale for the Australian guideline covering total microcystins is that blooms of *Microcystis aeruginosa*, which is the most common toxin producing cyanobacterium in Australia, generally contain a range of variants of microcystin in varying amounts. Experience indicates that the number of variants in an individual sample can range from a few to up to more than 20 in some cases. It is the cumulative toxicity of the microcystins in total that represents the potential hazard to human health from ingestion via drinking water. Therefore the unit recommended for the quantitative expression of this cumulative toxicity in the guideline is total microcystins expressed as toxicity equivalents of microcystin-LR.

At the time of revision of the NHMRC/NRMMC guidelines for toxins the NHMRC also carried out a review of the status of analytical methods to guidance on the selection of analytical techniques for interpretation and compliance with the guideline (Nicholson and Burch, 2001). The key finding of this review were as follows:

- The technique that provides the most reliable measurement for compliance with the ADWG for microcystins in water is high performance liquid chromatography (HPLC) with photo-diode array (PDA) detection. Liquid chromatography with mass spectral confirmation of toxin identity and quantification is also suitable if standards for the toxins present are available.
- For compliance monitoring in relation to the guideline, the concentrations of individual microcystins are determined by comparison against standards. The relative toxicity of microcystins other than microcystin-LR are then converted to microcystin-LR toxicity equivalents based on the ratio of their published LD₅₀ (mouse, i.p.) relative to that of microcystin-LR.
- In situations where standards are unavailable for particular toxins in a sample it is necessary to use HPLC with PDA detection for analysis and to estimate the concentration, and therefore toxicity, of these microcystins against microcystin-LR as the analytical standard. In this case a slight overestimate of total microcystins (as microcystin-LR toxicity equivalents) may result.

Implication of Drinking Water Guidelines for Management

It is important to recognise the purpose and definition of these guidelines in the Australian context. NHMRC/NRMMC (2004) state that

“The ADWG (Australian Drinking Water Guidelines) provide the authoritative Australian reference for use within Australia’s administrative and legislative framework to ensure the accountability of drinking water suppliers (as managers) and of state/territory health authorities (as auditors of the safety of water supplies). The ADWG are not, however, mandatory legally enforceable standards.”

Within these guidelines Microcystins are classified as a “health-related guideline value”, which is defined as “the concentration or measure of a water quality characteristic that, based on present knowledge, does not result in any significant risk to the health of the consumer over a lifetime of consumption.” Under this definition the guideline is intended to be used as follows: “If a value is exceeded, some form of immediate corrective action will generally be initiated. For example, if a guideline value for a health-related characteristic is exceeded, the response should be to take immediate action to reduce the risk to consumers, and, if necessary, to advise the health authority and consumers of the problem and the action taken” NHMRC/NRMMC (2004).

In many cases the Australian Drinking Water Guidelines are being adopted by water authorities, either in full or part, as their agreed levels of service for quality of water provided to con-

sumers. They are seen as useful targets and performance indicators for audits of process performance. In cases of outsourcing or private operation of particular components of the water supply system (eg. water treatment plants), the guidelines are often used as the starting point to set contractual performance indicators. The guideline values may then become contract conditions. In some cases tighter limits than the guidelines may be set for some parameters, depending upon local sensitivity and requirements.

Recreational Water

Australia has had guidelines for cyanobacteria in water used for recreation since 1993 (Johnstone, 1993). These guidelines recommended a single level for all cyanobacteria of 20,000 cells/mL without reference to known toxins, and were developed with a rationale as follows:

“On the basis of reports that have established links between skin contact with cyanobacteria and adverse health effects and the apparent variability in sensitivity it is reasonable to conclude that contact with visible levels of cyanobacteria may constitute a health risk for sensitive individuals.....20,000 cells/mL would correspond to a slight discolouration of water and would satisfy the criteria for accepting that discoloured water poses a potential health risk.” (Johnstone, 1993).

This guideline is still used by some state agencies however others have adopted variations of the WHO Guidelines (WHO, 2003); for example, see http://www.nrm.qld.gov.au/water/blue_green/recreation.html.

Currently however, the entire guidelines for water recreation, including for cyanobacteria are under revision by the National Health and Medical Research Council. The NHMRC released a draft document: “Guidelines for Managing Risks in Recreational Water” which include cyanobacteria and algae in both fresh, and coastal and estuarine water for public consultation in May 2004. This document is being reviewed to incorporate comments and prepared for publication during 2005. The approach taken in this revision was to consider recent research and evidence of the health significance of cyanobacteria and cyanotoxins in recreational water situations, in particular to include reference to WHO (2003).

In short the draft proposed that the Australian guideline for exposure to cyanobacteria in recreational water should be primarily based upon risk of exposure to known toxins via ingestion. The approach adopted in these Guidelines was to use animal toxicity data for microcystin toxin and conventional toxicological calculations to derive a guideline for sub-chronic exposure to microcystins via ingestion as the potential worst-case hazard in a typical recreational situation. This microcystin concentration was then converted to an equivalent worst-case cell density of *Microcystis aeruginosa*, based upon cell toxin quota data used in drinking water guideline derivation (NHMRC/NRMMC, 2004). This “guideline-equivalent” cell density can then also be translated into an equivalent biovolume of cyanobacterial material to gauge the potential hazard of other cyanobacteria in the first instance.

The draft guidelines have recommended that managers can use cell counts in addition to toxin concentrations to prompt management actions. This is because in the Australian context, for most practical purposes, cell counting is still used primarily by most authorities to monitor algal-related water quality problems. This is because cell counting is widely available and provides relatively rapid and cost-effective information. By contrast, toxin testing is not widely available and has a relatively slow turn-around time for analyses. Cell counts (and biovolumes) are recommended as an indicator or “surrogate” for a potential toxin hazard.

The draft guidelines also recommend a monitoring program that is based upon a three-tier “Alert Levels Framework” (ALF), which is a monitoring and management action-sequence that uses cell counts are used to prompt actions, such as additional sampling and toxin monitoring. A similar system has been in use in Australia for management of cyanobacteria in drinking water for many years.

A new approach developed for the application of these draft Australian guidelines is to use a risk assessment procedure for cyanobacterial growth to categorise each individual surface water body to determine its suitability for recreational use. This is done with a combination of “recreational water environment grading” based upon an assessment of prior monitoring data for cyanobacteria, as well as upon historical information on physico-chemical conditions that are used to identify risk factors for the development of cyanobacteria in that water body. The grading is intended to provide an indication of the susceptibility of the water body to cyanobacterial growth. For a grading of ‘Very Good’ the water body will almost always comply with the guideline values for recreation, and is at low risk of cyanobacterial growth. Water bodies graded as ‘Very Poor’ will be highly susceptible to cyanobacterial growth and may rarely pass the quantitative guidelines and their use for recreational activities is not recommended. For the remaining gradings (‘Good, Fair and Poor’) it is recommended that a monitoring program be introduced. The monitoring program that is then implemented is based on the Alert Levels Framework system described above.

Agriculture - Livestock Water

Australia has also developed Livestock Drinking Water Guidelines for Cyanobacteria. These form part of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC/ARMCANZ, 2000). The guidelines for livestock are referred to as trigger values, which have the following definition and application: “Below the trigger value there should be little risk of adverse effects on animal health. Above the trigger value, investigations are recommended (e.g. of other factors such as age, condition, other dietary sources) to further evaluate the situation” (ANZECC/ARMCANZ, 2000). The trigger values were developed using data on chronic and toxic effect levels on animals, taking into consideration animal weights, percentage intake from water, and safety factors for data not specific to the species.

The summarised advice in the livestock drinking water guidelines is as follows: “Algal blooms should be treated as possibly toxic and the water source should be withdrawn from stock until the algae are identified and the level of toxin determined. An increasing risk to livestock health is likely when cell counts of *Microcystis* exceed 11,500 cells/mL and/or concentrations of microcystins exceed 2.3 µg/L expressed as microcystin-LR toxicity equivalents. There are insufficient data available to derive trigger values for other species of cyanobacteria” (ANZECC/ARMCANZ, 2000).

The guidelines provide individual derivations of trigger values for cattle, sheep, pigs, chickens and horses which take into account interspecies sensitivity. The full details of the guidelines and their derivation are given at: <http://www.deh.gov.au/water/quality/nwqms/pubs/volume3-9-3.pdf>

Fish and Shellfish

Although there are no national guidelines for cyanotoxins in fish or shellfish in Australia, a ‘health alert’ level has been derived for toxins in fish, prawns and mussels in the state of Victoria, Australia (Van Buynder *et al.*, 2001). The health alert level for microcystins and nodularin toxins in seafood is as follows: fish (250 µg/kg), prawns (1,100 µg/kg) and mussels (1,500 µg/kg). They were derived by determining a tolerable daily intake level for adults and modified to be protective for short-term exposure.

Sampling of seafood samples for toxins during a bloom of *Nodularia spumigena* indicated that toxin levels correlated well with cell counts and were found to be significantly higher in the prawn and fish viscera and mussels than in the prawn and fish flesh. These health alert levels are used to place restrictions on commercial and recreational harvesting of seafood in Victoria during cyanobacterial blooms.

CYANOTOXIN MONITORING

A wide range of techniques have been used for cyanotoxin monitoring in Australia. The techniques used in the commercial routine area are listed in Table 2. The preferred techniques are instrumental analytical techniques based upon High Performance Liquid Chromatography (HPLC), often now with addition of Mass Spectrometry (LCMS). In addition mouse bioassays are also sometimes used for rapid screening of unknown or mixed samples as an indicator of toxicity, before proceeding to analytical methods.

Table 2: Analytical methods commonly used commercially for cyanotoxin detection and analysis in Australia

TOXIN	ANALYTICAL METHOD	DETECTION LIMIT (µg/L)
Microcystins	• Liquid Chromatography with Mass Spectrometry (LC-PDA/MS)	<0.05
	• Protein Phosphatase Inhibition Assay (PPIA)	N.A.
	• Mouse Bioassay	
Nodularin	• Liquid Chromatography with Mass Spectrometry (LC-PDA/MS)	<0.05
	• Mouse Bioassay	N.A.
Cylindrospermopsin	• Liquid Chromatography with Mass Spectrometry (LC-PDA/MS & LC MS-MS)	<1.0
	• Mouse Bioassay	N.A.
Saxitoxins (Paralytic Shellfish Poison – PSP's)	• Liquid Chromatography (HPLC) with post-column derivatisation	<1.0
	• Mouse Bioassay	N.A.
Anatoxin-a	• Liquid Chromatography with Fluorescence detection (HPLC/FLD)	<0.5

N.A.: Not applicable – screening only

A wide range of other methods are used within various research laboratories for screening and analysis including, ELISA methods for microcystins; neuroblastoma cytotoxicity assay, saxiphilin and single-run HPLC methods for saxitoxins; and protein synthesis inhibition assays for cylindrospermopsin. Research is also underway into development of rapid molecular genetic methods involving real-time PCR for rapid quantitative analysis of water for both cyanotoxin genes and gene products.

As part of the revision of the NHMRC guidelines for toxins a review of the status of analytical methods was carried out and a report gives guidance on the selection of analytical techniques (Nicholson and Burch, 2001; <http://www.nhmrc.gov.au/publications/pdf/eh22.pdf>) The key finding of this review considered the nature of the Australian guideline for microcystins which is expressed as microcystin-LR toxicity equivalents (TE), and recommended in summary that:

- The technique that provides the most reliable measurement for compliance with the ADWG for microcystins in water is high performance liquid chromatography (HPLC) with photo-diode array (PDA) detection. Liquid chromatography with mass spectral confirmation of toxin identity and quantification is also suitable if standards for the toxins present are available.
- This method offers a conservative over-estimation, where concentration-equivalent conversions for microcystins other than Microcystin-LR are required to be used.
- ELISA also meets most criteria for routine use.

ACKNOWLEDGEMENTS

The authors thank John Papageorgiou for providing currently unpublished research information on cyanobacterial LPS toxins, and Jenny House, who assisted in the preparation of the manuscript.

REFERENCES

- ANZECC/ARMCANZ, (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Volume 3, Primary Industries - Rationale and Background Information (Chapter 9). Australian and New Zealand Environment and Conservation Council (ANZECC) and Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ.). Web address: <http://www.deh.gov.au/water/quality/nwqms/volume3.html>.
- Baker, P. D., and Humpage, A. R., (1994). Toxicity associated with commonly occurring cyanobacteria in surface waters of the Murray-Darling Basin, Australia. *Aust. J. Mar. Freshwater Res.*, 45:773-786.
- Baker, P.D., (1996). Occurrence of *Cylindrospermopsis* in south-eastern Australia. In: *Cylindrospermopsis – A new toxic bloom challenge for Australia*. Proceedings of one-day symposium. Brisbane, 24 October, 1996. Agriculture and Resource Management Council of Australia and New Zealand.
- Buttke, T.M., and Ingram, L. O., (1975). Comparison of Lipopolysaccharides from *Agmenellum quadruplicatum* to *Escherichia coli* and *Salmonella typhimurium* by Using Thin-Layer Chromatography. *J. Bacteriol.*, 124:1566-1573.
- Chorus, I. and Bartram, J., (Eds.) (1999). Toxic Cyanobacteria in Water. A Guide to Public Health Consequences and Their Supplies. WHO series in Environmental Management, 320 pp. Routledge, London.
- Falconer, I. R., (1998). Algal toxins and human health. Pp 54-72. In J. Hrubec (Ed), The handbook of environmental chemistry. Volume 5, Part C., Quality and Treatment of Drinking Water II. Springer-Verlag, Berlin, Heidelberg.
- Falconer, I. R., (1991). Tumour promotion and liver injury caused by oral consumption of cyanobacteria. *Environ. Toxicol. Water Qual.*, 6: 177-184.
- Falconer, I. R., (2005). Cyanobacterial Toxins of Drinking Water Supplies: Cylindrospermopsins and Microcystins. CRC Press, Boca Raton, Florida.
- Falconer, I. R., Beresford, A. M. and Runnegar, M. T. C., (1983). Evidence of liver damage by toxin from a bloom of the blue-green alga, *Microcystis aeruginosa*. *Med. J. Aust.*, 1: 511-514.
- Falconer, I. R., and Humpage, A.R., (2001). Preliminary evidence for in vivo tumour initiation by oral administration of extracts of the blue-green alga *Cylindrospermopsis raciborskii* containing the toxin cylindrospermopsin. *Environ. Toxicol.*, 16: 192-195.
- Falconer, I. R., S. J. Hardy, A. R. Humpage, S. M. Froscio, G. J. Tozer, and Hawkins, P.R., (1999). Hepatic and renal toxicity of the blue-green alga (cyanobacterium) *Cylindrospermopsis raciborskii* in male Swiss Albino mice. *Environ Toxicol.*, 14: 143-150.
- Fawell, J. K., C. P. James, and James, H.A., (1994). Toxins from blue-green algae: Toxicological assessment of microcystin-LR and a method for its determination in water. Medmenham: WRc plc. Report number FR 0359/2/DoE 3358/2.
- Froscio, S. M., A. R. Humpage, P. C. Burcham, and Falconer, I.R., (2001). Cell-free protein synthesis inhibition assay for the cyanobacterial toxin cylindrospermopsin. *Environ. Toxicol.*, 16: 408-412.
- Froscio, S. M., A. R. Humpage, P. C. Burcham, and Falconer, I.R., (2003). Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. *Environ. Toxicol.*, 18: 243-251.
- Fitzgerald, D.J., Cunliffe, D.A. and Burch, M.D., (1999). Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. *Environ. Toxicol. Wat. Qual.*, 14: 203-209.

- Galanos, C., Luderitz, O., Rietschel, E.Th., Westphal, O., Brade, H., Brade, L., Freudenberg, M.A., Schade, U., Imoto, M., Yoshimura, H., Kusumoto, S. and Shiba, T., (1985). Synthetic and Natural *Escherichia coli* Free Lipid A Express Identical Endotoxic Activities. *Eur. J. Biochem.*, 148: 1-5.
- Gupta, S., (1998). Cyanobacteria toxins: microcystin-LR. pp 95-110. In Guidelines for Drinking Water Quality 2nd Edition. Addendum to Volume 2. Health Criteria and Other Supporting Information. World Health Organization, Geneva.
- Hawkins, P. R., Runnegar, M. T. C., Jackson, A. R. B. and Falconer, I. R., (1985). Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.*, 50: 1292-1295.
- Hawkins, P. R., N. R. Chandrasena, G. J. Jones, A. R. Humpage, and Falconer, I.R., (1997). Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon*, 35: 341-346.
- Holst, O., Ulmer, A.J., Brade, H., Flad, H.-D. and Rietschel, E.Th. (1996). Mini Review: Biochemistry and Cell Biology of Bacterial Endotoxins. *FEMS Immunol. Med. Microbiol.*, 16: 83-104.
- Humpage, A. R., Rositano, J., Bretag, A. H., Brown, R., Baker, P. D., Nicholson, B. C. and Steffensen, D. A., (1994). Paralytic shellfish poisons from Australian cyanobacterial blooms. *Aust. J. Mar. Freshwater Res.*, 45: 761-771.
- Humpage, A.R., and Falconer, I.R., (1999). Microcystin-LR and liver tumour promotion: Effects on cytokinesis, ploidy and apoptosis in cultured hepatocytes. *Environ. Toxicol.*, 14: 61-75.
- Humpage, A. R., M. Fenech, P. Thomas, and Falconer, I.R., (2000a). Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin. *Mutat. Res.*, 472: 155-161.
- Humpage, A. R., S. J. Hardy, E. J. Moore, S. M. Froscio, and Falconer, I.R., (2000b). Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon. *J. Toxicol. Environ. 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. *Environ. Toxicol.*, 18: 94-103.
- Humpage, A. R., Fontaine, F., Froscio, S. M., Burcham, P., and Falconer, I. R., (2004). Cylindrospermopsin genotoxicity and cytotoxicity: Role of cytochrome P450 and oxidative stress. Accepted for publication in the *Journal of Toxicology and Environmental Health*.
- Johnstone P., (1993). Guidelines for the Recreational Use of Water Potentially Containing Cyanobacteria. ARMCANZ Occasional Paper. Agriculture and Resource Management Council of Australia and New Zealand, Canberra.
- Kao, C. Y., (1993). Paralytic shellfish poisoning. In: I.R. Falconer (Ed.), *Algal Toxins in Seafood and Drinking Water*. Academic Press, London, pp 75-86.
- Llewellyn, L. E., A. P. Negri, J. Doyle, P. D. Baker, E. C. Beltran, and Neilan, B. A., (2001). Radioreceptor assays for sensitive detection and quantitation of saxitoxin and its analogues from strains of the freshwater cyanobacterium, *Anabaena circinalis*. *Environ. Sci. Technol.*, 35: 1445-1451.
- Maheshwari, B.L., Walker, K.F. and McMahon, T.A., (1995). Effects of regulation on the flow regime of the River Murray, Australia. *Regulated Rivers: Research and Management*, 10: 15-38.
- MDBMC, (1994). The Algal Management Strategy for the River Murray. Murray-Darling Basin Ministerial Council, Canberra.
- Negri, A.P., Jones, G.J., Blackburn, S.I., Oshima, Y. and Onodera, H., (1997). Effect of culture bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*. *J. Phycol.*, 33: 26-35.
- NHMRC/NRMMC, (2004). Australian Drinking Water Guidelines. National Health and Medical Research Council/Natural Resource Management Ministerial Council, Canberra.

- Nicholson, B.C. and Burch, M.D., (2001). Evaluation of Analytical Methods for Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines. Occasional Paper. National Health and Medical Research Council. Canberra, Australia
- Nishiwaki-Matsushima, R., Ohta, T., Nishiwaki, S., Suganuma, M., Kohyama, K., Ishikawa, T., Carmichael, W. W. and Fujiki, H., (1992). Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. *J. Cancer Res. Clin. Oncol.*, 118: 420-424.
- Ohtani, I., Moore, R.E. and Runnegar, M.T.C., (1992). Cylindrospermopsin: A potential hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J. Am. Chem. Soc.*, 114: 7941-7942.
- Schembri, M. A., Neilan, B.A. and Saint, C.P., (2001). Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environ Toxicol.*, 16: 413-421.
- Seawright, A. A., C. C. Nolan, G. R. Shaw, R. K. Chiswell, R. L. Norris, M. R. Moore, and Smith, M. J., (1999). The oral toxicity for mice of the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska). *Environ. Toxicol.* 14: 135-142.
- Shaw, G.R., Sukenik, A., Livne, A., Chiswell, R.K., Smith, M.J., Seawright, A.A., Norris, R.L., Eaglesham, G.K. and Moore, M.R., (1999). Blooms of the cylindrospermopsin containing cyanobacterium, *Aphanizomenon ovalisporum* (Forti) in newly constructed lakes, Queensland, Australia. *Environ. Toxicol.*, 14: 167-177.
- Shen, X. Y., P. K. S. Lam, G. R. Shaw, and Wickramasinghe W., (2002). Genotoxicity investigation of a cyanobacterial toxin, cylindrospermopsin. *Toxicon*, 40:1499-1501.
- Sykora, J.L., Keleti, G., Roche, R., Volk, D.R., Kay, G.P., Burgess, R.A. and Shapiro, M.A., (1980). Endotoxins. Algae, and *Limulus* Amoebocyte Lysate Test in Drinking Water. *Water Res.*, 14: 829-839.
- Terao, K., S. Ohmori, K. Igarashi, I. Ohtani, M. F. Watanabe, K. I. Harada, E. Ito, and Watanabe, M., (1994). Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. *Toxicon*, 32: 833-843.
- 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.
- Walker, K.F. and Thoms, M.C., (1993). Environmental effects of flow regulation on the lower River Murray, Australia. *Regulated Rivers: Research and Management*, 8: 103-119.
- WHO, (1998). Guidelines for drinking water quality, Second Edition, Addendum to Volume 2, Health Criteria and other supporting information. World Health Organization, Geneva.
- WHO, (2003). Guidelines for safe recreational water environments. Volume 1: Coastal and Fresh Waters. World Health Organization, Geneva.

BELGIUM AND LUXEMBOURG:

Cyanobacterial Blooms

R. WILLAME & L. HOFFMANN

Public Research Center-Gabriel Lippmann, Environment and Biotechnologies Research Unit (CREBS), 41, rue du Brill, L-4422, Grand-Duchy of Luxembourg. E-mail: willame@lippmann.lu

Introduction

As most European countries, Belgium and Luxembourg do not escape toxic cyanobacterial bloom problems. So far, in both countries few data are available on the occurrence of massive cyanobacterial developments and in particular on their toxicity, although cyanobacteria may represent a serious problem for many water bodies used for drinking water production or recreational activities. Van Hoof et al. (1994) were the first to reveal the presence of toxic planktonic cyanobacteria in Belgium by demonstrating with the help of bioassays toxic and mutagenic effects of *Microcystis aeruginosa* samples collected in various water bodies. Few years later, Wirsing et al. (1998) reported a high microcystin content of 556 $\mu\text{g g}^{-1}$ DW for a *Microcystis* bloom occurring in a Belgian fishpond. A first evaluation of the extent of the occurrence of cyanobacterial blooms in Belgium was made by Willame & Hoffmann (1999) who reported on 21 Belgian cyanobacterial blooms as well as on the dominant species involved. Toxicological data for the assessment of human health risks in these countries were, however, lacking until the recent study by Willame et al. (in press). The latter authors conducted, from 1999 to 2001, a broad survey of cyanobacterial blooms in the south-eastern part of Belgium and in Luxembourg.

In addition to the monitoring of the presence of cyanobacterial blooms and cyanotoxins in water bodies used for various purposes in both countries, monitoring of cyanobacterial populations combined with microcystin analyses of the Haute-Sûre reservoir has been undertaken since 2003. This reservoir, located in the Grand-Duchy of Luxembourg, is of paramount importance as drinking water supply. It provides almost 50% of the available drinking water resources of the country and is also a highly frequented tourist site where recreational activities, including swimming, boating, fishing, etc., commonly take place.

Survey of cyanobacterial blooms

According to Willame et al. (in press), *Microcystis*, *Planktothrix*, and *Anabaena* were the main dominant bloom-forming genera of the south-eastern part of Belgium and Luxembourg. They (co-)dominated respectively in 34%, 34% and 20% of the investigated water bodies. *Aphanizomenon* and *Woronichinia* were rarely observed and (co-)dominated in 9% of the samples (Fig. 1). The statistical analysis of the results showed the central role of parameters related to the geology, which determines the natural water types, for the composition of the cyanobacterial bloom assemblages. Interestingly, *Microcystis* usually dominated in waters with low conductivity whereas *Planktothrix* was more frequently encountered in waters with higher conductivity.

Microcystin was detected in 53% of the analyzed bloom samples. The majority of the samples which contained microcystins were dominated by *Microcystis* (47%), followed by *Planktothrix* (29%), *Anabaena* (12%), *Woronichinia* (18%), and *Aphanizomenon* (6%) (see geographical distribution of hepatotoxic blooms in Fig. 2). These data do not necessarily indicate that the dominant cyanobacteria are the microcystin producer since species assemblages in blooms usually contain several cyanobacterial taxa. Total microcystin concentrations per

seston biomass ranged from 31-2231 $\mu\text{g g}^{-1}$ DW. The maximum microcystin concentration obtained in a *Woronichinia naegeliana* dominated bloom was 4 times higher than the one measured by Wirsing et al. (1998) in a Belgian *Microcystis* bloom. As in most European water bodies, microcystin-LR was the most frequently encountered microcystin variant.

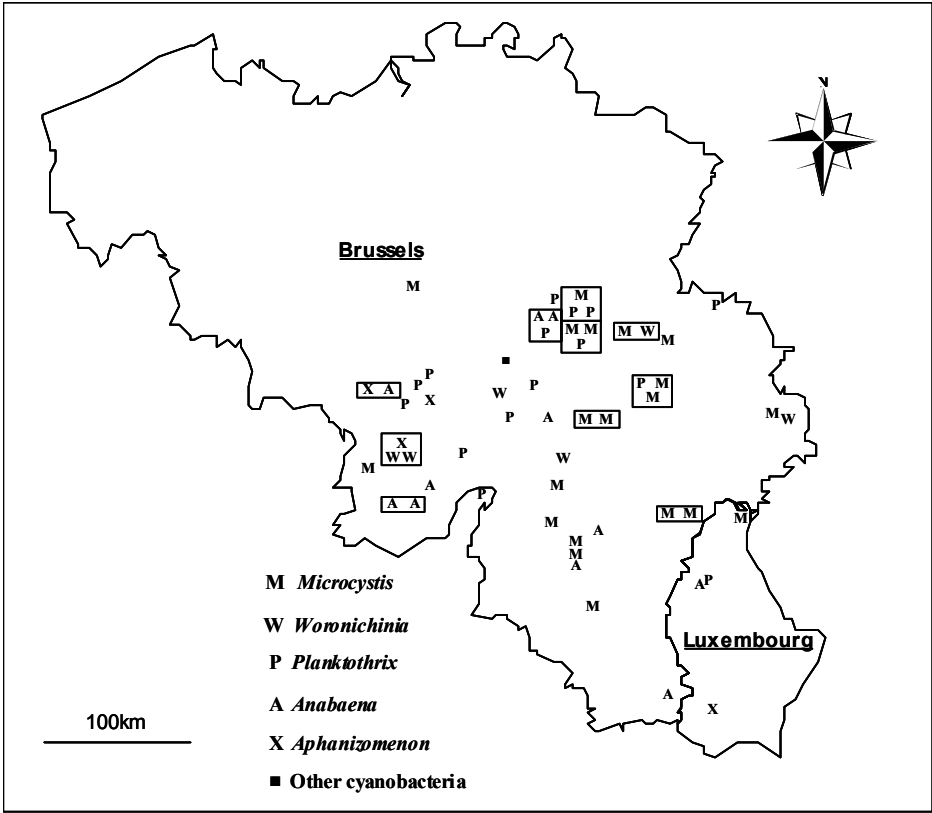


Fig. 1: Geographical distribution of the dominant cyanobacterial genera forming blooms (squares indicate sampling sites visited several times)

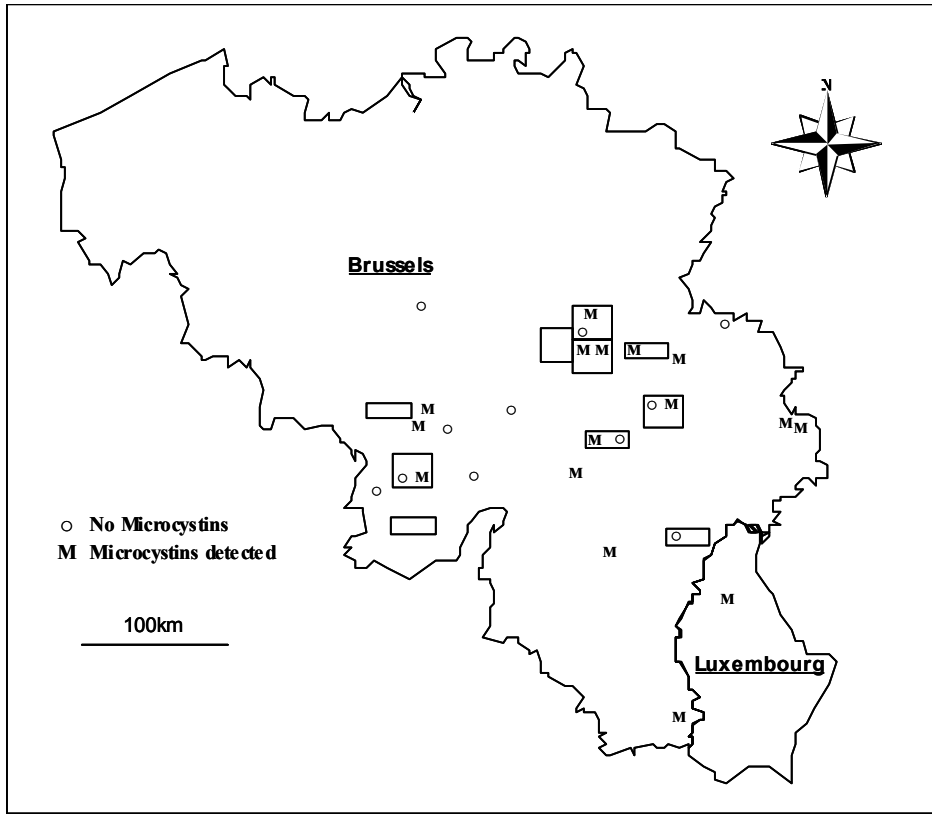


Fig. 2: Geographical distribution of microcystin-containing blooms (squares indicate sampling sites visited several times)

Long-term monitoring of the Haute-Sûre Reservoir (Luxembourg)

Monitoring of phytoplankton since 1988 revealed the recurrent presence of cyanobacteria in elevated densities during summer in the Haute-Sûre reservoir, the most important source of surface water for drinking water production in Luxembourg. Figure 3 shows the seasonal dynamics of cyanobacteria from January 1999 to December 2001. In this reservoir, the cyanobacterial assemblages are mainly dominated by the filamentous *Planktothrix* spp., *Anabaena planctonica*, coiled *Anabaena* spp. and *Aphanizomenon* spp. as well as by the colonial *Woronichinia naegeliana*. Although the main taxa remain similar from year to year, important differences in their relative development are observed. Statistical analyses of the seasonal phytoplankton dynamics indicate that silicate, nitrate and water transparency generally play an important role in determining the seasonal succession of the main phytoplankton groups while water temperature, ammonium and iron concentrations as well as the flow regime of the main inflow appear to be the major variables specifically controlling cyanobacterial assemblages during summer and autumn (Willame et al. in prep.).

Monitoring of cyanobacterial populations combined with microcystin analyses started in 2003. The results for 2003 (Fig. 4) show quite low microcystin concentrations, ranging up to $0.12 \mu\text{g g}^{-1}$ DW. Following WHO guidelines, the cyanobacterial biovolume levels found in this reservoir are generally below the first alert level for water bodies used for recreational purposes (Falconer et al., 1999). The main microcystin variant detected was microcystin-LR. The microcystin concentrations were not correlated to cyanobacterial biovolumes or species assemblages and were very variable between sampling stations.

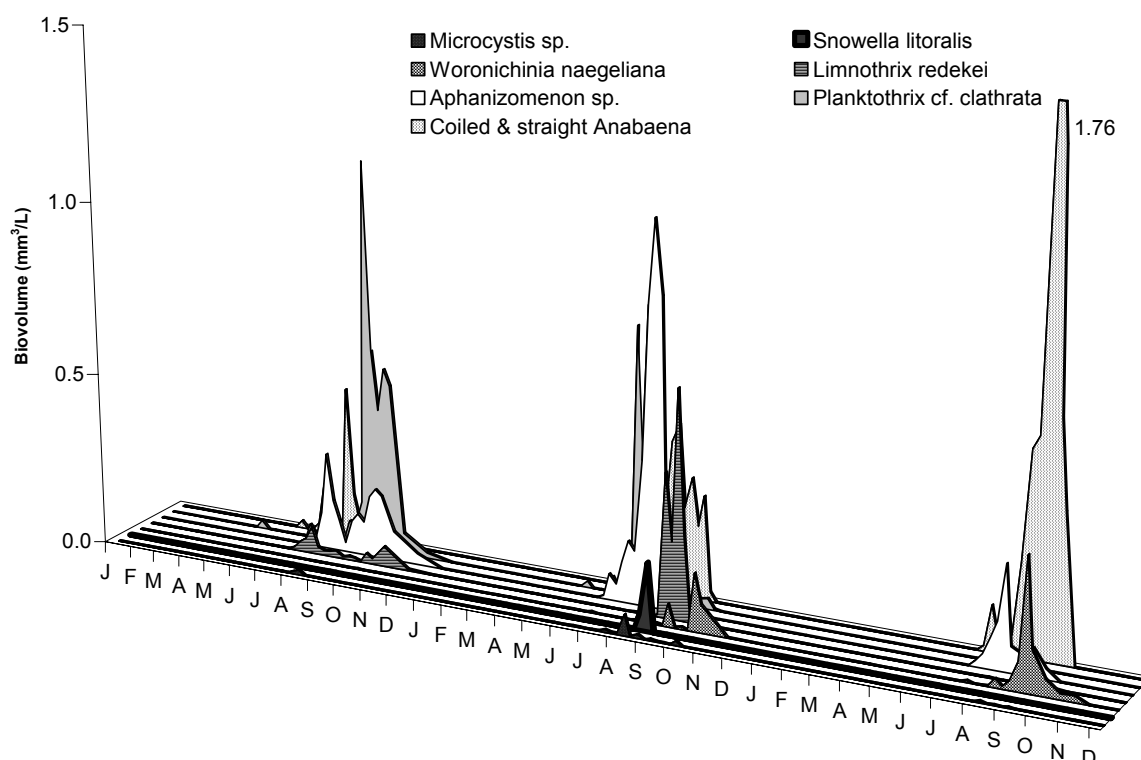


Fig. 3: Seasonal cyanobacterial dynamics in the Haute-Sûre reservoir 1999 – 2001

Note: "Biovolume" means the total volume of cellular material determined from cell counting and mean cell volume calculated from geometric dimensions of the cells. It corresponds to fresh biomass assuming a specific weight of 1, i.e. 1 mm^3 of biovolume $\approx 1 \text{ mg}$.

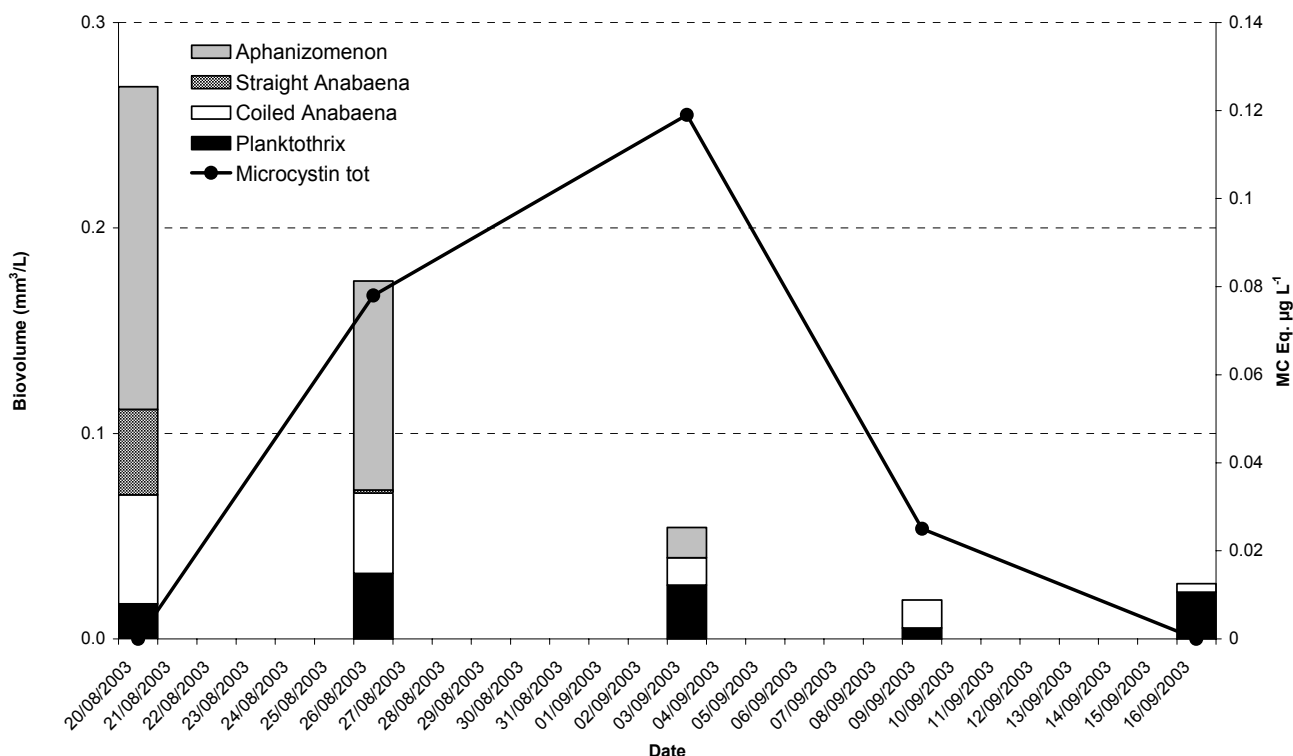


Fig. 4: Seasonal patterns of biovolume of cyanobacterial and the concentration of microcystin in the Haute-Sûre Reservoir, summer 2003 (Lultzhausen station)

Note: microcystins is given as the sum of all variants, determined as MC-LR concentration equivalents)

Public awareness and research in Belgium and Luxembourg

Belgian and Luxembourgish authorities have neglected the cyanobacterial bloom problems for a long time and thus significantly lack knowledge as compared to other European countries. Data obtained during the last decade demonstrate that the monitoring of cyanobacterial blooms should be on the agenda of the administrations responsible for water quality management in both countries. The national authorities are becoming increasingly aware of the problem and nationally funded projects were recently set up to evaluate the frequency, extent and harmfulness of cyanobacterial surface water blooms.

In Belgium, the Belgian Science Policy sponsors the multi-disciplinary B-Blooms project in order to develop the knowledge and tools required for a country-wide monitoring network and early-warning system of nuisance blooms. The specific objectives of this project are (website: <http://www.bblooms.ulg.ac.be/>): (1) to document the extent, nature and dynamics of toxic cyanobacterial blooms and to set up a national database; (2) to develop a predictive model as decision tool for regional authorities; (3) to develop tools for a rapid detection and identification of cyanobacterial blooms.

In Luxembourg, the Administration of Water Management funded a project to monitor the microcystin presence in the major drinking water reservoir, the Haute-Sûre reservoir in which cyanobacterial summer blooms regularly develop. The Luxembourg National Research Found (FNR) also currently sponsors a research project to (1) develop new molecular tools for rapid monitoring of the presence of cyanotoxin-producing cyanobacteria in surface water

and (2) to assess the adequacy and efficiency of the currently used water treatment processes in the presence of cyanotoxins in the Haute-Sûre reservoir.

The data obtained in the last years should help water management authorities in both countries to set up the necessary regulations for the use of surface water bodies for the production of drinking water or for recreational purposes in the presence of cyanobacteria, and thus also to better implement the EC Water Framework Directive in view of the protection of the aquatic environment and the improvement of the status of aquatic ecosystems.

REFERENCES

- Falconer, I.R., J. Bartram, I. Chorus, T. Kuiper-Goodman, H. Utkilen & G. Codd, 1999. Safe levels and practices. In: Chorus I. & J. Bartram (eds.). Toxic cyanobacteria in water: a guide to their public health consequence, monitoring and management. Published on the behalf of WHO by E & FN Spon, London, pp. 155-178.
- Van Hoof, F., P. Castelain, M. Kirsch-Volders & J. Vanderekom, 1994. Toxicity studies with blue-green algae from Flemish reservoirs. In: Codd, G.A., T.M. Jefferies & E. Potter (eds), Proceeding of the first international symposium on detection methods for cyanobacterial (Blue-green algal) toxins, 27-29 September 1993, University of Bath, UK: Detection methods for cyanobacterial toxins. Special Publication N° 149: 139-141. The Royal Society of Chemistry, Cambridge (UK).
- Willame, R. & L. Hoffmann, 1999. Bloom-forming blue-green algae in Belgium and Luxembourg. *Archiv für Hydrobiologie Supplement, Algological Studies* 94: 365-376.
- Willame, R., T. Jurczak, J. F. Iffly, J. Meriluoto & L. Hoffmann, 2005. Distribution of hepatotoxic cyanobacterial blooms in Belgium and Luxembourg. *Hydrobiologia* 551 (in press).
- Willame, R., I. Thys, V. Jacquet, H. M. Cauchie, M. Salvia-Castellvi, M. Verbanck & L. Hoffmann. Environmental variables involved in the development of bloom-forming cyanobacteria in a mesoeutrophic reservoir. *Algological Studies* (submitted).
- Wirsing, B., L. Hoffmann, R. Heinze, D. Klein, D. Daloze, J. C. Braekman & J. Weckesser, 1998. First report on the identification of microcystin in a water bloom collected in Belgium. *Systematic and Applied Microbiology* 21: 23-27.

BRAZIL:

Management and Regulatory Approaches for Cyanobacteria and Cyanotoxins

Sandra. M. F. O. AZEVEDO

Carlos Chagas Filho Biophysics Institute – Federal University of Rio de Janeiro, 21949-900 CCS – Bloco G Ilha do Fundão, Rio de Janeiro, RJ, Brazil. E-Mail: sazevedo@biof.ufrj.br

Occurrence of mass developments of cyanobacteria and of cyanotoxins in Brazil

The growth of agro-industry in some Brazilian regions has been very pronounced over the last 20 years. The large monospecific cultures and extensive use of fertilizers have caused a rapid eutrophication in rivers and reservoirs. The urbanization rate has also undergone rapid increase, including an increase in wastewater discharge without any previous treatment. These two large-scale processes are currently the main causes of eutrophication of rivers and reservoirs. This increasing eutrophication in Brazilian water bodies has led Cyanobacteria to become dominant in several natural freshwater supplies, brackish lagoons and reservoirs.

Records of cyanobacterial blooms in Brazil begin in the 1980's. A review of data in the Brazilian literature about phytoplankton ecology shows that aquatic environments located in areas with anthropogenic influence present a high percentage of cyanobacteria dominance and water bloom occurrence. On the average, almost 50% of these environments are already dominated by cyanobacteria. Part of this occurrence can be considered as a natural fact, but the number of environments showing cyanobacteria as the main group in the phytoplankton community has increased.



Fig. 1: Occurrence of toxic cyanobacterial blooms in Brazil

Open symbols: hepatotoxic blooms
Solid symbols: neurotoxic blooms

The occurrence of toxic cyanobacterial blooms has been registered in 11 of 26 Brazilian states, from the North to South. These blooms occur mainly in reservoirs but there are records of occurrence in several coastal lagoons, natural lakes, rivers and estuaries. According to a review by Sant'Anna and Azevedo (2000) the most common genera were *Microcystis* and *Anabaena*, but an increase in *Cylindrospermopsis* dominance has been detected in the last decade. (Bouvy, et al., 1999; Huszar et al, 2000).

In general, there is little information about cyanotoxin analyses or toxicity tests done with cyanobacterial bloom material. The most commonly detected cyanotoxins

are microcystins. These hepatotoxic heptapeptides were already confirmed in samples from different Brazilian regions. Besides this the confirmed occurrence of another group of cyanotoxins in continental waters has been reported. The production of saxitoxins by *Cylindrospermopsis* (Lagos *et al.* 1999; Molica *et al.* 2002) was reported for at least 5 different Brazilian states. In addition the production of anatoxin-a(s) by *Anabaena* was confirmed by Monserrat *et al.* (2001) and Molica *et al.* (2004) in cyanobacterial samples from two different states. Moreover, the presence of cylindrospermopsin was confirmed in filters from a dialysis clinic at Caruaru city (Pernambuco State, Brazil). However, the identification of the cyanobacterial organism responsible for cylindrospermopsin production was not possible. (Carmichael *et al.*, 2001).

Furthermore, the isolation of hepatotoxic nanoplanktonic cyanobacteria (*Synechocystis aquatilis*) from coastal areas and picoplanktonic cyanobacteria strains producing microcystin from reservoirs in Brazilian Northeast region (Domingos *et al.*, 1999; Komárek, *et al.*, 2001) define a new challenge for public health and water treatment authorities. Due to the very small size of these cells their identification requires special care and the removal by traditional methods of water treatment can be more difficult. Therefore, the potential toxicity of these species needs to be considered and the risk from picoplanktonic cyanobacteria in water supplies needs to be monitored in order to assess the hazards of cyanotoxins.

The adverse impacts on water supplies due to cyanobacterial blooms are usually underestimated. Reports about these impacts are mainly related to massive fish mortality that frequently are much more related to reduction of dissolved oxygen concentration. The loss of scenic quality, and consequent reduction of recreational activities with economic loss for the tourism business, is also described in some regions. However, the ecological impacts, such as effects on aquatic biodiversity or bioaccumulation of cyanotoxins through food chain, has been analyzed, estimated and described by only a few scientific papers (Magalhães, *et al.*, 2001, Panosso, *et al.*, 2003; Ferrão Filho, *et al.*, 2002 a,b). Moreover, this knowledge is still restricted to academic circles and consequently is used neither by aquaculture workers nor by authorities involved with water quality or food quality control.

The most serious incident involving cyanotoxins happened in 1996 in Caruaru city (Pernambuco State) when 130 patients of a hemodialysis clinic experienced intoxication symptoms related with hepatotoxins, 100 patients developed acute liver failure caused by microcystin intoxication and 60 of these died until December of 1996. Occurrence of toxic cyanobacteria in the reservoir and inadequate water treatment at both municipal water plant and the dialysis clinic allowed the occurrence of cyanotoxins in the water used for dialysis and facilitated this outbreak. (Jochimsen *et al.*, 1998; Carmichael *et al.*, 2001; Azevedo *et al.* 2002).

Regulation and management

Legislation for drinking water

In 2000, the Brazilian Ministry of Health with collaboration of Pan-American Health Organization (PAHO) coordinated a review of the Brazilian regulation for drinking water quality. Cyanobacteria and cyanotoxins were incorporated in this new regulation as parameters that must to be monitored for water quality control.

Since then the new federal Brazilian legislation for water quality control included the requirement for monitoring cyanobacterial populations in drinking water supplies, and microcystins analysis in raw and treated water. The biomass of cyanobacteria in raw water needs to be monitored monthly by cell counting up to a threshold of 10,000cells/ml (or 1mm³ /L of bio-volume). From 10,000 to 20,000 cells/mL of cyanobacteria the monitoring needs to be done weekly and above this threshold toxicity testing and/or quantitative cyanotoxin analyses in drinking water are necessary. The standard value of 1µg/L for microcystins was adopted as

mandatory and values of 3µg/L for equivalents of saxitoxins and 15µg/L for cylindrospermopsin were included as recommended guidelines. In addition, when the number of cyanobacterial cells is above 20,000/mL the use of algicides in the water supply or any other chemical compound during the water treatment process, which is able to promote the lyses of cyanobacteria cells (and therefore release of the cyanotoxins) is forbidden.

Management and educational actions:

Since in general the consequences of toxic cyanobacterial bloom occurrence are underestimated or undiscovered by different authorities responsible for the environment and water quality control, the management actions, preventive plans and remedial measures are usually only implemented when a serious event occurs. Besides, it is common to observe that these actions are restricted to a few weeks surrounding the event, depending on the “media attention” for the case.

The most common management action implemented is a phytoplankton-monitoring program on water bodies with frequent occurrence of cyanobacterial blooms. This has been done both by researchers working on limnology issues and also by technicians from water companies. Further, some isolated actions as prohibition of fishing and bathing, releasing reports from water treatment plants and use of powdered activated carbon are also described for some areas, mainly in big cities regions São Paulo, Porto Alegre and Belo Horizonte.

An important limitation for an effective management and the implementation of remedial measures in some regions are the laboratory capacities and staff training. Even where the theoretical knowledge about the local toxic cyanobacteria issues is good enough, the efficiency of a monitoring program for cyanotoxins is often limited by the lack of analytical resources. On the other hand, in some regions the main limitation is still staff training, which needs improvement of their background understanding for the issue.

In Brazil, the main educational activities are short training courses promoted by the Ministry of Health. They have been conducted in different Brazilian regions and technicians from state secretaries of health and from water companies are stimulated to participate. Besides this, the Ministry of Health published a manual about cyanobacteria and cyanotoxins including information about remedial measures and water treatment. It is being distributed in the entire country. Some state water companies are also involved with the production of educational material, but it is an isolated action restricted to a few states.

References

- AZEVEDO, S.M.F.O.; CARMICHAEL, W.W.; JOCKIMSEN, E.M.; RINEHART, K.I.; LAU, Sharon; SHAW, G.R.; EAGLESHAM, G.K. (2002). Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology*, 181: 441-446.
- BOUVY, M.; MOLICA, R.; OLIVEIRA, S.; MARINHO, M.; BEKER, B. (1999). Dynamics of a toxic cyanobacterial bloom (*Cylindrospermopsis raciborskii*) in a shallow reservoir in the semi-arid region of northeast Brazil. *Aquatic Microbial Ecology*. 20: 285-297.
- CARMICHAEL, W.W., AZEVEDO, S.M.F.O., AN, J., MOLICA, R.J.R., JOCHIMSEN, E.M., LAU, S., RINEHART, K.I., SHAW, G.R, EAGLESHAM, G.K. (2001) Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins *Environmental Health Perspectives* 109(7) : 663-668.
- DOMINGOS, P.; RUBIN, T. K.; MOLICA, R.J.R.; AZEVEDO, S.M.F.O. & CARMICHAEL, W.W. (1999) First report of microcystin production by picoplanktonic cyanobacteria isolated from a north-east Brazilian drinking water supply. *Environmental Toxicology* 14: 31-35.
- FERRÃO FILHO, A S; DOMINGOS, P.; AZEVEDO, S.M.F.O (2002a).. Influences of a *Microcystis aeruginosa* Kutzinger bloom on zooplankton populations in Jacarepaguá Lagoon (Rio de Janeiro, Brazil). *Limnologica*. 32:295-308.

- FERRÃO FILHO, A.S.; SUZUKI, B.K; AZEVEDO, S.M.F.O (2002b). Accumulation of microcystins by a tropical zooplankton community. *Aquatic Toxicology*, 59:201-208.
- HUSZAR, V.L.M.; SILVA, L.H.S.; MARINHO, M.; DOMINGOS, P.; SANT'ANNA, C.L. (2000). Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. *Hydrobiologia*. 424: 67-77.
- JOCHIMSEN, E.M.; CARMICHAEL, W.W.; AN, J.; CARDO, D.; COOKSON, S.T.; HOLMES, C.E.M.; ANTUNES, M.B.C.; MELO FILHO, D.A.; LYRA, T.M.; BARRETO, V.; AZEVEDO, S.M.F.O. & JARVIS, W.R.(1998). Liver failure and death following exposure to microcystin toxins at a hemodialysis center in Brazil. *The New England Journal of Medicine*. 36:373-378.
- KOMÁREK, J.; AZEVEDO, S.M.F.O.; DOMINGOS, P.; KOMÁRKOVÁ, J. & TICHÝ, M. (2001) Background of the Caruaru tragedy: a case taxonomic study of toxic cyanobacteria. *Algological Studies* 140(2): 9-29.
- LAGOS, N.; ONODERA, H.; ZAGATTO, P.A.; ANDRINOLO, D.; AZEVEDO, S.M.F.O. & OSHIMA, Y. (1999) The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii* isolated from Brazil. *Toxicon* 37: 1359-1373.
- MAGALHÃES, V.F.; SOARES, R.M, AZEVEDO, S.M.F.O. (2001) Microcystin contamination in fish from the Jacarepaguá Lagoon (RJ, Brazil): Ecological implication and human health risk. *Toxicol.* 39: 1077-1085.
- MOLICA, R. J R; ONODERA, H.; GARCIA, C.; RIVAS, M.; ANDRINOLO, D.; NASCIMENTO, S.; MEGURO, H.; OSHIMA, Y.; AZEVEDO, S.M.F.O; LAGOS, N. (2002) Toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii* (Cyanobhyceae) isolated from Tabocas reservoir in Caruaru, Brazil, including demonstration of a new saxitoxin analogue. *Phycologia*. 41(6):606-611.
- MOLICA, R.J.R.; OLIVEIRA, E. J. A. CARVALHO, P. V. V. C.; COSTA, A N. S. F.; CUNHA, M. C. C.; MELO, G. L. and AZEVEDO, S. M. F. O.(2004) Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. *Harmful Algae* (in press).
- MONSERRAT, J.M., YUNES, J. S., BIANCHINI, A.(2001). Effects of *Anabaena spiroides* (cyanobacteria) aqueous extracts on the Acetylcholinesterase activity of aquatic species.. *Environmental Toxicology and Chemistry*. 20(6):1228 – 1235.
- PANOSSO, R; CARLSSON, P; SUZUKI, B.K; AZEVEDO, S.M.F.O; GRANÉLI, E. (2003). Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacterial strains. *Journal of Plankton Research*. 25:1169-1175.
- SANT'ANNA, C.L. & AZEVEDO, M.T.P. (2000). Contribution to the knowledge of potentially toxic Cyanobacteria from Brazil. *Nova Hedwigia*. 71:(3-4):359-385.

CANADA:

The Development of a Microcystin Drinking Water Guideline

Rocio ARANDA-RODRIGUEZ¹, Frank M. BENOIT¹, Michele GIDDINGS²

¹ Chemistry Research Division, Health Canada, Tunney's Pasture, 0800C, Ottawa, ON, K1A 0L2, Canada,
E-mail: Rocio_Aranda-Rodriguez@hc-sc.gc.ca

² Water Quality and Health Bureau, Health Canada, 2720 Riverside Dr, 6604B, Ottawa, ON, K1A 0K9, Canada

Introduction

In Canada, the provision of safe drinking water requires the cooperation of all governments, particularly at the federal and provincial/territorial levels. More specifically, the Federal-Provincial-Territorial Committee on Drinking Water, made up of representatives from Health Canada, Environment Canada, and provincial and territorial departments responsible for water quality, recommends drinking water guidelines for a variety of chemical and physical parameters of health and aesthetic concern in Canadian drinking water (Federal-Provincial-Territorial Committee on Drinking Water, 1999, 2004). These guidelines are not enforceable standards unless they are embodied in provincial or territorial legislation. However, provinces and territories instead use the guidelines as the basis for assessing the quality of their drinking water supplies.

One of the chemical parameters for which a drinking water guideline has been developed in recent years is microcystin-LR. This paper briefly summarizes the incident that initiated the guideline development process for microcystin, Health Canada's concurrent development of an inexpensive analytical method and an easy-to-use field test kit for the cyanobacterial toxin, and the rationale behind the numerical guideline value.

The Manitoba Incident

Cyanobacteria grow in shallow, warm, slow-moving or still bodies of water common throughout Canada, particularly in the Prairie provinces of Alberta, Saskatchewan and Manitoba. Many rural water supplies used for domestic purposes and for livestock watering are subject to repeated blue-green algal blooms, particularly during the hot summer months. Cyanobacterial genera known to occur in Canada and most often associated with poisonings are *Anabaena*, *Aphanizomenon*, *Microcystis*, *Oscillatoria* and *Nodularia*.

In late August 1993, a large algal bloom developed in the Deacon Reservoir, the main storage facility in the city of Winnipeg, Manitoba, for water from Shoal Lake. The water from Shoal Lake was generally considered to be of high quality and required only disinfection with chlorine prior to distribution and consumption. Sampling determined that toxin-producing blue-green algae were present in Shoal Lake, but not in the Deacon Reservoir. In addition, analysis of water samples indicated that microcystin-LR was present in Shoal Lake and within the distribution system, but was not present at detectable levels ($>0.05 \mu\text{g/L}$) in the Deacon Reservoir. Maximum microcystin-LR concentrations measured in the raw water of Shoal Lake and in treated tap water were approximately $0.45 \mu\text{g/L}$ and $0.55 \mu\text{g/L}$, respectively.

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, Manitoba Environment became concerned that higher levels of microcystin-LR could develop in Shoal Lake during the more usual, relatively hot, dry summers. In addition, because Shoal Lake, a relatively nutrient-poor water supply, supported a toxic blue-green algal bloom, Manitoba Environment was also concerned that toxic blooms may be occurring in more nutrient-rich rural surface water supplies throughout southern Manitoba.

To address these concerns, Manitoba Environment, in cooperation with the City of Winnipeg, continued to monitor for microcystin-LR in Winnipeg's water supply, detecting it at concentrations ranging from 0.1 to 0.5 µg/L on six occasions between 1994 and the end of 1996. In addition, a comprehensive study of the quality of surface water supplies in rural southwestern Manitoba was conducted in 1995 and 1996. In 1995, 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%), suggesting that conventional treatment methods were only partially successful in removing the toxin. In 1996, seven rural surface water supplies were intensively sampled for microcystin-LR from June through December, and the hepatotoxin was found throughout the entire sampling period, sometimes at levels exceeding 0.5 µg/L.

Initiation of the Guideline Development Process

The finding of microcystin-LR in cyanobacterial blooms in the Deacon Reservoir in the summer of 1993 prompted Manitoba Environment to request that Health Canada develop an "Emergency Health Advisory" guideline for microcystins in drinking water. An emergency guideline of 0.5 µg/L was established in response to the 1993 incident, and Health Canada and Federal-Provincial-Territorial Committee on Drinking Water decided shortly thereafter to initiate the thorough evaluation and review process that would eventually lead to the development of a guideline for microcystin-LR in drinking water in Canada.

Before a guideline could be adopted a practical analytical method for monitoring and assessing the presence and toxicity of microcystins on a routine basis was required. At that time, in the 1990s, there was no readily available field test kit for rapidly determining the presence or absence of microcystins in a water supply, and laboratory-based analytical methods available for quantitative results were too complicated and expensive for regular monitoring of water supplies. To improve available detection and testing methods, Health Canada decided to develop an analytical method and examine the possibility of creating a prototype field test kit.

Development of New Detection and Testing Methods

In 1997, Health Canada was approached by Saskatchewan Health for help in developing an analytical method to detect and assess the toxicity of cyanobacterial blooms in an affordable, efficient, reliable and routine way. It was concluded, after considerable discussion, that the most suitable method would use high-performance liquid chromatography with ultraviolet detection (HPLC-UV). An HPLC-UV procedure published in the United Kingdom in 1998 was chosen for modification. This method was developed to detect and quantify dissolved microcystin-LR, microcystin-RR, microcystin-YR and nodularin in raw and treated waters. Several of the procedure's parameters were modified in an attempt to remove the high background and interference peaks in the HPLC chromatograms and to lower the detection limit. Using the modified analytical method for a 500-mL water sample, microcystin-LR could be detected at levels as low as 0.3 µg/L. The minimum quantifiable limit for microcystin-LR was 0.8 µg/L. The method underwent a thorough evaluation and testing phase, during which sampling was carried out at various sites in several provinces in Canada (Giddings, 2002).

A field test kit was developed by the University of Alberta Protein Phosphatase laboratory in collaboration with Health Canada 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. The field test kit is a non-radioactive assay that exploits the ability of microcystins to specifically inhibit type-1 protein phosphatase. 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 colour reaction against a control sample. The absence of toxin results in a clear yellow colour, whereas the presence of toxin results in a colourless solution.

This comparison also enables the individual performing the test to roughly estimate the amount of toxin present in the sample (positive control is 1.5 µg/L microcystin-LR). Although the prototype kit was validated during field trials in the summer of 2000, there have been delays in its commercialization.

Test kit results are to be used only as an initial indicator of the presence/absence of toxin. Final determination of water safety is based on a comparison of the toxin levels derived from a laboratory analytical method with the drinking water guideline developed for microcystin-LR.

Canadian Drinking Water Guideline for Microcystin

Health Canada (2002) has determined that microcystin-LR is possibly carcinogenic to humans and has placed it in Group IIIB (inadequate data in humans, limited evidence in experimental animals). For compounds in Group IIIB, it is considered appropriate to use a lowest-observed-adverse-effect level (LOAEL) or no-observed-adverse-effect level (NOAEL) from the most suitable chronic or subchronic study, divided by an appropriate uncertainty factor, to derive a tolerable daily intake (TDI). Such an approach has been used for microcystin-LR, the only microcystin for which there is sufficient information available to derive a guideline value.

For microcystin-LR, a TDI of 0.04 µg/kg body weight per day was derived from a NOAEL of 40 µg/kg body weight per day for liver changes in a 13-week mouse study (Fawell et al., 1994), using an uncertainty factor of 1000 (×10 for intraspecies variation, ×10 for interspecies variation and ×10 for the less-than-lifetime study). An additional uncertainty factor for limited evidence of carcinogenicity in animals was not considered necessary.

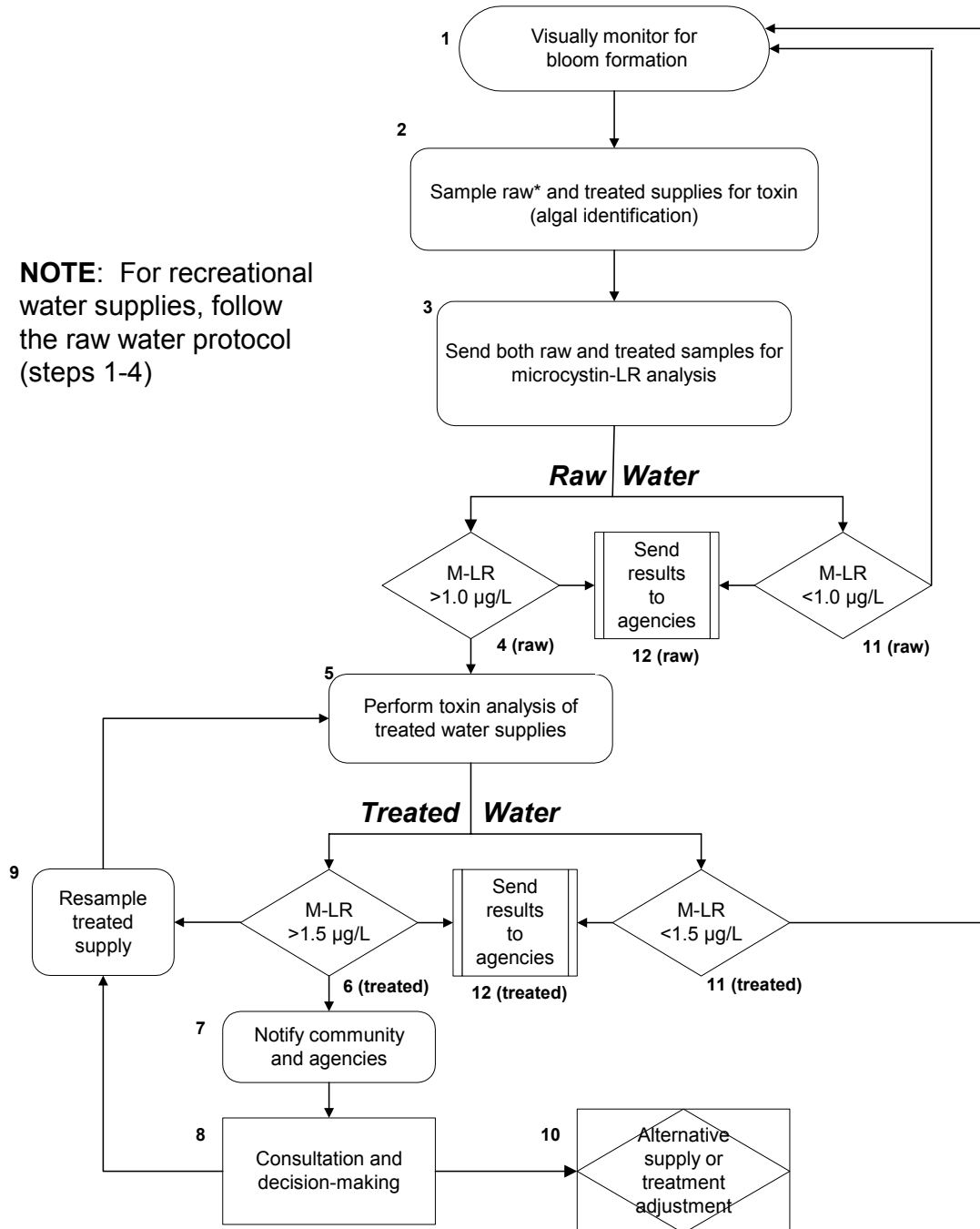
A maximum acceptable concentration (MAC) for microcystin-LR was calculated from the TDI by assuming a 70-kg adult consuming 1.5 L of water per day, as well as allocating 80% of the total daily intake to drinking water. The MAC thus derived is 1.5 µg/L. This MAC, derived for microcystin-LR, is believed to be protective against exposure to other microcystins (total microcystins, i.e., free plus cell bound) that may also be present. It is a conservative value, as it is derived on the basis of daily consumption of microcystin-LR over a full year. However, as there are spatial and temporal variations in the levels of microcystins within supplies and as there are also likely to be other microcystins present that could go undetected, this value is considered appropriate (Health Canada, 2002).

Also, as a precautionary note, Health Canada (2002) advises dialysis centres to be aware or informed if the source water from their local treatment plant is prone to blue-green algal blooms so that they may undertake to provide additional treatment of the water, if necessary. This treatment can range from granular activated carbon filtration followed by reverse osmosis to much more complex membrane filtration systems (e.g., ultrafiltration). The extent of additional treatment will depend entirely on the quality of the municipal water supply. Continuous monitoring of performance and equipment will be required to ensure adequate quality of the water. As well, it is important that all manufacturers' specifications be evaluated for the local conditions.

A flow chart illustrating those factors that should be considered by water purveyors and health and environment authorities during bloom events and actions that may be taken to address the issue is shown below.

**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.

Ongoing Analytical Research

The development of accurate methods for the analysis of freshwater toxins will allow decision-makers in water systems to react more rapidly and with increased confidence to occurrences of algal blooms that may pose threats to human health. The analysis of microcystins poses several challenges, which are listed below.

Availability of authentic microcystin congeners: *About 60 microcystin variants have been identified, including a number of microcystin-LR homologues, some of which have similar toxicity to microcystin-LR. The current analytical methods rely on the availability of authentic microcystin congeners for identification and quantification. Previous studies have shown the presence of additional microcystin congeners, in some instances at higher concentrations than microcystin-LR. However, in the past, only four variants (-LR, -RR, -LW and -LF) have been commercially available, thus limiting the number of microcystins that can be monitored accurately. Other microcystins can be only tentatively identified and their quantities only estimated.*

The microcystins that are commercially available are general reagents rather than analytical standards; thus, their purity has to be checked by HPLC-photodiode array (PDA) and liquid chromatography–mass spectrometry (LC-MS). It was found that microcystin-RR purity ranges from 70 to 80%. Some lots contained a mixture of microcystin-RR and its demethyl variant [Dha⁷]microcystin-RR; other lots contained [Dha⁷]microcystin-RR only (Kubwabo et al., 2004).

More recently, security concerns have given rise to additional regulations in the handling and shipping of microcystins. For example, in order to export microcystin from the United States, a licence from the U.S. Department of Commerce must be obtained. Moreover, some laboratory facilities may have to comply with the requirements for containment level 2 in order to receive cyanobacterial cultures. In the future, this may limit the number of laboratories capable of analysing microcystins.

Interferences in the determination of microcystins: The currently accepted analytical method consists of isolation of microcystins using solid-phase extraction (C₁₈ or hydrophilic-lipophilic balance [HLB] columns) followed by separation using LC and detection with either PDA or MS. The LC-PDA method works well with relatively clean samples such as drinking water or samples containing low natural organic matter (NOM). However, the method is prone to interferences with samples with high NOM content. Alternative isolation procedures based on immunoaffinity technology (Aranda-Rodriguez et al., 2003) have been developed to address these issues.

Health Canada has developed innovative and alternative methods for the analysis of microcystin in algal cells. An efficient and rapid method for the isolation of microcystin from cells based on accelerated solvent extraction has been developed (Aranda-Rodriguez et al., 2005).

Emerging Issues

While most of the work in Canada has focused on microcystin, the possible presence of toxins such as anatoxin-a and cylindrospermopsin cannot be ignored. The cyanotoxins (microcystin, anatoxin-a, cylindrospermopsin) are structurally and biologically different, such that concurrent analysis poses significant challenges. Several methods are available for the determination of microcystin, including screening (phosphatase inhibition test and enzyme-linked immunosorbent assay [ELISA]) and laboratory-based methods (extraction-HPLC-PDA or HPLC-MS). Anatoxin-a, an alkaloid-like neurotoxin, degrades within 24 hours under high pH and in the presence of pigments; therefore, it is important to look for degradation products when analysing for anatoxin-a. A method for the concurrent determination of microcystin and anatoxin-a has been developed recently and uses a common isolation strategy (C₁₈ columns) followed by a distinctive elution technique.

Cylindrospermopsis raciborskii is new to North America and dominates 23% of lakes in Florida, USA. It is reported that the majority of *C. raciborskii* or *Aphanizomenon ovalisporum* produce the cytotoxin cylindrospermopsin, a cyclic guanidine alkaloid. *C. raciborskii*, of subtropical and tropical origin, was recently discovered in a lake near Ottawa, Canada. Cylindrospermopsin is not commercially available. There is no Canadian guideline for cylindrospermopsin in drinking water, because there is insufficient evidence that cylindrospermopsin is an issue in Canada at this time.

Conclusion

In conclusion, a guideline for microcystin-LR in drinking water was developed in Canada through a successful federal-provincial-territorial cooperative effort. This effort involved more than just guideline development in this case, as can be seen by the project to develop improved tools to monitor the presence of 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 in Canada.

For more information on Health Canada's involvement in activities related to drinking water quality in Canada, please refer to the Water Quality and Health Bureau's web page at: <http://www.hc-sc.gc.ca/hecs-sesc/water/index.htm>.

References

- Aranda-Rodriguez, R., Kubwabo, C. and Benoit, F.M. 2003. Extraction of 15 microcystins and nodularin using immunoaffinity columns. *Toxicon* 42(6):587–599.
- Aranda-Rodriguez, R., Tillmanns, A., Benoit, F.M., Pick, F.P., Harvie, J. and Solenaia, L. 2005. Pressurized liquid extraction of toxins from cyanobacterial cells. Accepted for publication in the *Journal of Environmental Toxicology*.
- Fawell, J.K., James, C.P. and James, H.A. 1994. Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. Report No. FR 0359/2/DoE 3358/2, Water Research Centre, Medmenham, UK. 46 pp.
- Federal-Provincial-Territorial Committee on Drinking Water. 1999. Canadian Drinking Water Guidelines Development Process. Health Canada, Ottawa, Ontario. [Available at: http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/dwg_development_process.pdf]
- Federal-Provincial-Territorial Committee on Drinking Water. 2004. Summary of Guidelines for Canadian Drinking Water Quality. Health Canada, Ottawa, Ontario. [Available at: <http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/summary.pdf>]
- Giddings, Michèle. 2002. Cyanobacteria Toxins: The development and evaluation of methods to determine microcystin levels in Canadian water supplies. Proceedings of Health Effects of Exposure to Cyanobacteria Toxins: State of the Science. Sarasota, Florida., August 13-14, 2002. Florida Department of Health, the Center for Disease Control, Mote Marine Laboratory, and the University of South Florida, Editors: David Johnson, M.D., M.S., Raymond D. Harbison, Ph.D.
- Health Canada. 2002. Guidelines for Canadian Drinking Water Quality: Supporting Documentation — Cyanobacterial Toxins — Microcystin-LR. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. [Available at: <http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/microcys.pdf>]
- 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.
- Kubwabo, C., Vais, N. and Benoit, F.M. 2004. Identification of microcystin-RR and [Dha⁷]microcystin-RR in commercial standards by electrospray ionization mass spectrometry. *Journal of AOAC International* 87(4):1028–1031.

CZECH REPUBLIC:

Management and Regulation of Cyanobacteria and Cyanotoxins

B. MARŠÁLEK, L. BLÁHA, L. BLÁHOVÁ, P. BABICA

Center for Cyanobacteria and their Toxins, Institute of Botany, Czech Academy of Science, and RECETOX, Masaryk University, Kamenice 3, CZ-62500 Brno, Czech Republic, E-mail: marsalek@brno.cas.cz

Introduction

Cyanobacteria have become a health and ecological problem in many waterbodies in the Czech Republic including drinking water supplies, recreational reservoirs, fish ponds, technological reservoirs for nuclear power plants and also in 7 rivers downstream of reservoirs with dense scums. Although the problem has been recognized for relatively long time, until recently, health authorities did not control most activities related to toxic cyanobacteria and management was dependent on non-profit voluntarily activities and research funds. Since 1994 several scientific projects assessed microcystin concentrations in the CR. Recently, regulatory actions were adopted for both recreational and drinking water supplies. The state-of-the art, principles and problems in regulations of toxic cyanobacteria in the Czech Republic are described below.

Occurrence of toxic cyanobacteria in the CR –10 years of monitoring data

The survey of cyanobacteria 1994-2004 in the CR showed that about 80% of all reservoirs suffer from annual developments of heavy cyanobacterial water blooms (Maršálek et al. 2001a, 2002b, 2004). Concentrations of cyanobacterial cells in the Czech reservoirs typically reach up to several millions of cells per ml during the summer development. Almost exclusively microcystins were determined in the samples from the CR - no other cyanotoxins (such as anatoxin-a, anatoxin-a(S), PSPs) were studied. According to our 10 years data, 90% of all samples contained microcystins, microcystin-LR was the most abundant variant in the cyanobacterial blooms in the Czech Republic (present in 98% of samples which contain microcystins) typically forming about 45% of total microcystins. Cell-bound concentrations of total microcystins ranged from 3 – 5800 µg/g dry weight with the median value of 702 µg/g d.w. Dissolved microcystins were also determined in the raw waters as well as in tap drinking water samples. As of 2004, dissolved microcystins in the surface raw waters (determined by ELISA) ranged from 0.2 µg/L up to 20 µg/L. Tap water samples from selected drinking water treatment plants (18 samples collected during 1999, 2003 and 2004 episodes) contained up to 8 µg/L of microcystin-LR (Bláha et al. 2003, and unpublished data). Recent monitoring activities by the newly established National Centre for Cyanobacteria and their Toxins (see also below) focus on an integration of methods for analyses of other types of cyanotoxins such as neurotoxins, nodularin and cylindrospermopsin.

Management and regulations of recreational waters

About 70% of the natural lakes and ponds or "natural bathing sites" that are primarily devoted for recreational purposes in the CR, tend to regularly develop cyanobacterial blooms during the summer months (July-September). Risk assessment of the recreational water quality is based on the determination and quantification of cyanobacterial biomass, i.e. cell counts and/or chlorophyll-a content. There are no requirements to analyse cyanotoxins in the recreational waters in the CR. The warning/alert categorization system has been adopted in the CR according to the ordinance of National Health Institute (Methodical advice CSN 757712, TNV 757717) that follows the recommendations of WHO (Chorus and Bartram, 1999). Based on this system, more than 20,000 cells/mL is considered the first warning level. If more than 100,000 cells/mL are present, the reservoir should be closed for public recreation. The responsibility to monitor water quality lies with the owners or managers of the recreational sites. They should regularly organize sampling and determination of cyanobacterial cells on their own expenses. The control organ, National Health Institute, should guarantee the quality of water to the public and conduct sampling at problematic sites. However, a number of the natural recreational reservoirs in the CR do not clearly fit to the definition of "public bathing site" (as covered by the ordinance), and consequently the conclusions of the National Health Institute are not compulsory, and they are often used only as "recommendations". It is not rare that people seeking recreation ignore such a recommendation and are swimming in the cyanobacterial scum. More adult and general education activities in this area are needed.

Regulations of cyanobacterial toxins in drinking waters

As a number surface drinking water supplies in the Czech Republic is loaded with annual developments of massive cyanobacterial water blooms, the urgent need to regulate cyanotoxin concentrations in both raw and tap waters arose. Although there was no regulation at that time, several drinking water treatment plants were temporarily closed at 1999 and 2000 by owners due to increased microcystin levels. In 2004, the provisional WHO guideline was implemented into the Czech legislation (government ordinance No. 252/2004). Monitoring of microcystins is required according to this ordinance, and the concentrations of microcystin-LR in the tap waters below 1 µg/L are considered safe.

In spite of existing law there are several problems that limit the quality of ordinance and its practical application. First, the ordinance focuses only on finished tap water, no monitoring microcystins in the raw water and/or during the treatment process is required. Additionally, the frequency of microcystin assessment is not clearly defined. Second, there is significant lack of equipped laboratories as well as educated experts in the CR that could provide qualified assessments of microcystins. Third, not only drinking water producing companies but also authorities (National Health Institute) do not have methodological tools to effectively meet their surveillance responsibility by checking microcystin concentrations in order to guarantee water quality to public. Fourth, according to the national law no. 281/2002 and the ordinance 274/2002, microcystins and anatoxin-a are considered toxins of high risk and special attention. Correspondingly, all persons and institutions using these toxins for any purpose must be registered at State Office for Nuclear Safety (SONS). Both the bureaucracy related to the SONS requirements as well as the SONS politics to limit number of registered user complicates the practical application of ordinance on microcystin regulation. Fifth, the narrow specification of the ordinance No. 252/2004 on microcystin-LR does not reflect known risks associated with toxic cyanobacteria as other variants of microcystins or other cyanotoxins are not covered.

This brief overview indicates that several serious problems in the CR must be solved to fully implement quality regulation of cyanotoxins. As a help in this situation the upgrade of ordinance No. 252/2004 is going to be prepared for 2005. It gives some alternatives to microcystin analyses such as quantification of cyanobacterial biomass in raw water by pigment

fluorescence or application of ecotoxicological bioassays combined with cell counts. The major idea of this upgrade is to require microcystin analyses only when the WHO recommended threshold for cyanobacterial biomass is exceeded.

National Centre for Cyanobacteria and their Toxins

To utilize existing experience in the field of toxic cyanobacteria in the Czech Republic, the new "Centre for Cyanobacteria and their Toxins" was established at the beginning of 2005. The Centre is as a joint venture of research laboratories (Institute of Botany and Masaryk University), non-profit organization (Association Flos-Aquae), regulatory bodies (grant from Ministry of Education) as well as private sector (drinking water companies). The Centre should become an expert national contact point for the problem of cyanobacteria that integrates information and guarantees quality education, information transfer and consultancy services to both experts and public. The Center also actively cooperates with international bodies as UNESCO, supported by CYANONET project. All relevant information can be found at <http://www.sinice.cz>.

References

- Bláha, L., and Maršálek, B. (2003). Contamination of drinking water in the Czech Republic by microcystins. *Arch Hydrobiol* 158, 421-429.
- Chorus, I., and Bartram, J., eds. (1999). *Toxic Cyanobacteria in Water: A Guide to Public Health Significance, Monitoring and Management*. E&FN Spon, London.
- Maršálek, B. (2001a). Toxiny sinic a současná realita v ČR (Cyanotoxins and the reality of Czech Republic, in Czech). *SOVAK* 10, 1-3.
- Maršálek, B., Bláha, L., Turánek, J., and Neča, J. (2001b). Microcystin-LR and total microcystins in cyanobacterial blooms in the Czech republic 1993-1998. In *Cyanotoxins - Occurrence, Causes, Consequences* (I. Chorus, ed., pp. 56-62. Springer-Verlag, Berlin.
- Maršálek, B. et. al. (2004). *Cyanobakterie v roce 2004* (Cyanobacteria in the year 2004, in Czech). Report for Czech Ministry of Environment, 112 p.

DENMARK:

Occurrence, Monitoring and Management of Toxic Cyanobacteria

Kirsten CHRISTOFFERSEN

Freshwater Biological Laboratory, University of Copenhagen, DK-3400 Hillerød, Denmark,
Email: kchristoffersen@bi.ku.dk

Introduction

Denmark is cultural landscape with numerous water bodies; approx. 1% of the area is occupied by lakes, ponds and streams, which receive water that is often enriched with inorganic nutrients from agricultural, industrial and domestic activities. These nutrients will affect the wax and wane of most aquatic biota and eventually appear in the coastal areas surrounding the whole country - despite very strong regulatory acts and intensive water treatment taken by order of the authorities. Therefore numerous water bodies are subject to frequent blooming of planktonic cyanobacteria especially during summer periods where temperatures are relatively high and water columns are stable. It appears from the recent report on the status of Danish freshwaters (Jensen et al. 2004) that almost half of the phytoplankton standing stock in 2003 (as average over the growing season) in the monitored Danish freshwater lakes was cyanobacteria (Fig. 1).

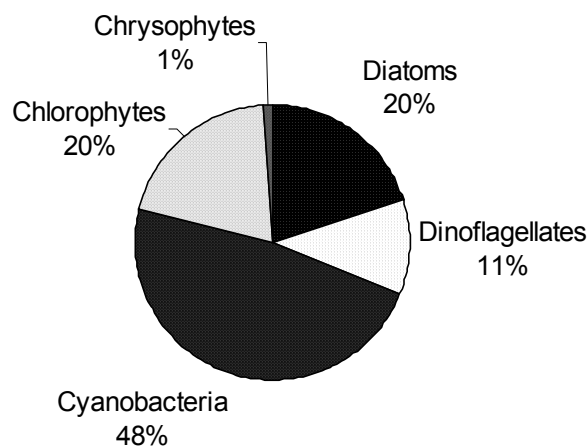


Fig. 1. The average distribution of phytoplankton groups (percentage of total phytoplankton biovolume averaged over the growing season) in the monitored Danish freshwater lakes in 2003. Information from Jensen et al. (2004).

Quite a number of lakes but also neritic coastal zones have a long history of nuisance cyanobacterial blooms. When toxic – or potentially toxic - blooms occur it often implies restrictions for the typical recreational usage of the water bodies.

Most aquatic sites are accessible to the public for swimming, boating, sports fishing and similar recreational activities. A small number of water bodies are used for drinking water intake to supplement the groundwater resources. Approx. 10% of the water consumption for the capital (Copenhagen) is from surface water during summer. Two full scale pilot infiltration plants have been established during the last 10 years to investigate if such systems can provide sustainable drinking water supplies in the near-future.

Occurrence of cyanobacteria and their toxins

Surveys on the occurrence of phytoplankton, in addition to numerous other biological, chemical and physical parameters in freshwater and coastal waters have been carried out on a routinely and standardized basis by all the counties under surveillance of the Danish Environmental Protection Agency since 1988. The number of monitored sites has changed through the years (Jensen et al. 2004). The current lake survey program includes 37 freshwater lakes and 4 brackish lakes (Fig. 2). A marine monitoring program where phytoplankton is assessed includes 26 pelagic stations located along the entire coastline as well as some open-sea stations.

The data obtained from these surveillance programs are used for regional management of the environment by the local counties and municipalities, and are also compiled into a national archive of environmental data. These data are presented to the public through annual reports by the Danish National Environmental Research Institute. All reports since 1990 are available electronically as PDF-files (<http://www.dmu.dk/Udgivelser/Faglige+rapporter/>)(in Danish).



Fig. 2. The freshwater (1-37) and brackish lakes (1-4) which are included in the national lake survey program at present. Information from Jensen et al. (2004).

Freshwater lakes: 1 Søby Sø, 2 Holm Sø, 3 Magesø, 5 Nors Sø, 6 Ravn Sø, 7 Søholm Sø, 8 Kvie Sø, 9 Bastrup Sø, 10 Hornum Sø, 13 Ørn Sø, 14 Furesø, 15 Fårup sø, 16 Damhussøen, 17 Bryrup Langsø, 19 Hinge Sø, 20 Tissø, 21 Engelsholm Sø, 22 Bagsværd Sø, 23 Borup Sø, 24 Arreskov Sø, 25 Tystrup Sø, 30 Arresø, 31 Vesterborg Sø, 33 St. Søgård Sø, 35 Utterslev Mose, 36 Søgård Sø, 37 Gundsømagle Sø. Brackish lakes: 1 Ketting Nor, 2 Ferring sø, 3 Ulvedybet, 4 Nakskov indrefjord.

The most frequently appearing cyanobacterial genera in Danish lakes and coastal areas are *Microcystis* (6-8 potentially toxic species), *Anabaena* (at least 3 potentially toxic species), *Aphanizomenon* (2 potentially toxic species), *Planktothrix* (2 potentially toxic species), and *Nodularia* (1 potentially toxic species).

However, while surveys on the occurrence of cyanobacteria are carried out on a regular basis, there is no such monitoring of the occurrence of the toxins (i.e., microcystins, nodularin, PSP and anatoxins) that many of these blooms can cause. Instead, a number of recent investigations carried out by several governmental research institutions (i.e., University of Copenhagen and Danish National Environmental Research Institute) as well as private consulting companies can provide information on the frequency of toxic cyanobacteria and the amount of toxins that may occur in water bodies.

The results from these surveys undertaken in Denmark from 1992 to 2003 (Table 1) have illustrated that not only are most (or all) cyanobacteria blooms toxic but also that the toxin content can vary highly among localities and over the season. The most frequent toxins are microcystins, but other types have also been found, including anatoxin, nodularin and saxitoxin. The most recent survey of a large number of lakes showed that the toxin content on a volumetric basis can easily exceed the $1 \mu\text{g l}^{-1}$ guideline as suggested by WHO (Christoffersen 2000).

Table 1: Overview of surveys of toxic cyanobacteria undertaken in Denmark, 1992 – 2003.

Abbreviations: dw = dry weight. MLR = microcystin LR equivalents, STX = saxitoxin equivalents

Year	Number of locations	Sampling period (overall)	Range of particulate toxin content	Reference
1992-93	1 lake	May to Sept.	0 – 16 MLR $\mu\text{g l}^{-1}$	Unpublished data
1993-95	122	May to Nov.	5 – 1900 $\mu\text{g MLR g dw}^{-1}$ < 40–160 $\mu\text{g STX g dw}^{-1}$	Henriksen 2001, Henriksen and Moestrup 1996 Kaas and Henriksen 2000
1994	14 lakes	July to Oct.	0.04 – 102 $\mu\text{g l}^{-1}$	Sørensen and Christoffersen 1996
1995	1 lake	March to Oct.	0.1 – 118 MLR $\mu\text{g l}^{-1}$	Unpublished data
1997	55 lakes	July to Oct.	0.2 – 25856 MLR $\mu\text{g l}^{-1}$	Christoffersen 2000a
1997	2 coastal sites	August	0.15 MLR $\mu\text{g l}^{-1}$	
2002	1 lake	July to Oct.	0.04 – 1.6 MLR $\mu\text{g l}^{-1}$	Unpublished data
1998-2000	1 pilot infiltration plant	Full year cycles	0.003 – 14 MLR $\mu\text{g l}^{-1}$	Christoffersen 2000b
2001-2004	2 infiltration plant	Full year cycles	0.02 – 38 MLR $\mu\text{g l}^{-1}$	Christoffersen 2001
2003	7 lakes	July to Nov.	0.02 – 2.1 MLR $\mu\text{g l}^{-1}$	Unpublished data

Surveillance, warning and guidelines

The authorities have an alarm group which makes decisions on what is to be done and by whom when larger blooms occur. An immediate action plan may include initiation of defense mechanisms (e.g., floating barrage, posting of warning signs at the site, and warnings to the public in newspapers), providing information to the relevant authorities (e.g., medical officer of public health, veterinary centers, doctors) and direct information to local interest groups (e.g., kindergartens, water sport clubs and scouts).

There are no official safe-guard guidelines for the amount of toxic algae in bathing water but the local authorities (counties and/or municipalities) will put up signs at the waterfront warning the public that the water may contain harmful algae and that people are advised not to swim in or otherwise get in contact with the water. People are advised to get out of the water and avoid further contact, in the case that they cannot see their feet when wading in the littor-

ral zone. It is also advised not to let children play in the water and not to let pets drink the water.

There is also no official guideline for drinking water but the provisional guideline by WHO of 1 µg microcystin l⁻¹ (Chorus and Bartram 1999) is used when needed. Recent studies of the potential transport of toxins with surface waters that are infiltrated through sand and/or soil layers to the ground water have indicated that such a transport is possible (Christoffersen 2000 a and b, Christoffersen 2001).

Cyanobacterial toxins may accumulate in aquatic organisms, e.g. crustaceans, mussels, and fish which may impose a human risk problem (Chorus 2001). The Danish Veterinary and Food Administration control the designated coastal areas where commercial mussel (*Mytilus* and *Ostrea*) harvesting takes place. Harvesting and distribution of mussels is only allowed if analyses of water and mussels show no presence of algal toxins (all known types). If the concentrations are above the limits, immediate action will be taken to close the area concerned and all mussels collected will be destroyed. A closed area can only be re-opened if repeated tests ensure that the toxin concentration is yet again on an acceptable level. Information on the actual situation for mussel harvesters is placed on a website hosted by the Danish Veterinary and Food Administration (<http://www.uk.foedevarestyrelsen.dk/forside.htm>). The information can also be obtained from a service telephone line (automatic message) (Phone no +45 8728 1571).

Private mussel gatherers are encouraged to watch the media for any critical situations and have to take special care if collections take place outside the routinely monitored site.

Management

Denmark has implemented an Action Plan on the Aquatic Environment since 1987 and re-evaluated it in 1998 and 2004. Due to these actions it has been possible to reduce the nutrient loadings to many water bodies. As a national average the phosphorous loading has decreased from approx. 5.5 t year⁻¹ to approx. 2 t year⁻¹ since 1989 corresponding to an actual phosphorous content in inflow waters of around 0.15 mg l⁻¹. However, the water quality is not yet at desired levels in approximately two thirds of the Danish lakes studied (Jensen et al. 2004). Other measures such as biomanipulation, chemical treatments and aeration are also applied.

To ensure that information about toxic cyanobacterial blooms reach the authorities there is an informal information network which includes environmental administrative units as well as research institutions including the Danish Environmental Protection Agency, the Danish National Environmental Research Institute, the Royal Veterinary University, and the Danish Institute for Fisheries Research and University of Copenhagen. Information about actual bloom situations is provided by the counties through their web sites and a national overview is given by the Danish National Environmental Research Institute through the website <http://alger.dmu.dk>.

When massive blooms occur, the Bathing Water Instruction requires that the algal material is investigated, and that a risk assessment of the situation takes place. According to the Danish Bathing Water Instruction and the Guide to Control of Bathing Water the local authorities (counties and/or municipalities) are responsible for surveillance of the water quality at the bathing sites (lakes and coastal zones) which includes test for pathogenic bacteria, but cyanobacteria will also be considered when relevant. One of the counties (Sønderjylland) and the private research institution DHI Water & Environment host a web site (<http://www.badevand.dk>) with all relevant information on the bathing water quality in Denmark.

Toxic bloom situations are likely to occur every year in Denmark and often require immediate response by several authorities. This implies that access to information and relevant data as well as the actual measures to protect the public need to be coordinated. It has therefore

been suggested to implement an Algal Protocol (Kaas and Garde 2002) to support the local management efforts by compiling guidelines, existing data, outline official action plans, and provide contact networks and procedures for information of the public. It is recommended that the Protocol should be prepared in cooperation between the county, the local authorities, and the health inspector.

References

- Christoffersen, K. 1996. Ecological implications of cyanobacterial toxins in aquatic ecosystems. *Phycologia* 36 (6, suppl.): 42-50
- Christoffersen, K. 2000a. Are cyanobacteria a problem in Danish lakes? *Vand og Jord* 3: 104-109 (in Danish).
- Christoffersen, K. 2000b. Investigations of algae toxins from cyanobacteria and occurrence of heterotrophic bacteria in the infiltration plat at Tissø. Report to County of Vest Sjælland, pp. 22 (in Danish).
- Christoffersen, K. 2001. Investigations of microcystins in the infiltration plant at Arrenæs. Report to Københavns Vand, pp. 17 (in Danish).
- Christoffersen, K. and Olrik, K. 1996. Toxic algae in Esrum Sø and Esrum Å. *Vand og Jord* 1: 21-23 (in Danish).
- Christoffersen, K., Riemann, B., Klysner, A., & Søndergaard, M. 1993. Potential role of natural populations of zooplankton on plankton community structure in eutrophic lake water. *Limnology and Oceanography* 38: 561-573.
- Christoffersen, K., Lyck, S. and Winding, A. 2002. Microbial activity and bacterial community structure during degradation of microcystins. *Aquatic Microbial Ecology* 27: 125-136.
- Chorus, I. and J. Bartram, J. 1999. Toxic cyanobacteria in water. A guide to public health significance, monitoring and management. E&FN Spon, London.
- Chorus, I. 2001. (Ed.) Cyanotoxins – occurrence, causes consequences. Springer Publishers, Berlin, pp. 49-56.
- Henriksen, P. 2001. Toxic freshwater cyanobacteria in Denmark In: Chorus, I. (Ed.) Cyanotoxins – occurrence, causes consequences. Springer Publishers, Berlin, pp. 49-56.
- Henriksen, P. and Moestrup, Ø. 1997. Seasonal variations in microcystin content of Danish cyanobacteria. *Natural Toxins* 5: 99-106.
- Jensen, J.P., Søndergaard, M., Jeppesen, E., Lauridsen, T.L., Liboriussen, L., Landkildehus, F. and Sortkjær, L. 2004. Lakes 2003. NOVA 2003. Report no. 515 from Danish National Environmental Research Institute, pp. 88 (in Danish).
- Kaas, H. and Henriksen, P. 2000. Saxitoxins (PSP toxins) in Danish lakes. *Water Research* 34: 2089-2097.
- Kaas, K. and Garde, G. 2002. Handbook on toxic algae in bathing water. Miljøprojekt nr. 716 to the Danish Environmental Protection Agency, pp. 68 (in Danish).
- Sørensen, O. and Christoffersen, K. 1996. Algae toxins in lakes. *Vand og Jord* 1: 17-20 (in Danish).

Relevant websites

Danish Environmental Protection Agency
Danish National Environmental Research Institute
The Danish Veterinary and Food Administration
Ministry of Food, Agriculture and Fisheries
Danish Institute for Fisheries Research

<http://www.mst.dk>
<http://www.dmu.dk>
<http://www.uk.foedevarestyrelsen.dk/forside.htm>
<http://www.fvm.dk/>
<http://www.dfum.dk/dk/index.asp>

FINLAND:

The Network of Monitoring Cyanobacteria and their Toxins in (1998–2004)

Jarkko RAPALA¹, Johanna ISSAKAINEN², Jaana SALMELA³, Kalle HOPPU³, Kirsti LAHTI⁴ and Liisa LEPISTÖ²

¹ National Product Control Agency for Welfare and Health (STTV), P.O.Box 210, FIN-00531 Helsinki, Finland.
E-mail: jarkko.rapala@sttv.fi

² Finnish Environment Institute (SYKE), P.O.Box 140, FIN-00251 Helsinki, Finland.

³ Poison Information Centre, Helsinki University Hospital, P.O.Box 340, FIN-00029 Helsinki, Finland.

⁴ The Water Protection Association of the River Vantaa and Helsinki Region. Asemapäällikönkatu 12 C, FIN-00520 Helsinki, Finland.

Systematic monitoring of cyanobacteria

Launching of the new monitoring system in 1998

Data on nuisance algae have been collected in Finland by authorities and private citizens since 1967. The respective species have traditionally been determined microscopically, and the results recorded in the database of harmful algae provided by the national environmental administration. However, until exceptionally extensive and long-lasting cyanobacterial blooming in the summer 1997 the data were not collected systematically.

In summer 1998 a new nation-wide observation system was launched to monitor cyanobacteria throughout the country. The aim was to provide up-to-date data about the cyanobacterial situation and information about their spatial and temporal variation during the summer months. Since then, the monitoring programme has been a joint-venture of local and regional authorities and the research institutes Finnish Environment Institute (SYKE) and Finnish Institute of Marine Research (FIMR).

Observation sites and methods

Observation sites have been selected by regional environment centres to represent various water types: eutrophic waters with frequent cyanobacterial mass occurrences, mesotrophic waters with less frequent water blooms and oligotrophic waters with no or few observations of nuisance algae or cyanobacteria. Several observation sites are situated in the vicinity of cities or public beaches. Minor changes in the number and place of the sites have occurred during the years. In summer 2004 the observation net included altogether 328 sites, of which 262 were in lakes and rivers and 66 in coastal waters.

The cyanobacterial abundance is monitored weekly in June–August from the pre-determined sites. Municipal health or environmental authorities or volunteer citizens visit the sites by Monday–Tuesday, and estimate the cyanobacterial abundance by visually examining the water area from the shore. In order to harmonize the estimates the observers have received prior pre-training from SYKE. The observations are classified into four classes (0-3), and the term 'algae' is used to encompass both algae and cyanobacteria:

0: No algae. No algae on the water surface or on the shore line. The Secchi depth is normal.

1: Observed. Greenish flakes detected in the water or when taken into a transparent container, or narrow stripes on the shore. The Secchi depth is reduced by algae.

2: Abundant. The water is clearly coloured by algae, small surface scums or cyanobacterial mass on the beach are detected.

3: Very abundant. Wide and heavy surface scums or thick aggregates of cyanobacteria are detected on the shore.

Collection and reporting of the weekly data

The regional environment centres (n=13) collect the observation data and send them to SYKE by Wednesday. The report is prepared at SYKE on Wednesday a.m. The report consists of four different parts:

1. The summary which is a short one-page description of the weekly situation.
2. The map which shows by colour codes the situation at each observation site (Fig. 1).
3. The cyanobacterial "abundance barometer" which allows comparison of the current situation to previous years (Fig. 2). The barometer is calculated as the balanced mean of the observation sites and cyanobacterial abundance.
4. Also descriptions of regional situations may be included in the report.

The report is published each Wednesday at noon or early in the afternoon at the Internet pages of SYKE (<http://www.environment.fi/SYKE>) and by giving a press release. Data on the Baltic Sea areas is also published at the Internet pages of Finnish Institute of Marine Research (<http://www.fimr.fi>).

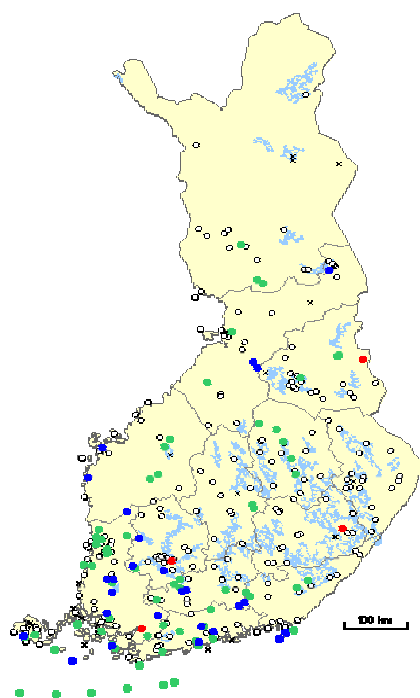


Fig. 1. Example of the weekly map of cyanobacterial abundance.

○ not detected, ● detected,
● abundant, ● very abundant

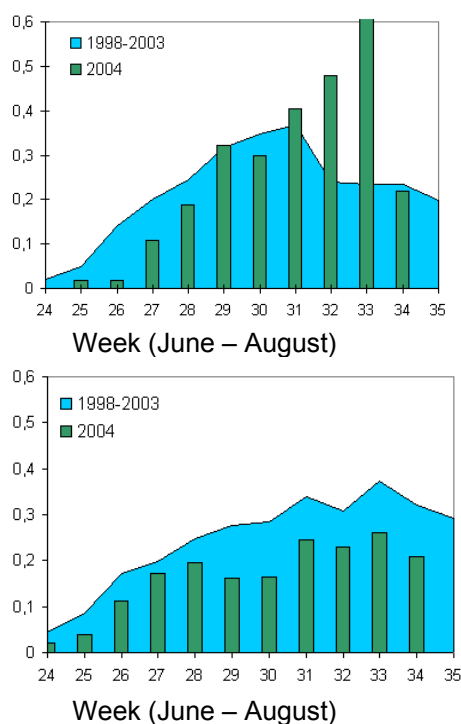


Fig. 2. The cyanobacterial "abundance barometer" in 2004 at the coastal Baltic Sea area in Finland (upper figure) and in the freshwater sites (lower figure).

If cyanobacteria are estimated as abundant or very abundant a water sample is taken and sent to SYKE or regional environment centres for further microscopical investigation.

tion. The species composition of the cyanobacterial mass occurrences is recorded in the national database of harmful algae.

In addition, in the open Baltic Sea areas (see Fig. 1) cyanobacteria are monitored by using unattended recording, sampling on passenger ferries, and by coastguard from the air. Also satellite images are used in estimating the size of the surface blooms.

Recent research projects and monitoring of toxins

More systematic monitoring of microcystins was started in Finland in the early 1990's. Since then, methods have been taken into use to detect also anatoxin-a, PSP-toxins and anatoxin-a(S). However, the monitoring of toxins has been totally dependent on research funding.

Recent research funding on the monitoring of cyanobacteria and their toxins has included e.g. research on the removal of cyanobacteria and toxins at operating drinking water treatment plants (funding from National Technology Agency through The Finnish Research Programme on Environmental Health in 1998-2001, The Ministry of Environment and The Ministry of Agriculture and Forestry) and research on cyanobacteria and associated health effects in recreational waters (funding from the Academy of Finland, Research Programme Microbes and Man, 2003-2005).

The on-going research on recreational waters also includes monitoring of toxins in cyanobacterial water blooms. From the monitoring network described above, samples have been collected from sites when the abundance of cyanobacteria has been estimated as high or very high. In the preliminary screening in 2002, approximately 50 and in 2003 approximately 130 bloom samples were studied for the occurrence and concentrations of microcystins, anatoxin-a, anatoxin-a(S), PSP-toxins and lipopolysaccharide endotoxins. Occasionally the toxin concentrations in the blooms were very high: microcystins 5 mg l⁻¹, saxitoxin 1 mg l⁻¹, anatoxin-a and its degradation products 0.87 mg l⁻¹ and endotoxins 67x10³ EU ml⁻¹ (Lepistö et al. 2005a, Rapala et al. 2005 and unpublished data). Such an intensive screening on cyanobacterial toxins was previously conducted 20 years ago, in 1985-1986 (Sivonen et al. 1990). Since that time, more sensitive methods have become available.

On the basis of research and the long experience in monitoring of cyanobacteria, a national monitoring scheme has been proposed (Fig. 3). The scheme presented is suitable for risk management purposes as well in recreational waters as in raw waters of drinking water treatment plants.

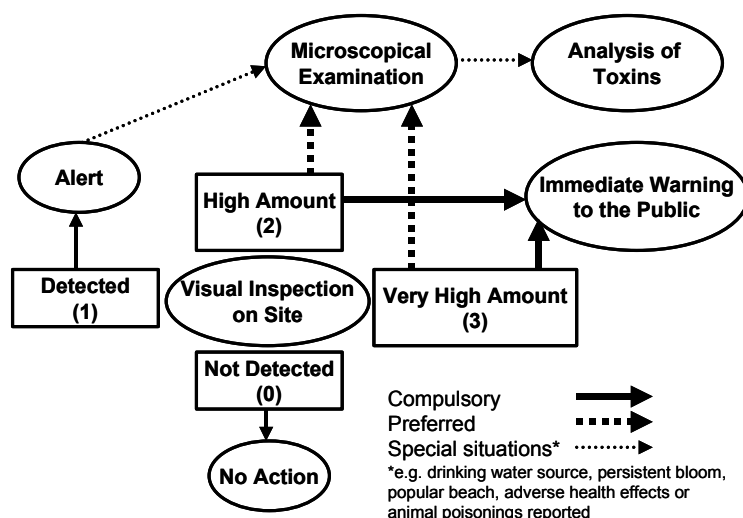


Fig. 3. The proposed monitoring scheme for cyanobacteria.

The proposed monitoring scheme for recreational waters is largely based on visual monitoring on the site, as described in chapter 1. In the monitoring of raw water (in particular, the incoming water to the treatment plant) and treated drinking water, microscopical examination for cyanobacteria is preferred.

If the water bloom is persistent or if cyanobacterial cells are detected in the treated drinking water, microcystin analysis using detection methods which are as rapid as possible, such as ELISA or protein phosphatase inhibition assay, are advised. Depending on the species composition other toxins may also be analysed. It is not necessary to analyse cyanobacterial neurotoxins if only *Microcystis* spp. are detected, but in the case of *Anabena* spp. or *Planktothrix* spp. the need for neurotoxin analyses should be considered.

The rationales for such approaches include e.g.:

- In the monitoring of recreational waters the importance of immediate warning of water users of the risk caused by cyanobacteria should be the main target. This target can be achieved only by rapid and thus relatively simple means.
- In the monitoring of raw water and treated drinking water microscopical examination reveals if cyanobacteria are present in the incoming water, and if the cells break through the treatment process. Visual observation of the raw water source does not necessarily reveal the presence of cyanobacteria, since e.g. *Planktothrix* may occur in masses in the deeper water layers.
- According to research results, the majority of cyanobacterial occurrences are toxic. Thus, the presence of cyanobacteria alone is a strong indication of potential health hazard.
- Analysis of specific toxins from bathing water samples is too time consuming in order to meet the need of rapid assessment and action. If e.g. microcystins were analysed from the water samples, there might still be other hazardous compounds present in the water, because cyanobacteria produce a variety of different toxins and yet still unidentified compounds.
- Microscopical examination of raw and treated drinking water can easily be included in the regular monitoring at drinking water treatment plants. Cyanobacterial cells are easy to identify under microscope. Special expertise is needed for identification to species level, but this is not necessary in routine monitoring.
- The proposed monitoring scheme is based on scientific research and risk assessment.
- The cost of such monitoring is relatively low.

Other actions

Regular monitoring of cyanobacteria in selected lakes

Selected lakes, those of special interest, have been routinely monitored for the presence of cyanobacteria during summer months by microscopically identifying cyanobacteria and counting their biomass. If the resources have allowed, toxins (mainly microcystins) have been included in the analyses. In most cases, the results show that cyanobacterial biomass and toxin concentrations correlate well (e.g. Fig. 4). Also this implies that visual or microscopical examination of water is able to give a sufficiently reliable estimate of the hazard posed by cyanobacteria.

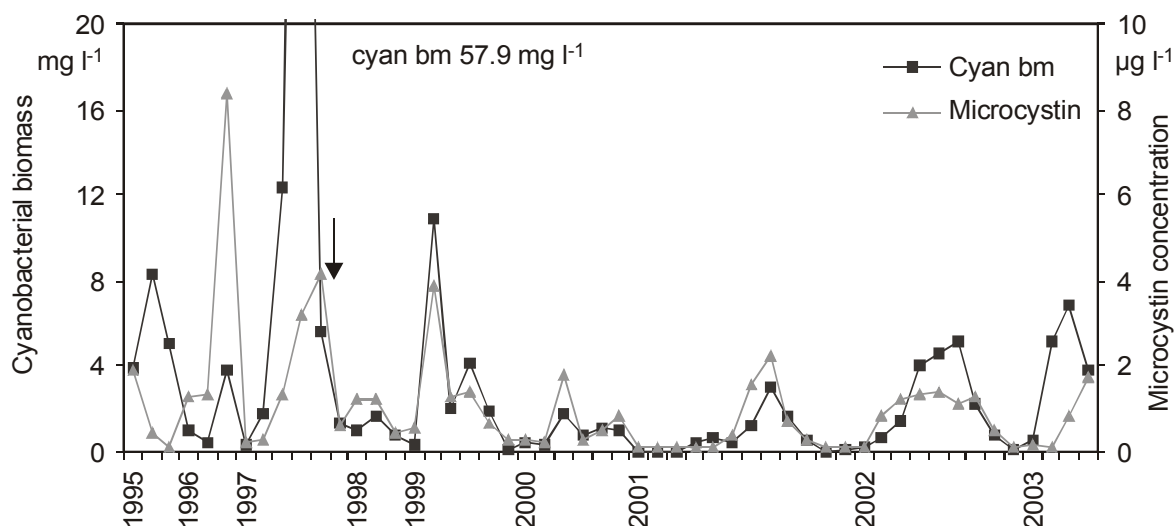


Fig. 4. Long-term monitoring since 1995 of cyanobacterial biomass (cyan bm) and total microcystin concentration in the 0–2 m water layer in Lake Tuusulanjärvi, Southern Finland (Lepistö et al. 2005b)

Telephone services

During summer months (June–August) since 1998 there has been at SYKE a continuous (Monday–Friday, 8.00–16.00) telephone service ("Citizens Algaline") from which the public can get information about cyanobacteria. This service was launched after the heavy and long-lasting cyanobacterial water blooms in 1997. On the average 350 telephone calls have been received each summer. In the dry and hot summer 2002 altogether over 670 calls were recorded. A similar telephone service ("Algaline"), mainly for the coastal areas, is also provided by Finnish Institute of Marine Research.

The Poison Information Centre at Helsinki University Hospital provides a 24-hour telephone service, which gives medical advice for persons in acute poisonings of any kind. Yearly, approximately 100 telephone calls have been received concerning adverse health effects suspected to be caused by cyanobacteria. In 1997 there were more than 350 contacts concerning cyanobacteria. In a preliminary study in 1999 the reports to the Poison Information Centre were connected to the data on the occurrence of cyanobacteria obtained through the nationwide observation network described above. Several symptoms were reported, and simultaneous dermal and respiratory exposure to cyanobacteria appeared to cause the most severe symptoms (Fig. 5). According to the results in 2003, *Anabaena lemmermannii* was the main dominant blooming cyanobacterium in Finland connected to the health reports and to microcystins and neurotoxins (Lepistö et al. 2005a). In three cases of the adverse health effects of children (fever, eye irritation, abdominal pain and skin rash) the site of exposure was directly associated with samples that contained relatively high (33–530 $\mu\text{g l}^{-1}$) saxitoxin concentrations (Rapala et al. 2005).

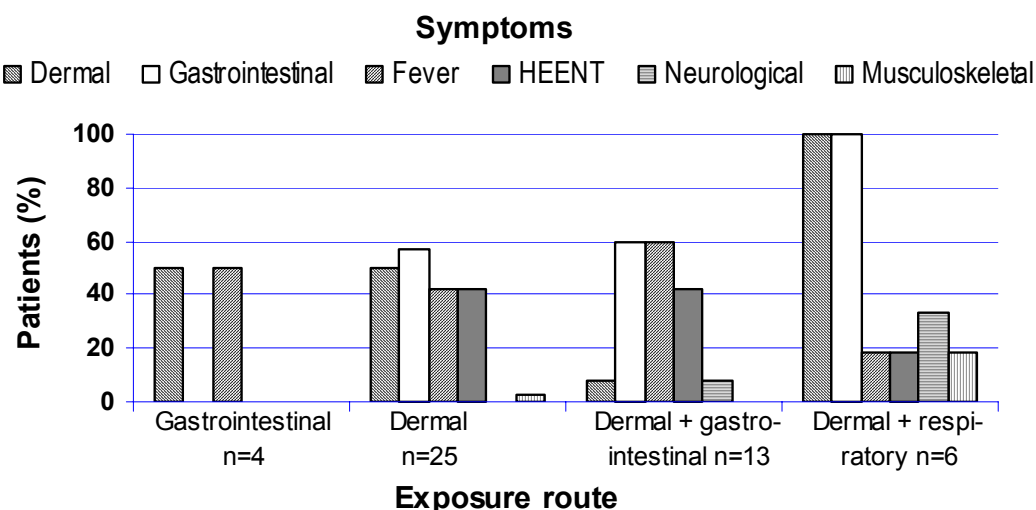


Fig. 5. Dermal, gastrointestinal, fever, HEENT (head, ear, eyes, nose and throat), neurological and musculoskeletal symptoms reported by patients in Finland after exposure to cyanobacteria. The symptoms are divided into groups on the basis of the exposure route (gastrointestinal, dermal, simultaneous dermal and gastrointestinal and simultaneous dermal and respiratory exposure) (Salmela et. al. 2001).

Monitoring of public beaches

Since the late 1980's the local health authorities have visually monitored cyanobacteria at the public beaches. If cyanobacteria are detected the health inspectors have been advised to immediately place warning signs by the shore. Also beach supervisors are well aware of cyanobacteria, and they readily tend to notify local health authorities if cyanobacteria are detected in the water.

Conclusions

Visual monitoring of cyanobacteria in water bodies allows a cost-effective, rapid and sufficiently reliable warning of water users for risk management purposes. In Finland, a cost-effective monitoring network has been developed. It has arisen as a consequence of public awareness and interest, actions taken by the authorities and targeted national research funding. The network produces information for health authorities for decision making, information on the ecological condition of the water bodies, and it also meets the needs of informing the public. Health authorities have been given guidelines in the late 1980's to monitor cyanobacteria in public beaches and place warning signs if cyanobacteria have been detected. The major disadvantage is that toxin analyses and methodological development has thus far been exclusively based on temporary research projects with no guarantee of continuity.

References

- Lepistö, L., Rapala, J., Lyra, C., Berg, K. A., Erkomaa, K. and Issakainen, J. 2005a. Occurrence and toxicity of cyanobacterial blooms dominated by *Anabaena lemmermannii* P. Richter and *Aphanizomenon* spp. in boreal lakes. Arch Hydrobiol – Algological Studies (in press).
- Lepistö, L., Lahti, K., Rapala, J., Villa, L. and Sammalkorpi, I. 2005b. Effects of fish removal on biomass of cyanobacteria and their toxicity in Lake Tuusulanjärvi, Southern Finland. Verh. Internat. Verein. Limnol. (in press).
- Rapala, J., Robertson, A., Negri, A. P., Berg, K. A., Tuomi, P., Lyra, C., Erkomaa, K., Lahti, K., Hoppu, K. and Lepistö, L. 2005. First report of saxitoxin and possible associated health effects in lakes of Finland. Environ. Toxicol. (in press).
- Salmela, J., Lahti, K. and Hoppu, K. 2001. Sinilevöpitoinen saunavesi voi aiheuttaa oireita ihmisille. Finnish Medical Journal 56(27-29):2891-95 (in Finnish). Also published as an abstract: Hoppu, K., Salmela, J. and Lahti, K. 2002. High risk for symptoms from use of water contaminated with cyanobacteriae in sauna. J Toxicol – Clin Toxicol 40(3):309-10.
- Sivonen, K., Niemelä, S. I., Niemi, R. M., Lepistö, L., Luoma, T. H. and Räsänen, L. A. 1990. Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal waters. Hydrobiol. 190:267-275.

FRANCE:

The Occurrence of Cyanobacteria in Management and Regulatory Approaches

Luc BRIENT

U.M.R. Ecobio CNRS 6553, Université de Rennes I, 35042 Rennes, France. E-mail : Luc.brient@univ-rennes1.fr

Awareness of the issue of the presence of potentially toxic cyanobacteria started to develop in France in 1995 following work in the Brittany area¹ and in 1998 in all of the French territory². Given the extent of the proliferations – nearly 70 % of the freshwater lakes and reservoirs examined contained hepatotoxins – the French authorities, since 2003, took various measures for the management of water bodies used for swimming, watersports and for the production of drinking water. Since 1999 some local communities have already applied the WHO decision tree³ for freshwater recreational sites.

LEGISLATION

Drinking-water

Since 2001 a decree⁴ set the maximum limit at 1 µg/L of microcystin-LR in drinking water to be applicable from December 2003. It does not detail actions to be taken in the event of exceedence. Microcystin analyses is required in treated water in the event of algal proliferation in the raw water. There is no limit set for this parameter in raw water.

Freshwater recreational sites

Following a recommendation by the public health services, in 2003^{5,6,7} a decision diagram was set up for the managers of the sites in collaboration with the health services responsible for routine surveillance. In water bodies with persistently high algal concentrations, a stricter monitoring system is implemented which is based on visual and on microscopic observations. Three levels of management responses are identified in reaction to levels of cyanobacteria:

- Level 1: At < 20 000 cells/mL ± 20 %, of which the majority are cyanobacteria, recreational activities are allowed to continue and users are informed by posters on site. There is however no standardised information. Monitoring is intensified to fortnightly sampling, counting and species identification.
- Level 2: at between 20 000 and 100 000 cells/mL: microcystins are analysed. If the concentration LR-equivalents is > 25 µg/L swimming is prohibited. The only watersports allowed are those with minimum contact like rowing. There are however no restrictions at all for professional athletes.
- Level 3: where cyanobacterial scum appears in bathing areas, all activities in the water are prohibited.

Algicide treatments

Authorization to use an algicide must be requested by the departmental prefect and national authorities for preventive use. Its use is prohibited at the time of the proliferation of cyanobacteria⁸.

MONITORING AND MANAGEMENT

Drinking water resources

There is currently no official protocol for the monitoring of water bodies for cyanobacteria. Nevertheless, as the majority of the resources are liable to algal proliferation, there is an ongoing awareness of eutrophication potential. Monitoring is based on monthly chlorophyll-*a* analyses, counts and identification of the dominant algae. Intervals are intensified to weekly as soon as cyanobacteria (5000 to 10000 cells/mL) appear. A recent interregional study¹⁰ in France recommended that the weekly frequency could be fortnightly for high performing drinking-water treatment plants (i.e. ozonation and activated carbon). The health risk from cyanobacteria in raw water used for the production of drinking water is managed either by the installation of activated carbon (filters) and /or ozonation in the water treatment systems, or by interrupting the use of the affected raw water source.

Since 2004 the following monitoring strategy is being tested: the manager of the drinking-water supply informs the health services when a twice-weekly visual inspection reveals a marked change in the water environment or a pH > 9. If the cyanobacteria levels are > 5000 cells/mL the treatment level is increased and there is a check for the presence of microcystins in the raw water. If these are above 1 µg/L, microcystins are also analysed in the treated drinking water. The controls include algal cell counting. If microcystin-LR concentrations in treated water is > 1 µg/L, water use is restricted – in particular it must not be used for patients undergoing haemodialysis – and the public is informed by the local managers and the health authorities. Decisions taken include the distribution of bottled water or the use of alternative water resources.

Freshwater recreational sites

Information on the number of cyanobacteria cells for the public is published on the Internet within 5 days by the departmental health service. Most of the algal identification results are available within 48 hours, and the results of microcystin analyses within 72 hours. The 3 recommended analytical methods are the ELISA, PP2A and HPLC /UV or /MS. Currently there are no other routine checks for cyanotoxins. The time required for the analyses is still a difficulty for the managers and the availability of a probe with fluorescence specific to phyco-cyanin could make it possible to deal with the risks inherent to the appearance of cyanobacteria more quickly.

There are currently no centralized data in France relating to closure of sites in reaction to the levels of cyanobacterial contamination. On the basis of monitoring carried out in the Western part of France on more than 40 water bodies, nearly 25 % of the sites were subject to partial or total closure for all the summer period. The first closures in 2000 were badly received by water owners and made it necessary to supply specific information on the potential risks caused by cyanobacteria. The public was informed by warning notices and also by the press, local television and radio. At the same time, cases of dermatitis which may have been caused by cyanobacteria were reported by swimmers and watersports professionals. The death of twenty dogs at a site with a high level of (benthic blue-green) algae on a river led the health services to put up panels forbidding the access of dogs to the river. Another example of the death of a dog was associated with the presence of a molecule similar to anatoxin-a.⁷

The total closure of certain rivers or lakes has had an important financial and economic impact on water sports. Some of these, such as windsurfing, were transferred to sites as yet unaffected by cyanobacteria. The reopening of some lakes frequented by large numbers of people during the summer showed that these closures had no lasting impact in discouraging the public from taking up their usual watersports activities again.

These closures or potential closures have made it possible to accelerate the implementation of actions to decrease the eutrophication of freshwater: for the urban areas this is by in-

creased introduction of dephosphorization systems in wastewater treatment as well as by increasing the share of separate drainage networks for sewerage and rain water. In the catchment areas it is by management of mineral and organic fertilization.

Freshwater shellfish

There is no information available concerning freshwater shellfish contaminated by cyanobacterial toxins and their consumption.

THE SCIENTIFIC INTEREST GROUP

The scientists concerned are collaborating in an organization called the Scientific Interest Group (GRISCYA) which brings together 20 national scientific institutions from the various fields of research relating to cyanobacteria. The French Agency for the Safety of Food with respect to Health (AFASSA) has set up a group of national and international experts on cyanobacteria. Some difficulties with the application of the currently implemented methods and practices have come up and are currently being discussed, in particular the following issues: sampling for water used for swimming and water sports, definition of 'proliferation', thresholds in terms of cell numbers for allowing preventive algicide treatment, the expression of the results in microcystin LR or LR-equivalents, and the various methods of analyses which are currently not standardized.

The current monitoring of a single sampling site in a water body does not give a good overview of the extent of the cyanobacterial problem. A substantial improvement of risk management would come from using methods which estimate spatial variations in algal biomass. The recent development of a probe specific to detect phycocyanin by fluorescence is a step in this direction. This method would replace the time consuming and expensive counting of cells by relating the biomass of cyanobacteria to the concentration of phycocyanin.

The management of cyanobacteria in France falls within the scope of the E.U. water framework directive which puts forward a good ecological state of the water resources for 2015¹⁵

REFERENCES

- 1 VEZIE C., BRIENT L., SIVONEN K. et al., Occurrence of microcystin-containing Cyanobacterial blooms in freshwaters of Brittany (France), *Arch. Hydrobiol.*, 1997, vol. 139, n°3, pp. 401-413.
- 2 SARAZIN, G., C. QUIBLIER-LLOBÉRAS, G. BERTRU, L. BRIENT, C. VEZIE, C. BERNARD, A. COUTÉ, M.C. HENNION, C. ROBILLOT & N.TANDEAU DE MARSAC (2001) Première évaluation du risque toxique lié aux cyanobactéries toxiques d'eau douce en France : Le programme " EFFLOCYA ". *Revue des Sciences de l'Eau*.15/1, 315-326
- 3 CHORUS I., BARTRAM J. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*, published on the behalf of the World Health Organization, London, Spon/Chapman&Hall, 1999, 416 p.
- 4 Décret n°2001-1220 du 20 décembre 2001 relatif aux eaux destinées à la consommation humaine, à l'exclusion des eaux minérales naturelles (transposition en droit français de la directive européenne 98-83/CE).
- 5 Avis du Conseil Supérieur d'Hygiène Publique de France : recommandations pour la gestion des situations de contamination d'eaux de baignades et de zones de loisirs nautiques par prolifération de cyanobactéries, 6 mai 2003.
- 6 Circulaire DGS 7a n° 2033-270 du 4 juin 2003, relative aux modalités d'évaluation et de gestion des risques sanitaires face à des proliférations de micro algues (cyanobactéries) dans les eaux de zones de baignades et de loisirs nautiques.

- 7 GUGGER M., LENOIR S., BERGER C., LEDREUX A., DRUART J.C., HUMBERT J.F.,
GUETTE C., BERNARD C. First identification in a French river of the benthic cyanoact-
erium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis.(*In*
Press) 2005
- 8 <http://www.afssa.fr/Ftp/Afssa/24119-24120.pdf>
- 9 <http://bretagne.sante.gouv.fr/1024.html>
- 10 http://ecobio.univ-rennes1.fr/Fiches_perso/Banque/publi1_LBrient.pdf
- 11 Van HULLEBUSCH E., CHATENET P., DELUCHAT V., CHAZAL P., FROISSARD D.,
BOTINEAU M., GHESTEM A.,BAUDU M. (2003), Copper accumulation in a reservoir
ecosystem following copper sulfate treatment (St Germain les Belles, France), *Water Air
Sol Pollution*, 150, 3-22.
- 12 Latour-Duris, D., Giraudet, H. & J.-L. Berthon (2002). Effet d'une expérimentation de bras-
sage artificiel épilimnique par aération sur les poussées cyanobactériennes dans la rete-
nue hypereutrophe de Grangent (France). *Rev. Sci. Eau* 15 (1) : 289-299.
- 13 Briand J.-F., Robillot C., Quiblier-Lloberas C., Humbert J.-F., Couté A. & Bernard C.
(2002). Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms
in a shallow pond in France. *Water Res.* 36 (13) 3314-3322.
- 14 DELPHINE LATOUR, ODILE SABIDO, MARIE-JOSE SALENSON and HERVE GIRAU-
DET (2004) Dynamics and metabolic activity of the benthic cyanobacterium *Microcystis*
aeruginosa in the Grangent reservoir (France) *Journal of Plankton research* vol 26, N°6, 1-
8
- 15 <http://europa.eu.int/comm/environment/water/index.html>

GERMANY:

APPROACHES TO ASSESSING AND MANAGING THE CYANOTOXIN RISK

Ingrid Chorus

Dept. for Drinking-water Hygiene, Federal Environmental Agency, 14195 Berlin, Corrensplatz 1, Germany
E-mail: ingrid.chorus@uba.de

INTRODUCTION

Approximately 1/3 of Germany's drinking-water supply is based on surface water, i.e. from rivers and reservoirs, either through direct use or indirectly as bank filtrate. Additionally, a number of water supplies are influenced by surface water through artificial groundwater re-charge. Recreational use of inland freshwater resources is very popular, and in face of Germany's high population density, waterbodies are generally intensively frequented for swimming, boating, camping and fishing.

With the exception of some drinking-water reservoirs in mountain regions, few water-bodies have escaped anthropogenic eutrophication, although in Germany the general trend during the last 2-3 decades has been a reduction of nutrient loading: particularly phosphorus loads have been reduced to 1/3 between 1985 and 2000 through improved sewage treatment (Behrend et al. 2000). Mass developments of cyanobacteria are thus widespread problem.

OCCURRENCE OF TOXIC CYANOBACTERIA

Although phytoplankton data exist for many water-bodies, they are fragmentary and not compiled on a national level. Cyanobacterial occurrence in Germany can thus only be inferred from some surveys as well as an understanding of the literature and ongoing investigations. This suggests three toxic taxa to be the relevant most frequently: In shallow eutrophic lakes and rivers *Planktothrix agardhii* is very common, often showing seasonal succession patterns with other filamentous cyanobacteria. *Planktothrix rubescens* is found in deeper reservoirs, where it concentrates in deeper water layers during thermal stratification in summer. *Microcystis* spp., often forming surface scums, are also widespread, sometimes associated with *Aphanizomenon* spp., whereas massive occurrence of *Anabaena* spp. seems to be less common.

Two early studies provided evidence of cyanobacterial toxicity in Germany (Kalbe et al. 1976; Henning and Kohl 1981). A large survey conducted from 1995-1997 included more than 600 samples from a total of 133 water-bodies (Fastner et al. 1999 and 2001, Bumke-Vogt et al. 1999 and Chorus et al. 2001). Key results were:

- It is unlikely to find natural populations of *Planktothrix agardhii* or *P. rubescens* without microcystins or measurable toxicity to primary rat hepatocytes.
- Microcystin was detected in 97 % of the samples dominated by *Microcystis* spp, and 88 % of them showed toxicity to primary rat hepatocytes. Median microcystin concentrations in dry matter from > 400 samples were around 300 µg/g, with maxima ranging up to 6000 µg/g.
- Concentrations in relation to water volume outside of scums in most samples amounted to only a few µg per litre, but several 100 µg/L were also found with dense populations of *Planktothrix agardhii*.

- As the concentration of chlorophyll-a in conjunction with rough estimates of the share of cyanobacteria in total phytoplankton is a useful measure of cyanobacterial occurrence, this survey included an investigation of the ratio of microcystin to chlorophyll-a. Median ratios were 0.13 for samples dominated by *Microcystis* spp., 0.3 for dominance of *P. agardhii* and 0.4 for dominance of *P. rubescens*. However, maximal ratios reached 3 µg of microcystin per µg of chlorophyll-a.
- Scum concentrations were found in the range of 1000 and up to 10000 µg/L
- In contrast, Anatoxina and Saxitoxins were found in less than ¼ of the samples and *water-bodies investigated*

The occurrence of cylindrospermopsin was first investigated in Germany in 2000. Results identified this toxin for the first time in Europe with concentrations ranging up to 18 µg per g dry weight in field samples. (Fastner *et al.* 2003). *Cylindrospermopsis raciborskii*, however, has already been observed in Germany in 1990 and from then on has regularly contributed to cyanobacterial biomass with up to 8 mm³/L (biovolume) in some lakes (Wiedner *et al.* 2002).

REGULATORY APPROACHES

Awareness of a cyanotoxin risk in Germany emerged in 1991, triggered by international publications, particularly from the UK National Rivers Authority after army cadets exposed to toxic *Microcystis* through swimming and canoe training had become ill with atypical pneumonia. It was quickly evident that the widespread prevalence of cyanobacteria in Germany necessitated attention to the issue. Initial assessments suggested that risks for human health, potentially even acute, would particularly arise from recreational exposure to scums and to high densities of dispersed cells. The federal government therefore published a recommendation in 1992 (Bundesgesundheitsblatt 1992), designed to supplement the monitoring programmes following the EU Bathing-water Directive (EU 1976). However, this recommendation received fairly little attention, probably because of a lack of widespread discussion, awareness and participation in its development. This situation changed as results from the large survey (see above) emerged and were intensively communicated to audiences in public health as well as in research. A revised publication of this guidance document 5 years later (Bundesgesundheitsblatt 1997) then triggered intensive discussion, including the (justified) request for improved state participation in the development of such guidance.

This is important in the federal structure of Germany, in which water management as well as public health surveillance is largely a state responsibility. The role of the federal government includes responsibility for the legal water management framework as well as compilation of reports to the European Union on compliance to the EU directives on drinking water and bathing water from each of the 16 states ("Länder"). Whereas implementation of the EU Bathing Water Directive is totally up to the states, roles are shared for the implementation of the EU Drinking-water Directive: the federal government revised the German Drinking-Water Ordinance for compliance to the EU-Directive, the states representation in the Upper House of Parliament confirmed it, and the states are responsible for monitoring and surveillance.

Drinking-water

For chemicals, the approach taken by the German Drinking-water Ordinance is broad and risk-based by stating that beyond the parameters listed in the articles and annexes, drinking-water should contain no substances in concentrations that may be harmful to human health. For cyanotoxins, the WHO Guideline value provides a clear criterion for assessing potentially harmful microcystin concentrations. The availability of this Guideline as basis for setting national targets has thus proven to be very valuable.

The ordinance also defines an approach to process control by stating that for collecting, abstracting, treating and distributing drinking-water, the generally acknowledged technical rules and standards should be met. For surface water sources, the German technical standards and rules require treatment (including filtration) and disinfection. Most waterworks which depend on eutrophic surface water either use effective close-to-natural treatment methods such as bank filtration or have installed advanced treatment (ozone, GAC) due to problems with other contaminants such as high DOC and pesticides. Emerging international research results as well as pilot studies in Germany (Chorus, ed., 2001) indicate that such treatment is effective in removing cyanobacteria and cyanotoxins. Last but not least, part of good practice for surface water supplies is the regular monitoring of phytoplankton population development in the waterbody. Thus, a waterworks director who neglects cyanobacterial risk assessment and management would stand little chance of winning a court case charging him with this negligence.

On this specific legal and technical background for the drinking-water supply situation in Germany, a specific parametric value for cyanotoxins was not perceived as adequate. The advantage of such an approach is its flexibility in reacting both to emerging new evidence (such as the recent discovery of cylindrospermospin in German water-bodies) and to site-specific issues. However, refraining from explicit regulations requires particular attention to maintaining and further developing the awareness of the cyanotoxin hazard as well as the provision of effective guidance for situation-specific hazard analysis and risk assessment. The Federal Environmental Agency is therefore currently targeting the establishment of a national reference centre for this purpose.

Bathing-water

The 2003 revision of the “Recommendation for the Protection of Bathers from Cyanobacterial Toxins” was developed in a joint working-group of the federal and the state governments. Its legal status is not mandatory, but it defines good practice and in a court case would serve as reference for expert opinion. Many of the states are currently developing institutional structures and expertise for its implementation.

This structured surveillance scheme endeavours to focus efforts on situation in which a health risk is likely by following three steps: (i) visual inspection and assessment, taking ecological characteristics into account, (ii) assessment of cyanobacterial occurrence, and (iii) microcystin analyses (Figure 1). Results of steps 1 or steps 1 and 2 may suffice as basis for either excluding a risk or taking preliminary measures to protect public health. The relevance of step 3 is particularly to avoid undue restrictions of recreational use. It is explicitly pointed out that if institutional capacity and competence is available for all 3 steps, this sequence is likely to be most efficient, but there is no need to undertake step 2 before step 3 if e.g. competence for microscopy is lacking whereas access to an immunoassay for rapid microcystin screening may be more readily available. The scheme should therefore be adapted to local needs and options.

The recommendation gives 4 pages of background information for risk assessment, including evidence of health impact, an explanation for non-experts of cyanobacterial seasonality, and the variability of surface scum occurrence and of cellular toxin content. The particular relevance of microcystins in relation to other cyanotoxins is explained with its widespread occurrence and chronic toxicity. It is emphasised that a sound ecological understanding of the phytoplankton in the water-bodies is invaluable for assessing the risk of cyanobacterial proliferation and predicting occurrence, and collaboration between health and environmental authorities is therefore recommended. The recommendation then proceeds to describe the three steps shown in Figure 1, including the time and equipment needed and recommendations for methods.

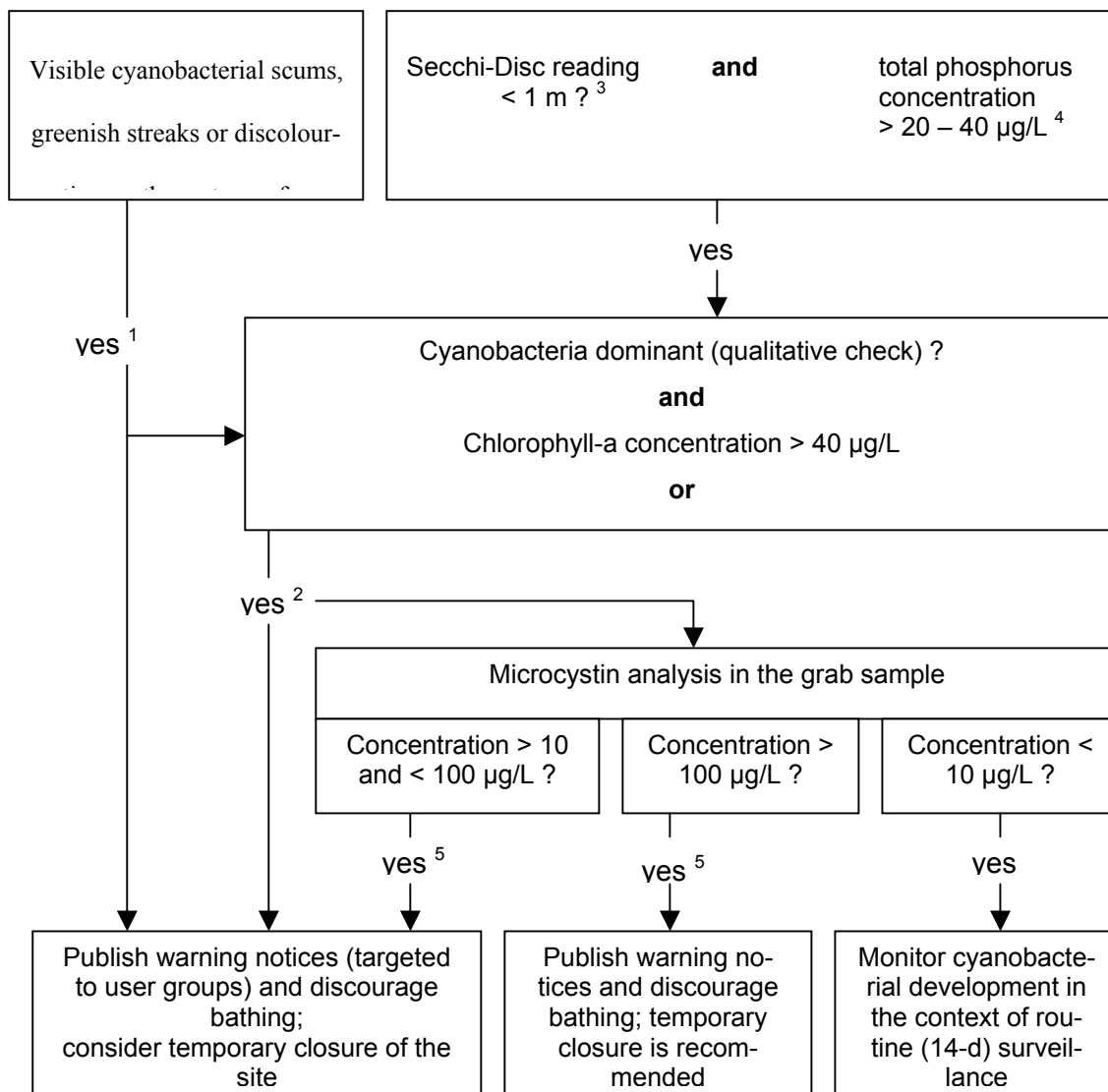


Figure 1: Scheme for surveillance of cyanobacteria and microcystins in bathing water (translated from Bundesgesundheitsblatt 2003)

Note: This links into monitoring at 14-day intervals following the EU Bathing-water Directive (EC-Directive 1977)

- ¹ direct warning if the water-body is well understood and cyanobacterial mass development appears to be likely
- ² depending on the assessment of the situation, proceed directly to warning and intensify observation of the site
- ³ applicable in shallow water-bodies; colony-forming species can also form blooms at transparencies of up to approximately 2 m
- ⁴ in thermally stratified and particularly in large water-bodies, cyanobacteria may dominate at > 20 µg/L (and sometimes even less); in small and turbulent water-bodies, more likely at > 30-40 µg/L
- ⁵ intensify frequency of observation of cyanobacterial development.

Step 1: Visual inspection and assessment of the site, and assessment of the water-body's capacity for mass developments of cyanobacteria

Scums or Secchi-disc readings < 1 trigger taking samples for microscopy. It is emphasised that microscopical assessment of whether or not these phenomena are caused by cyanobacteria can be performed in 5-10 minutes, and that professional staff

familiar with microscopy can easily learn to recognise cyanobacteria down to the genus level, which is sufficient for a preliminary risk assessment. If microscopy confirms cyanobacterial mass proliferation, the public should be immediately informed on site and through the media.

Furthermore, experienced site inspection and sampling staff, particularly if familiar with the water-body, can differentiate between cyanobacterial scums and other green surface layers (such as *Lemna minor*) already when performing the inspection, and can react immediately by posting warnings when scums occur. It is pointed out that in shallow water-bodies with dominance of *Planktothrix agardhii*, no scums are to be expected, but these typically show an olive-green to bluish-green discoloration. The recommendation describes in some detail how to correctly perform a Secchi disc reading and how to conserve samples for microscopy if they need to be stored for more than 1 day.

Step 1 includes guidance on determining the capacity of a water-body for cyanobacterial mass proliferation on the basis of analysing the concentration of total phosphorus (nitrogen is rarely the limiting nutrient in Germany). It is emphasised that this information substantially improves hazard analysis because it provides an assessment on whether cyanobacterial dominance is likely, and how much cyanobacterial biomass might develop: Depending on size and depth of the water-body, cyanobacterial dominance may be expected above 20 – 40 $\mu\text{g/L}$ total P, and cyanobacterial biomass expressed in terms of chlorophyll-a is unlikely to exceed 1 μg per μg of total P. It is pointed out that seasonal variability of total P is rarely as pronounced as that of phytoplankton, and therefore total P needs to be analysed less often (e.g. once per season). The recommendation suggests that depending on lab equipment around 24 samples can be analysed per day.

Step 2: Quantitative Analysis of Cyanobacterial Occurrence

The recommendation explains that two approaches may be used, either determination of cyanobacterial biovolume from cell counting and cell volume measurements under the microscope, or analysing the concentration of chlorophyll-a in conjunction with a brief microscopical check as to whether the phytoplankton largely consists of cyanobacteria or of algae.

Based on the survey results discussed above, the recommendation suggests to expect microcystin concentrations in a range similar to those found for chlorophyll-a when cyanobacteria are dominant. Therefore in such situations warnings should be issued at $> 40 \mu\text{g/l}$ chlorophyll-a or the site be temporarily closed at $> 100 \mu\text{g/L}$.

For the biovolume approach, two references in German on enumeration and cell volume determination using inverted microscopes are given, and the time estimate is 2, sometimes 4 hours per sample. Necessary equipment includes a microscope (preferably inverted) with 200- 400-fold magnification and counting chambers.

Step 3: Quantitative Analysis of Microcystin Concentrations

Both the immunoassay and HPLC with photodiode array detection are recommended, and the choice will depend on access to laboratory instruments (ELISA reader or HPLC with PDA) and expertise. The immunoassay is recommended particularly for authorities without ready access to HPLC, particularly because it rapidly provides results. Time needs are estimated with 1,5 – 2 hours for conducting the ELISA and 25 samples per day with HPLC.

Measures to take

The recommendation emphasises the primary importance of public information and points out that acceptance is likely to be better if this is based on regular general information on the recreational water quality as determined following the EU Bathing-water Directive. In contrast to other 'invisible' hazards, potentially toxic cyanobacteria can be discerned by site users if public information is good, and users can be provided with criteria for taking responsibility for making their own decisions on whether conditions are adequate for safe swimming or not. This is particularly important in settings with many recreational sites and rapid variability of cyanobacterial proliferation.

Where elevated cyanobacterial occurrence or microcystin concentrations are detected, intensified surveillance by public authorities is recommended, and in scum situations or above microcystin concentrations of 100 µg/L, temporary closure of the site is proposed. Also, the risk of exposure through sailboarding and sailing, particularly in rough weather with exposure through spray and capsizing, is pointed out.

At Secchi Disc readings > 2 m, cyanobacteria can hardly reach levels hazardous to health through recreational exposure. The recommendation points out that this can be achieved by reducing concentrations of total phosphorus to less than 20 – 40 µg/L P, through managing sewage effluent, stormwater effluents and agricultural practices.

Experience with the Recommendation to protect Bathers from Cyanobacterial Toxins

Feed-back on the implementation of this recommendation or elements of it is currently being compiled by the Federal Environmental Agency. Fragmentary first impressions are that it works well in practice, and can be rather effective. A major barrier in some settings is the distribution of necessary expertise and competence across different public authorities – e.g. environment and public health – and the need to overcome such structural obstacles for organising collaboration in sampling, analyses, assessing results and taking action. A successful example of implementation of the recommendation already in its 1997 version is given below from the state of Berlin. This not only demonstrated very high public acceptance of the information channels provided, but also shows that monitoring for surveillance of sites may accumulate a valuable data set which allows improved assessment of the water-bodies and potentially prediction of problem areas.

THE BERLIN EXAMPLE

Roswitha Kröger (LAGetSi Berlin, Roswitha.Kroeger@lagetsi.verwalt-berlin.de);

Antje Koehler (BBGes-ILAT, Koehler@bbges.de)

The city of Berlin, one of the 16 Länder (i.e. states of Germany), has a rich network of slowly-flowing lowland rivers and lakes (Fig. 1) which are extremely popular for recreation, and substantial pressure for being able to enjoy their use pressure is exerted from a population of 3.5 million. Recovery from many decades of heavy nutrient loading has begun in the last 1-2 decades, but most of the water-bodies are still quite eutrophic and regularly harbour extensive cyanobacterial populations. The public authorities responsible for surveillance of bathing sites have therefore implemented a long-term programme to monitor nutrient concentrations and the resulting phytoplankton biomass.

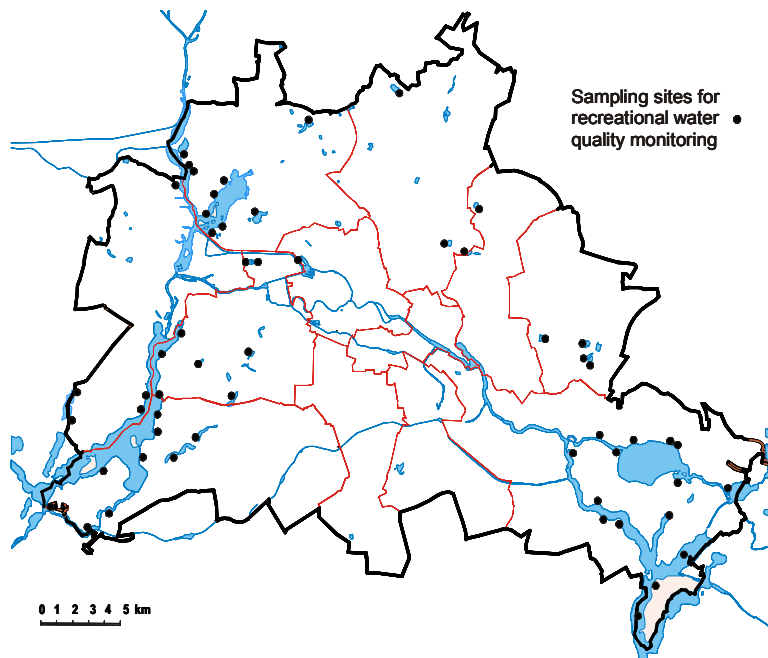


Fig. 1: Sites included in the recreational water quality monitoring programme of the city of Berlin along

- the Havel River System (west),
- the Spree-Dahme River System (south-east) and
- at a number of lakes

Since 6 years, this monitoring programme includes not only cyanobacterial biovolume, but also microcystin analyses. Results show regular patterns, indicating where which cyanobacterial taxa may be expected, e.g. in the Dahme River system dominance of *Planktothrix agardhii* and sometimes also occurrence of *Aphanizomenon flos-aquae*, in Müggelsee large colonies of *Aphanizomenon flos-aquae*, sometimes forming surface scums, and in the lakes of the River Havel predominantly *Microcystis* spp., sometimes alternating with *P. agardhii*.

Concentrations of cell-bound microcystin, particularly in scum situation, sometimes increase very rapidly and exceed the German guideline value for recreational water use of 100 µg/L. In some settings, cellular toxin content roughly follows cyanobacterial population development, e.g. for the populations of *Planktothrix agardhii* in Lake Zeuthen (Fig. 2). In others, microcystin concentrations are much more variable: for example at Wannsee, some *Microcystis* blooms showed very low microcystin concentrations while during other, sometimes extended periods high concentrations were observed (Fig. 2). For efficient monitoring in this situation, the structured approach of the Guideline for Protecting Bathers from Toxic Cyanobacteria discussed above was implemented: a component of routine surveillance of 42 bathing sites within the city now is sampling in response to scums and/or a threshold for Secchi disc read-

ings of < 1 m, and if microscopy shows cyanobacterial dominance at chlorophyll-a concentrations above 40 µg/L or cyanobacterial biovolumes above 1 mm³/L, microcystins are analysed.

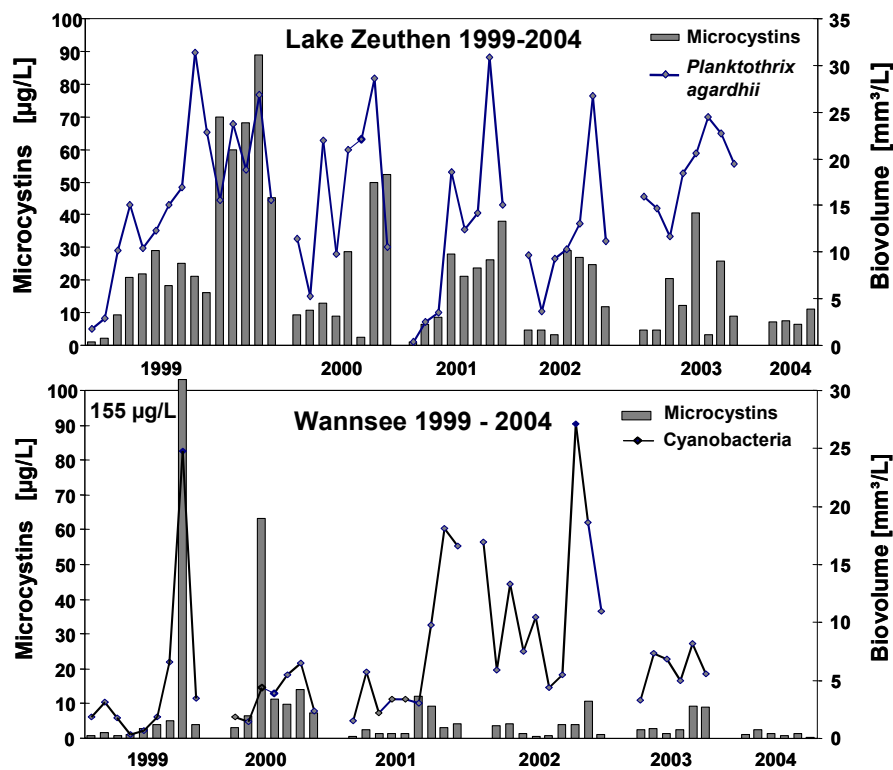


Figure 2: Seasonal patterns of cyanobacterial biomass

(determined with microscopy as biovolume) and microcystin concentrations (analysed by ELISA) in

Lake Zeuthen (dominated by *Planktothrix agardhii*) and Wannsee (dominated by *Microcystis* spp.)

An important criterion when sampling for toxin analysis in bloom situations is the exposure scenario to be assessed. Fig. 3 shows that toxin concentrations in samples taken in shallow areas (mostly used by children) can be several-fold above those in the area for swimmers.

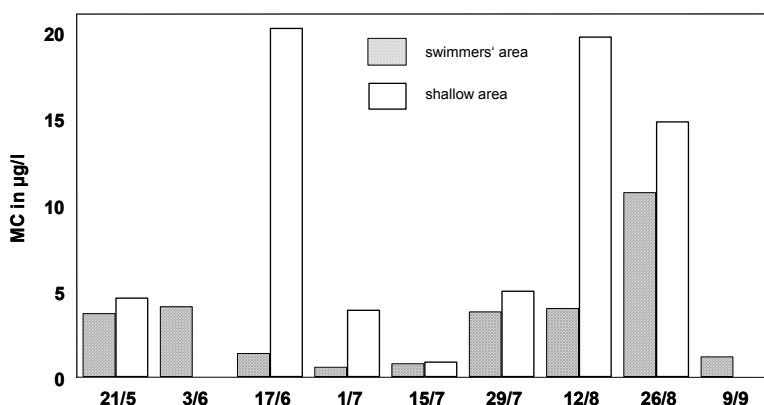


Figure 3:

Microcystin concentrations in the shallow area of the bathing site at Wannsee and in the swimmers' area (analyses with ELISA)

A major component of the programme is public information. This is targeted through several media:

- The homepage of the public authority: <http://www.badegewaesser.berlin.de>
- Press releases
- The "Badegewässer-Telefon" (bathing-water hotline): +41 30 9021 6000 which includes a voice information system for calls outside of office hours
- Posters at the recreational sites
- Information programmes for school classes (7th to 9th year).

Feed-back from the population is very positive, particularly for the bathing-water hotline, with up to 1000 phone calls per season, about 250,000 hits on the website and fewer bathers observed in scum situations.

REFERENCES

- Behrendt, H., et. al. 2003. Quantifizierung der Nährstoffeinträge der Flussgebiete Deutschlands auf der Grundlage eines harmonisierten Vorgehens. UBA/Texte 82/03, 194 Seiten.
- Bumke-Vogt, C., W. Mailahn & I. Chorus: Anatoxin-a and neurotoxic cyanobacteria in German lakes and reservoirs. *Env. Tox.* 14 (1), 117-126
- Bundesgesundheitsblatt 1992: Schutz von Badenden vor Algentoxinen und Allergenen in Badegewässern durch Einhaltung der Anforderungen nach EG-Richtlinie. *Bundesgesundheitsblatt* 35: 320
- Bundesgesundheitsblatt 1997: Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. *Bundesgesundheitsblatt* 7/97, 261-264
- Bundesgesundheitsblatt 2003: Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. *Bundesgesundheitsblatt – Gesundheitsforschung – Gesundheitsschutz* 6/2003, 530-538/6
- Chorus, I., Bumke-Vogt, C., Lindenschmidt, K.-E., Jaime, E. 2001: Cyanobacterial Neurotoxins. In: I. Chorus (ed). *Cyanotoxins: Occurrence, Causes, Consequences*. Springer Verlag, Berlin, Heidelberg, 37-45
- EC Directive 1977: 76/160/EWG Directive on the quality of bathing water, Nr. L 311/1-7
- Fastner, J., B. Wirsing, C. Wiedner, R. Heinze, U. Neumann, and I. Chorus (2001): Microcystins and hepatocyte toxicity . In: I. Chorus (ed). *Cyanotoxins: Occurrence, Causes, Consequences*. Springer Verlag, Berlin, Heidelberg, pp. 22-37.
- Fastner, J., R. Heinze, A.R. Humpage, U. Mischke, G.K. Eaglesham and I. Chorus (2003). *Cylindrospermopsis* occurrence in two German lakes and preliminary assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates. *Toxicon* 42:313-321.
- Fastner, J., U. Neumann, B. Wirsing, J. Weckesser, C. Wiedner, B. Nixdorf and I. Chorus (1999): Microcystins (hepatotoxic heptapeptides) in German fresh waterbodies. *Environ. Tox.* 14, 13-22.
- Henning M. and Kohl J-G (1981): Toxic blue-green algae water blooms in some lakes in the German Democratic Republic. *Int. Revue ges. Hydrobiol.* 66, 553-561
- Kalbe L, Duty J, Heise H, Putzke H-P, Schulz E, Tiess D (1976): Zu einer Blaualgen-Wasserblüte (*Nodularia*) der Ostsee im August 1974. *Zeitschrift ges. Hygiene und Grenzgebiete* 22: 250-255
- Mohaupt, V.; Herata, H.; Bach M.; Behrendt H., Fuchs S. 2001. Kläranlagen saniert - Woher kommen Gewässerbelastungen heute? *wasserwirtschaft-wassertechnik*, .
- Wiedner, C., Nixdorf, B., Heinze, R., Wirsing, B., Neumann, U., Weckesser, J., 2002. Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. *Arch. Hydrobiol.* 155:383-400.

GREECE:

Cyanotoxin Risk Assessment, Risk Management and Regulation

C. M. COOK¹, M. MOUSTAKA-GOUNI², S. GKELIS², T. LANARAS²

¹ National Agricultural Research Foundation (NAGREF), Agricultural Research Centre of Macedonia-Thrace, P.O. Box 60458, GR-570 01 Thermi, Greece (cook@pp-mail.bio.auth.gr);

² Department of Botany, Aristotle University of Thessaloniki, P.O. Box 109, GR-541 24 Thessaloniki, Greece (mmustaka@bio.auth.gr; sgkelis@pp-mail.bio.auth.gr; lanaras@pp-mail.bio.auth.gr)

INTRODUCTION

Studies on phytoplankton in some Greek lakes (Vegorit, Volvi, Mikri Prespa, Doirani and Kastoria) have shown that prolonged cyanobacterial blooms can occur, lasting up to 8 months, which are dominated by potentially toxic species (Moustaka-Gouni and Nikolaidis 1990; Moustaka-Gouni 1993; Tryfon and Moustaka-Gouni 1997; Temponeras et al. 2000; Vardaka et al. 2000). Generally, cyanobacterial water blooms in Mediterranean countries may be expected to be of extended or even continuous duration throughout the year, particularly in eutrophic freshwaters experiencing high temperatures and irradiance, stratification of the water column, high retention time of water and low zooplankton grazing pressure.

In Greece from 1987 to 2000 hepatotoxic cyanobacterial blooms were observed in 9 out of 33 freshwaters examined (Lanaras et al. 1989; Vardaka 2001; Gkelis et al. 2001a,b). Microcystins (MCYSTs) were detected by HPLC in 7 of these lakes and the total MCYST concentration per scum dry weight ranged from 42.2 to 2564 $\mu\text{g g}^{-1}$ (Gkelis et al. 2005). Cyanobacterial genera (*Microcystis*, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Cylindrospermopsis*) with known toxin producing taxa were present in 31 freshwaters. The two most abundant toxin producing species encountered were *Microcystis aeruginosa* and *Anabaena flos-aquae* (Gkelis et al. 2001b). However, there have been no reported incidents of adverse health effects to humans or animals, although perhaps as a result of unawareness.

Several MCYST variants have been identified in the cyanobacterial blooms. The most abundant variants are MCYST-LR and MCYST-RR, while MCYST-LA, MCYST-YR and desmethylated derivatives of MCYST-LR and MCYST-RR have also been found (Gkelis et al. 2001a). In addition, other bioactive peptides (anabaenopeptins A and B) have been identified and quantified in some Greek lakes (Gkelis et al. 2005). There is no evidence to date for the occurrence of neurotoxic cyanobacterial blooms in Greece (Cook et al. 2004).

The greater awareness concerning cyanotoxins at a scientific level in Greece is not reflected at a national governmental level in terms of the instigation of monitoring programmes and legislation. The occurrence of cyanotoxins in Greek freshwaters may have serious consequences for drinking water resources, due to the reliance on surface waters for domestic drinking supplies. To date, low concentrations ($<1 \mu\text{g L}^{-1}$) of MCYSTs have been reported in drinking water sources (Gkelis et al. 2004). However, a persistent bloom of *Microcystis aeruginosa* (biovolume $>10 \mu\text{L L}^{-1}$) has been observed in 2003 in Polyphytos Reservoir (unpublished data). Despite the known presence of cyanotoxins at certain times of the year, waterbodies (lakes and reservoirs) used for recreation, drinking-water supplies, aquaculture, irrigation and wildlife refuges, are not monitored. Only at the academic and research level has the cyanotoxin risk for waterbody users been assessed and the need for management action considered.

RISK ASSESSMENT: Cyanobacterial biovolumes, microcystin concentrations, and bioaccumulation

Cyanotoxin risk has been assessed in Lake Kastoria after studying seasonal patterns of cyanobacterial and MCYST-LR occurrence (Vardaka 2001; Cook et al. 2004). Cyanobacterial biovolume was high ($>11 \mu\text{L L}^{-1}$) throughout the year (Fig. 1) and was in excess of Guidance Level 2 ($10 \mu\text{L L}^{-1}$) proposed by WHO for recreational waters and Alert Level 2 for drinking water (Bartram et al. 1999). From April to November, cyanobacterial biovolumes in some of the surface water samples exceeded Guidance Level 3, with the potential for acute cyanobacterial poisoning. Intracellular MCYST-LR concentrations (max $3186 \mu\text{g L}^{-1}$) exceeded the WHO guideline for drinking water ($1 \mu\text{g L}^{-1}$) from September to November (Fig. 2) with a high risk of adverse health effects following ingestion or contact with lake water (Cook et al. 2004; Vardaka 2001). Based on toxicological data the involuntary ingestion of 2 mg MCYST-LR during swimming in such water could cause liver disease in a 10 kg child (Falconer et al. 1999).

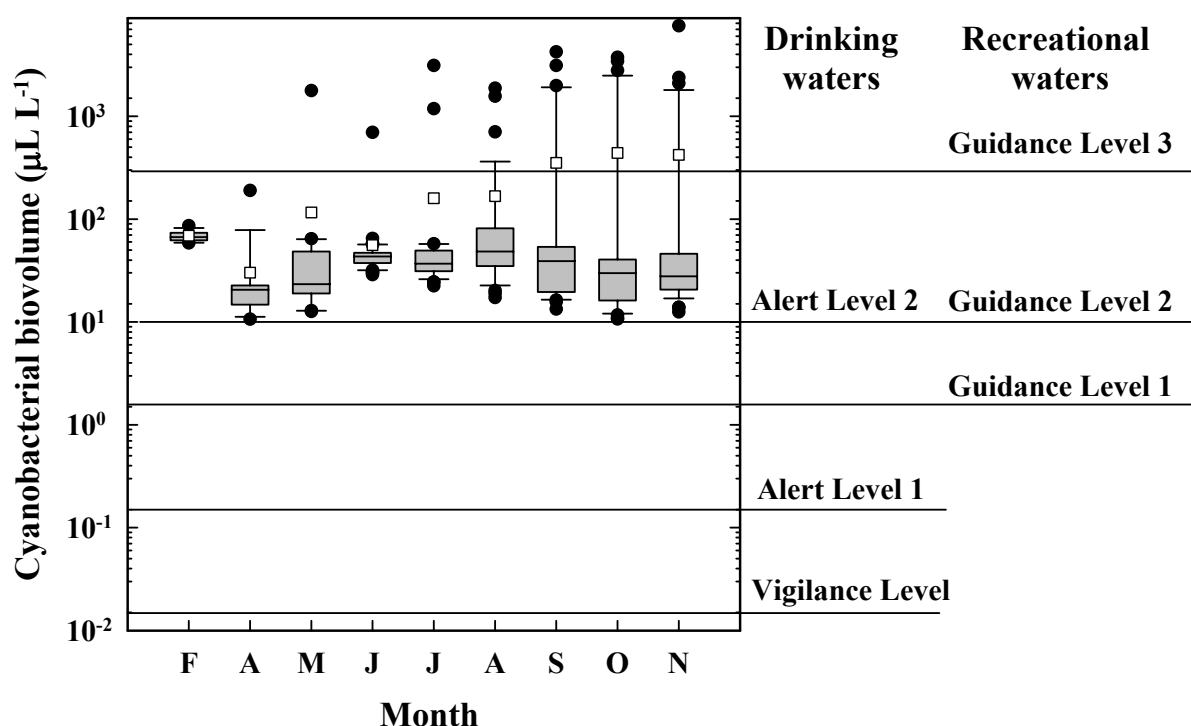


Fig. 1. Distribution of cyanobacterial biovolume values (volume of cyanobacteria per volume of lake water) represented by box and whisker plots, measured in Lake Kastoria from 1994 to 1997

(for experimental details see Vardaka 2001 and Cook et al. 2004) and Alert and Guidance levels proposed by the World Health Organization (Bartram et al. 1999) for drinking water supplies and recreational waters, respectively.

Outlying values (circles) and the mean value (squares) for each month independent of year are given. The total number of samples collected for a particular month ranges from 15 to 35. (Modified from Cook et al. 2004).

Low MCYST–LR concentrations (mean values $<1 \mu\text{g L}^{-1}$) with the exception of the individual samples indicated, are observed from February to June with no associated health risks (Fig. 2). In July and August MCYST–LR concentrations tend to increase, with about 14 % of the samples posing a moderate to high risk of adverse health effects. MCYST–LR concentrations are maximal in September, October and November, with 14 % of samples indicating the potential for a high risk of adverse health effects. From August to November MCYST–LR concentrations are higher than $2 \mu\text{g L}^{-1}$, the threshold recommended by WHO for recreational waters above which, appropriate actions should be taken (Falconer et al. 1999). In L. Kastoria 41 % of the samples from the surface water (0–0.2 m) had MCYST–LR concentrations higher than $1 \mu\text{g L}^{-1}$, the WHO provisional guideline value for drinking water.

MCYST–LR concentration was positively and significantly correlated with the total cyanobacterial biovolume ($r^2=0.724$, $P<0.05$, $n=277$) (Cook et al. 2004; Vardaka 2001). Therefore, cyanobacterial biovolume can be used as an indicator of the *in situ* MCYST–LR concentration in L. Kastoria. MCYST–LR concentrations $>1 \mu\text{g L}^{-1}$ were observed when *Microcystis* species (mainly *Microcystis aeruginosa*) constituted $>50\%$ (v/v) of the cyanobacterial biovolume (Cook et al. 2004; Vardaka 2001). *Microcystis* strains isolated from L. Kastoria had total MCYST concentrations per dry weight cyanobacterial cells, of 90 to $1200 \mu\text{g g}^{-1}$ and structural variants –LR, [D-Asp³] –LR, –RR and –LA were detected (Gkelis et al. 2001a).

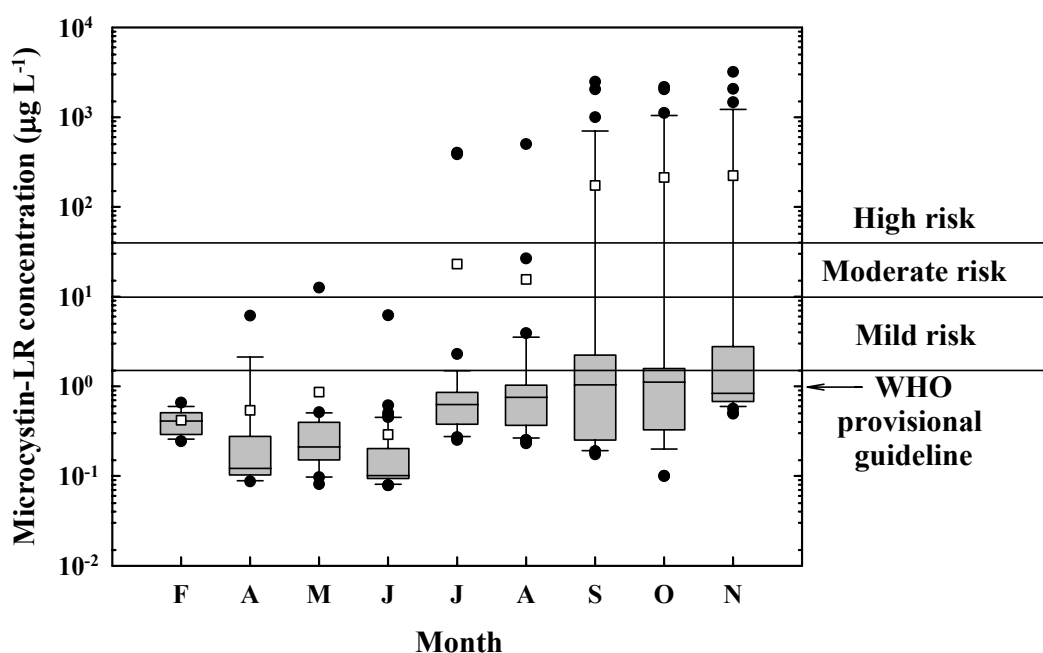


Fig. 2. Distribution of intracellular Microcystin–LR concentrations (MCYST–LR per volume of lake water) in Lake Kastoria from 1994 to 1997

(for experimental details see Vardaka 2001 and Cook et al. 2004) compared with guidelines for assessing adverse health risks in drinking-water (Falconer et al. 1999). Outlying values (circles) and the mean value (squares) for each month, independent of year, are given. The total number of samples collected for a particular month ranges from 15 to 35. (Modified from Cook et al. 2004).

Preliminary evidence indicates that microcystins are accumulated in some aquatic fauna, with average MCYST concentrations in fish and frog muscle of 225 and 125 ng per g, respectively, (Gkelis et al. 2002). A Tolerable Daily Intake (TDI) value for MCYSTs of $0.04 \mu\text{g}$ per kg body weight per day has been proposed by the WHO (Kuiper–Goodman et al. 1999). If an adult human (60 kg) was to consume 300 g of fish or frog from the lakes examined, the

Estimated Daily Intake of MCYSTs ingested would exceed the TDI on average by 28 and 15 times, respectively (Gkelis et al. 2002). Therefore, lake products targeted for human consumption should be monitored for MCYST content. A more comprehensive study of the cyanotoxin concentrations in lake fauna is necessary, in order to implement lake management policies for protecting human health and averting economic damage to lake-related industry.

The situation described for L. Kastoria is representative of other Greek lakes which also have high cyanobacterial biovolumes (Cook et al. 2004) and microcystin concentrations (Gkelis et al. 2005) in the summer and autumn. The combination of high cyanobacterial biovolumes and MCYST concentrations in water samples and the presence of MCYSTs in the food chain indicate elevated risks of acute toxicosis and adverse human health effects in several Greek lakes.

RISK MANAGEMENT

Inland water bodies are managed in terms of water usage, but are not managed yet with respect to their ecological water quality and cyanotoxin concentrations. Furthermore, there are no specific regulations concerning cyanotoxins. Subsequently, lakes experiencing cyanobacterial blooms may be used for recreational activities and athletic events, such as swimming, rowing and water-skiing and also for aquaculture. Lake products, such as fish, frogs and mussels are not monitored for cyanotoxins despite research evidence for the presence of microcystins in the tissues of lake fauna (Gkelis et al. 2002). In some instances local authorities are not willing to acknowledge the hazards that cyanotoxins present and resent the characterisation of a waterbody as potentially toxic. In the absence of a serious problem or national governmental intervention this attitude will probably continue to prevail.

The absence of cyanotoxin management policies for the lakes where cyanotoxins occur, presents a potential hazard for human health and wildlife concerning the consumption of lake products and water. The instigation of monitoring programs for the presence of MCYSTs in the waterbodies and the quality control of lake products are required. To date cyanotoxins have been monitored only for the duration of funded research programmes and these have not included the monitoring of commercial aquaculture products.

RESPONSIBILITY AND REGULATION

The legal situation concerning inland water administration is not always clear and several local administrative bodies and authorities are responsible, often with conflicting interests. None of these authorities alone has at present the resources for cyanotoxin work and the co-ordination between them has proven difficult.

Local Departments of the Ministry of Agricultural Development and Food are responsible for the safety of aquaculture products, produced in areas under their jurisdiction, targeted for human consumption. This Ministry also monitors the characteristics of the surface and ground waters which are used for irrigation and they acknowledge that there are problems in some lakes, for example, pollution and decreasing water levels (Greek Parliament 2001a). Environmental issues and ecological water quality are the responsibility of the Ministry for the Environment, Physical Planning and Public Works. Legally, this Ministry is not obliged to regularly monitor phytoplankton (including cyanobacteria) in waterbodies (Greek Parliament 2001b). The surface waters used for human consumption are of very good quality (Greek Parliament 2001b) in accordance with Directive 75/440/EEC. Currently, implementation of the Water Framework Directive (2000/60/EC) is underway, for the improvement of the ecological water quality of inland waters. Protected areas of the environment, including inland waters, are managed by newly created Protected Areas Management Bodies. The potability of drinking water is the responsibility of the Ministry of Health and Welfare.

The State Electricity Company is in control of water resources (lake water, reservoirs) used by hydroelectric power plants. The Athens Water Company draws drinking water from some surface water sources, such as the eutrophic Lake Yliki (phytoplankton biovolume $>10 \mu\text{g L}^{-1}$; Moustaka-Gouni, unpublished data), and phytoplankton abundance is monitored. There are no scientific publications to our knowledge on this monitoring. The presence of *Cylindrospermopsis raciborskii* in L. Yliki has been reported (see Cook et al. 2004) and also low concentrations ($<0.1 \mu\text{g L}^{-1}$) of MCYSTs (Gkelis et al. 2004). The Thessaloniki Water Company draws water from the River Aliakmonas which is linked to the outflow of freshwaters (e.g. Polyphytos Reservoir, Lake Kastoria) in which toxic cyanobacterial blooms (e.g. *Microcystis aeruginosa*) are known to occur. Currently cyanotoxins are not monitored by the company. Only a primitive test for the presence of algae is used. Low concentrations of MCYSTs ($<1 \mu\text{g L}^{-1}$) have been independently detected (Gkelis et al. 2004) in the Aliakmonas River. An increase in cyanotoxin concentrations in these water sources may cause adverse human health effects.

Municipalities and Local Councils have occasionally initiated phytoplankton studies in water-bodies under their jurisdiction. The General Secretariat of Research and Technology, Athens, and the EU have awarded research grants for the study of phytoplankton and cyanobacterial toxins, however these grants have been limited.

There is no central infrastructure for the study of cyanotoxins at present. However, the National Reference Laboratory for the monitoring of marine biotoxins (1993/383/EEC; amendment 1999/312/EC) has been established. Their work at present is focused on the monitoring of marine phytoplankton abundance and composition.

CONCLUDING REMARKS

To date there is no legislation in Greece concerning cyanotoxins. In addition, to the best of our knowledge, at a national government level the responsible Ministries have not taken issue with the cyanotoxin case. Presently in general, only the research community in Greece is aware of the seriousness of the cyanotoxin situation. An EU lead awareness campaign would be most beneficial for informing governmental and local authorities in Greece about global incidents, scientific and applied developments. In addition, educating young scientists, training students and informing the general public are also critical steps which should be taken.

The easiest and most cost effective approach, currently feasible, for cyanotoxin risk assessment in Greece, in the absence of national infrastructure and coordination, is the monitoring of cyanobacterial biovolume. Ideally, species identification would additionally indicate if known toxin producing cyanobacteria were present in the biomass, however this requires more highly skilled manpower. The correlation between total cyanobacterial biovolume and MCYST-LR concentration in L. Kastoria enables the prediction of cyanotoxin risk levels, even though this may underestimate the total cyanotoxin concentration. Such a method could be successfully implemented particularly for monitoring during periods of known high cyanotoxin risk. In a country with a relatively limited infrastructure the routine monitoring of samples for cyanotoxin requires the local implementation of quick, cost-effective methods. Additionally, the establishment of a national laboratory for cyanotoxins is necessary to make the Greek efforts more efficient and effective for the benefit of the public.

REFERENCES

- Bartram J., Burch M., Falconer I. R., Jones G., Kuiper–Goodman T. (1999). Situation assessment, planning and management. In: Chorus, I., Bartram, J. (Eds): *Toxic Cyanobacteria in Water*. E & FN Spon, London, New York, 1999, pp. 179–209.
- Cook C. M., Vardaka E., Lanaras T. (2004). Toxic cyanobacteria in Greek freshwaters, 1987-2000: Occurrence, toxicity and impacts in the Mediterranean region. *Acta Hydrochim. Hydrobiol.* 32, 107-124.
- European Community. (1993). Decision 93/383/EEC on reference laboratories for the monitoring of marine biotoxins. In: *Official Journal of the European Communities*, 8-7-1993, L 166/33-35.
- European Community. (1993). Council Decision 1999/312/EC: Amendment to Decision 93/383/EEC on reference laboratories for the monitoring of marine biotoxins. In: *Official Journal of the European Communities*, 8-5-1999, L 120/37-39.
- European Community. (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the Community action in the field of water policy. In: *Official Journal of the European Communities*, 22-12-2000, L 327/1-72.
- European Community (1975). Council Directive of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in Member States (75/440/EEC). In: *Official Journal of the European Communities*, 25-7-1995, L 194/26-31.
- Falconer I. R., Bartram J., Chorus I., Kuiper–Goodman T., Utkilen H., Burch M., Codd G. A. (1999). Safe levels and safe practices. In: Chorus, I., Bartram, J. (Eds): *Toxic Cyanobacteria in Water*. E & FN Spon, London, New York, 1999, pp.155–178.
- Gkelis S., Harjunpää V., Lanaras T., Sivonen K. (2005). Diversity of hepatotoxic microcystins and bioactive anabaenopeptins in cyanobacterial blooms from Greek freshwaters. *Environ. Toxicol.* (in press).
- Gkelis S., Lanaras T., Sivonen K. (2004). Occurrence and concentration of microcystins in waterbodies of Greece. Sixth International Conference on Toxic Cyanobacteria. Bergen, Norway, June 21-27, 2004, pp. 46.
- Gkelis S., Vardaka E., Lanaras T., Sivonen K. (2002). The presence of microcystins in aquatic fauna collected from Greek lakes. International Conference on Advances in the Understanding of Cyanobacterial Toxins. Occurrence, Controlling Factors and Analysis, Porto, Portugal, March 10–11, 2002.
- Gkelis S., Harjunpää V., Vardaka E., Lanaras T., Sivonen K. (2001a) Occurrence of microcystins in Greek water blooms and isolated cyanobacterial strains. 5th International Conference on Toxic Cyanobacteria, Noosa, Queensland, Australia, July 16–20, 2001.
- Gkelis S., Vardaka E., Moustaka–Gouni M., Lanaras T. (2001b). The two most abundant toxic cyanobacteria in Greek lakes and their impact on water quality. *Phycol.* 40, 123.
- Greek Parliament (2001a). Reply by the Minister for Agriculture to written question 1344/10-9-2001 (in Greek).
- Greek Parliament (2001b). Reply by the Minister for the Environment, Physical Planning and Public Works to written question 1344/10-9-2001 (in Greek).
- Lanaras T., Tsitsamis S., Chlichlia C., Cook C. M. (1989). Toxic cyanobacteria in Greek freshwaters. *J. Appl. Phycol.* 1, 67–73.
- Moustaka–Gouni M. (1993). Phytoplankton succession and diversity in a warm monomictic, relatively shallow lake: Lake Volvi, Macedonia, Greece. *Hydrobiologia* 249, 33–42.
- Moustaka–Gouni M., Nikolaidis G. (1990). Phytoplankton of a warm monomictic lake-Lake Vegoritis. *Arch. Hydrobiol.* 119: 299-313.
- Temponeras M., Moustaka–Gouni M., Kristiansen J. (2000). Seasonal variation in phytoplankton composition and physical-chemical features of the shallow Lake Doirani, Macedonia, Greece. *Hydrobiologia* 424: 109-122.

- Tryfon E., Moustaka–Gouni M. (1997). Species composition and seasonal cycles of phytoplankton with special reference to the nanoplankton of Lake Mikri Prespa. *Hydrobiologia* 351, 61–75.
- Vardaka E., Moustaka–Gouni M., Lanaras T. (2000). Temporal and spatial distribution of planktic cyanobacteria in Lake Kastoria, Greece, a shallow, urban lake. *Nord. J. Bot.* 20, 501–511.
- Vardaka E. (2001). Toxic cyanobacteria and cyanobacterial toxins in Lake Kastoria and in other freshwaters in Greece. Doctoral dissertation, Scientific Annals of the School of Biology of the Faculty of Sciences, Appendix, Aristotle University of Thessaloniki, 2001 (in Greek with English summary).

Hungary:

Regulation on Drinking Water and Bathing Water Quality including Cyanobacteria

Andrea TÖRÖKNÉ

National Institute for Environmental Health, Department of Hygienic Water Biology, P.O.Box 26 H-1450, Budapest, Hungary, e-Mail: toroka@okk.antsz.hu

Introduction

A survey on the cyanotoxin situation in Hungary covering 30 ponds and lakes used for recreation, swimming, fishing and water supply by water treatment began in 1983. The results showed that 50 of 79 water samples contained cyanobacterial scums and blooms and 33 of these 50 cyanobacterial samples (66%) were toxic to mice (Törökné et al 1986, Törökné and Mayer, 1988, Törökné 1991). The main species causing waterblooms were *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Anabaena* sp. and *Cylindrospermopsis raciborskii*. *Aphanizomenon flos-aquae* samples were collected from late spring till early summer as well as in late September and October. *Microcystis* blooms and scums were dominant in summer till September and *Cylindrospermopsis raciborskii* blooms occurred in August and September until early October. Several hundred samples were analysed by HPLC for toxins, especially for microcystins. The highest amount of total microcystin was 7.8 mg/g dry weight in biomass mainly composed of *Microcystis aeruginosa* collected from Lake Velencei, which is used for recreation. The highest microcystin content in water was 260 µg/L sampled from Kis-Balaton, the wetland upstream of Lake Balaton.

Only three regions of Hungary are supplied with drinking water chiefly based on surface water treatment plants. Two of them have some problems concerning toxin producing Cyanobacteria. One of them is the northern part of Hungary where reservoirs are used for water supply. The other is the area of Lake Balaton. This is also a very popular lake for recreation because of high water temperature in summer (25-28 °C). Seven drinking water treatment plants are situated around the lake. In Lake Balaton *Cylindrospermopsis raciborskii* caused water blooms in 1982, 1992 and 1994 with chlorophyll-a content of more than 200 µg/L. Although this species proved to be toxic in mouse assay (Minimal Lethal Dose MLD: 400 mg/kg) and in the Thamnotox test (Törökné 1999) the toxin analyses could not detect any cylindrospermopsin in the samples.

Among the natural bathing waters in Hungary, many are affected by cyanobacterial blooms. A further public health concern is the development of contact dermatitis, asthma-like symptoms and symptoms resembling hay fever during bathing in cyanobacterial blooms. A retrospective epidemiological study in a youth camp found that 100-150 children suffered from skin and eye irritation in a heavy bloom of *Microcystis aeruginosa* in Lake Velencei in 1994 (unpublished data). The first experimental evidence on allergenic effects of cyanobacteria is described in Törökné et al. (2001). These animal experiments demonstrated the allergenic effects of natural biomass containing cyanobacterial bloom material and non-axenic strains, but the six axenic cyanobacterial strains were not allergenic at all. Furthermore, microcystins did not effect allergenicity even in high amounts (1.5 µg/mL). In conclusion it is proposed that the associated heterotrophic bacteria may cause the symptoms of allergy. These findings are interesting in the light of the results of Pilotto et al. (1997): this prospective epidemiological study in Australia involved 852 persons to examine the occurrence of the symptoms of diarrhoea, vomiting, hay fever, eye irritation within 7 days after bathing in freshwater with cyanobacteria in relation to the toxin content, cyanobacterial cell density as well as the time spent

in the water. Interestingly, while a significant correlation was observed between the symptoms mentioned above and the number of cyanobacteria together with the time spent in the water, these authors also found no correlation between the toxin content and the symptoms.

Legislation on drinking water

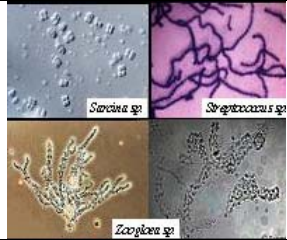
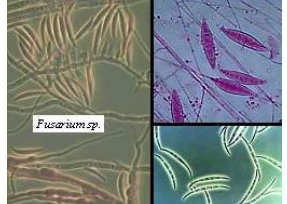



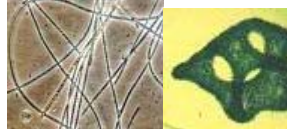
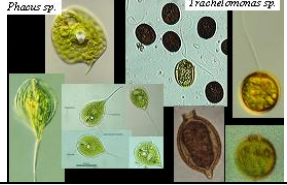
As the water supply in Hungary originates mainly from deep wells and bank filtration and only 8% of the total drinking water demand is covered by surface water treatment plants, legislation does not include cyanotoxin limits and monitoring. However, the Governmental Decree on Requirements of Drinking Water Quality and the Order on Monitoring which harmonize our legislation with the 98/83 EU Directive 201/2001(X.25) include some requirements for drinking water quality which are stricter than the EU directive, e.g.: for trihalomethanes a limit of 50 µg/l, the minimum hardness must reach 50 mg/l CaO, the limit for bound active chlorine 3 mg/l and the health authority can state individual limit values for Heterotrophic Plate Counts at 22°C and 37°C. Additionally, it includes “biological parameters” which can be determined by microscopy. This is a very useful and quick method for informing on problems in distribution networks and in the treatment plants. The required frequency of microscopical examinations is based on the amount of water supplied by the treatment plant, but every network must be examined at least once a year.

Postgradual education is organised by our institute to promote the effectiveness and quality of the microscopical examinations among the water treatment experts. For cyanobacteria, particularly laboratory staff from surface water treatment plants are educated on the basis of the WHO book on Toxic Cyanobacteria in Water (Chorus and Bartram 1999) to recognise the risks of water blooms and to manage this. Since 2000, in every year two-day workshops have been held to improve knowledge on new challenges in water treatment management. Additionally interlaboratory comparison exercises are organised twice a year for analyses of samples of drinking water collected from different parts of Hungary.

Legislation on natural bathing water

In the 273/2001(XII.21) Governmental Ordinance on the Quality of Natural Bathing Waters the only parameter used as basis for regulations concerning cyanobacterial blooms is the chlorophyll-a concentration. The proposed limit for chlorophyll-a is 25 µg/L and the still acceptable limit is 75 µg/L. As the 76/160 EU Directive is under revision (and this was the basic document during the harmonisation process), hopefully it will contain some instructions on managing cyanobacterial events at natural bathing sites.

Table 1. Biological parameters for drinking water

Parameters	Limit	Unit	Comment	View
Sediment	<0,1	mL/L	Amount of sediment after 0.45 µm membrane filtration of 1 L water	
Bacteria indicating contamination with waste water	0	Number/L	distuingishable, clearly recognizable bacteria	
Fungi	0	Number/L	Sediment must not contain fungi at all	
Protozoa	0	Number/L	Indicating pollution or pathogens	
Helmints	0	Number/L	Species of Nematodes, Oligochaetes, Ascarides, Gastrotriches and their eggs	
Iron and manganese bacteria	$2 \cdot 10^4$	Number/L		
Sulphur bacteria and cyanobacteria	10^2	Number/L		
Algae	10^4	Number/L	Just for surface water treatment plants	

References

- Törökné K.A., Schiefner K., Mayer G., Dura Gy., Deák Zs.: Természetes vizeinkben előforduló algaprodukciónak állatkísérletes vizsgálata, *Egészségtudomány* 30, 301-308. (1986) (The examination of surface water algal production in mice) with English summary
- Törökné K.A., Mayer G.: Toxikus cianobaktériumok hazai felszíni vizekben, *Hidrológiai Közlöny* 68, 49-54 (1988) (Toxic cyanobacteria in Hungarian fresh waters) with English summary.
- Törökné K. A.: Hazánkban előforduló toxintermelő cianobaktériumok tulajdonságainak vizsgálata. Doktori értekezés. 1991. ELTE TTK.(Toxin producing cyanobacteria in Hungary. Doctoral dissertation)
- Törökné K. A.: A new culture-free microbiotest for routine detection of cyanobacterial toxins *Environ.Toxicol.* 14(5). 466-472. (1999)
- Chorus I, Bartram J: Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management. London, New York. 1999.
- Pilotto, L.S., Douglas, R.M., Burch, M.D., Cameron, S., Beers, M., et al. 1997. Health effects of recreational exposure to cyanobacteria (blue-green) during recreational water -related activities. *Australian and New Zealand Journal of Public Health.* 21: 562-566.
- A Torokne, A Palovics, M Bankine: Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria - Experimental evidence *Environ. Toxicol.* 16(6):512-516 (2001)

ITALY:

Cyanobacteria in Surface Water

Enzo FUNARI¹, Liana GRAMACCIONI², Simona SCARDALA³

¹ Istituto Superiore di Sanità, Viale Regina Elena, 299 Roma, Italy. E-mail: funari@iss.it

² Istituto Superiore di Sanità, Viale Regina Elena, 299 Roma, Italy. E-mail: simonas@iss.it

³ Ministero della Salute, D.G. della Prevenzione Sanitaria, Via della Civiltà Romana 7, 00144 Roma, Italy.
E-mail: l.gramaccioni@sanita.it

Introduction

Cyanobacteria are natural microorganisms which occur in surface waters throughout the world. Italy of course does not escape from this general rule and as other countries it shares the phenomenon of cyanobacterial blooms especially in eutrophic superficial waters.

Due to the marginal consideration given to this issue in the national (as well as current European) legislation, no adequate data are available on the occurrence of these microorganisms and their toxic contents in water bodies.

National legislation

In Italy, the quality of bathing waters is ruled by a national Decree (DPR 470/1982), which enforces the Council Directive 76/160/EEC. The Decree establishes different tasks for the Ministry of Health, Regions and Municipalities to be accomplished in order to reach the objectives and fulfil the requirements. The main task of the Ministry of Health is to coordinate the activities for the implementation of this Decree, and update parameters, limits and methods on the basis of new scientific knowledge. This Decree sets the limit values for a number of microbiological, physical and chemical parameters. Yet, as the currently valid European Directive, it doesn't consider toxic algal species and cyanobacteria. However, it does include dissolved oxygen, and for this parameter a subsequent national Decree (DL n. 109 of 1993 and further updating) has established the conditions for which derogation from the limit values can be permitted. These limits may be exceeded substantially after demonstration that their levels are not associated with toxic algal proliferation. In order to obtain permission for such derogation the Regions have the obligation to prepare a specific surveillance plan on marine algae (in the sea) or cyanobacteria (in internal waters) aimed at controlling their density and toxicity. The results of these activities are then sent to the Ministry of Health, as Regional Reports. This Ministry expresses its opinion on the possibility to admit the requested derogation. In 1998, the Ministry of Health provided a list of toxic algae and cyanobacteria of concern and analytical methodologies, and recommended a limit value of 5.000.000 cells/L for toxic algal species as a safe level for bathing activities.

As to drinking waters, the national decree (DL 31/01) which enforces the Council Directive 98/83 EEC considers algae as an accessory parameter. This means that monitoring activities are conducted only in case local authorities estimate that there is a potential risk to human health. In conclusion, no limit value has been established for cyanotoxins in drinking water in Italy; the only reference used by local authorities is the provisional WHO guideline of 1 µg/l for the microcystin-LR in drinking waters (WHO 2004).

National situation

With reference to the problem of cyanobacteria, the analysis of the above mentioned Regional reports of the last three years and of literature data shows that there are many lakes affected by recurrent high densities, sometimes in form of blooms, mostly due to genera of *Planktothrix*, *Microcystis* and *Anabaena*. Fig. 1 shows the Italian regions for which, to our knowledge, data on cyanobacteria in fresh waters are available.



Figure 1 Regions for which monitoring data are available

The largest Italian lakes are in northern Regions. Many of them are used for bathing activities, some for commercial fishing and also as drinking waters supply. Their waters are often affected by eutrophication phenomena and are generally monitored for the presence of cyanobacteria. One important bloom of *Leptolyngbya* sp. occurred in August 1997, in Lake Varese. It was associated with the death of small fish and bivalves; in a study aimed at identifying the cause of these deaths, saxitoxin was determined in water and tissue samples (Giovannardi et al. 1999). This phenomenon occurred again the following year with less dramatic consequences. In summer 1997, Lake Iseo was infested by an intense *Anabaena flos-aquae* bloom. In 1999, this lake was affected by a large *Planktothrix rubescens* bloom, which developed in June and lasted all summer reaching very high densities (up to 70.000 cell/ml). The waters of this lake are also used as drinking water supply (Funari et al. 2000). In 2003, a bloom of *Anabaena* occurred in the Viverone Lake, with levels up to 20.000cell/ml.

In the central Regions many lakes are used both for recreational purposes and as drinking-water source. In some of these lakes high cyanobacterial density frequently occurs. In the last years, Lake Fiastrone has been characterised by a recurrent presence of cyanobacteria and in winter 2003 a bloom of *Planktothrix rubescens* reached a density of some 100.000cell/ml. Lake Trasimeno has been affected by the occurrence of *Planktothrix*, *Microcystis* and *Anabaena* and, very recently, this list has been enlarged with *Cylindrospermopsis raciborskii*. Lake Massaciuccoli (brackish water) harbours, at varying densities, *Aphanizomenon*, *Microcystis*, *Cylindrospermum* and *Cylindrospermopsis* species. In summer 2000, microcystins –YR and –LR were determined at levels of 0.74-7.6 µg/l (Simoni et al. 2002). From a preliminary study conducted by a research group of the National Institute of Health (data not yet published), microcystins –LR and –RR were found at levels always <1ug/l in drinking waters derived from 5 lakes.

As to the south, Sardinia and Sicily are particularly affected by this problem. Sardinia has many eutrophic lakes which represent some 90% of the drinking water supplies of the Region. This problem is not a new one in Sardinia, where in 1985 a *Planktothrix rubescens* bloom caused a brick-red discoloration in lake waters. The water treatment plant was not capable of intercepting the big amounts of cyanobacteria, which were found also in drinking water (Sechi 1992). Since then several surveys have been carried out to monitor these microorganisms. At present, *Microcystis*, *Anabaena*, *Aphanizomenon* and in the cold months the *Planktothrix rubescens* represent the cyanobacterial taxa most frequently found. In Sicily, which is characterized by a semi-arid climate, water supplies are mostly derived from artificial

lakes and reservoirs. Especially in summer these are often affected by cyanobacterial blooms, the most common genera being *Planktothrix*, *Microcystis*, *Anabaenopsis* and *Anabaena* (Rapporto Istisan 2000).

Further development

At present, the Ministry of Health in collaboration with the National Institute of Health is promoting an Observatory on Water and Health, where a section will be devoted to the problem of cyanobacteria in bathing and drinking waters. In this context, the main objectives are to build up a national database on cyanobacterial and cyanotoxins occurrence and to provide guidelines to local authorities for the activities of risk assessment and risk management.

References

- Council Directive 98/83/CE Concerning the Quality of Drinking Water, G.U.C.E. n.L330 of 5 december 1998 amendment G.U.C.E. n. L 111 of 20 april 2001
- DL 1993: Decree-Law n. 109 of 13 april 1993, G.U. 15.4.1993, n. 87
- DL 2001: Decree-Law n. 31 of 2 February 2001, G.U. n.52, 3 March 2001 Ordinary Supplement
- DPR 1982: Decree of the President of the Republic n. 470 of 8 June 1982, G.U. n.203 of 26 july 1982.
- EEC 1976: Directive 76/160/EEC Concerning the Quality of Bathing Water, G.U.C.E. 5 February 1976 n. L31
- Funari E., Cavalieri M., Ade P., Barone R., Garibaldi L., Pomati F., Rossetti C., Sanangelantoni A.M., Sechi N., Tartari G., Ventura S. (2000). Il problema ambientale e sanitario delle fioriture di cianobatteri nelle acque superficiali in riferimento alla situazione italiana. *Ann. Ig.*, 12: 381-400.
- Giovannardi S, Pollegioni L, Pomati F.(1999). Toxic cyanobacterial blooms in Lake Varese (Italy): a multidisciplinary approach. *Environ. Toxicol.* 14 (1): 127-134.
- Rapporto Istisan 2000/30. Workshop:Aspetti sanitari della problematica dei cianobatteri nelle acque superficiali italiane. Istituto Superiore di Sanità. Roma, 16-17 dicembre 1999. Atti a cura di Enzo Funari.
- Sechi N. Le problematiche legate al fitoplankton nelle acque lacustri destinate ad uso potabile (1992). *Giorn. Bot. Ital.* 126(2): 237-251.
- Simoni F., Di Paolo C., Coli A., Macchia S. (2002). Eutrofizzazione e tossine algali in un lago poco profondo con acque salmastre: un anno di monitoraggio. *Acqua & Aria*. 6: 67-75.
- WHO (2004) *Guidelines for drinking-water quality*. 3th edition. Volume I, Recommendations. Geneva: World Health Organization, pp.515.

NETHERLANDS:

Risks of toxic cyanobacterial blooms in recreational waters: guidelines

Bas W. IBELINGS

Netherlands Institute of Ecology (NIOO-KNAW) – Centre for Limnology – Department of Foodweb Studies.
Rijksstraatweg 6, 3631 AC, Nieuwersluis, The Netherlands. E-mail: b.ibelings@nioo.knaw.nl

Introduction

God created the earth, but the Dutch created Holland. This old proverb nicely emphasises the crucial role of watermanagement in the Netherlands. Protection against flooding has always been crucial for survival of the country. The earliest regional waterboards were already founded in the 14th century, and Rijkswaterstaat, responsible for watermanagement at the national level celebrated its 200th anniversary in 1998. Traditionally the focus of watermanagement has been purely on management of water quantity, but since 1970 water quality management was added to the responsibilities of the waterboards. Nowadays policies are firmly founded upon integrated watermanagement in which the all functions of water systems (e.g. production of drinking water, shipping, recreation, nature) are weighed and balanced. The current central aim of water management in the Netherlands has been described as: “to obtain and maintain a safe country and to preserve and strengthen healthy, resilient water-systems that ensure sustainable use of water”. The use of surface waters for recreation, which is the subject of this paper, is thus only one of a number of functions of lakes and rivers in the Netherlands. The specific responsibility for the quality and safety of recreational water lies with the provinces and not with the waterboards, although waterboards have the responsibility to carry out the monitoring.

Most of the lakes in the Netherlands are man-made. In the densely populated western part of the country these lakes are the result of extensive peat-digging in the 19th century. Almost all of these lakes are highly eutrophic, despite large and costly efforts to reduce nutrient loads to the surface waters. Especially concentrations of phosphorous (P) have dropped strongly over the last decade or so, partially as a result of the reduction in P levels in the Rhine to which most of these peat lakes are connected. Lake restoration has been successful in some cases, but many lakes are still turbid and without the extensive submerged vegetation that once characterized them. Blooms of cyanobacteria are a conspicuous attribute of many of the lakes. Both more or less permanent blooms of filamentous cyanobacteria (genera like *Planktothrix* and *Limnothrix*) and (summer)blooms of colonial genera like *Microcystis* occur frequently. Other cyanobacteria that are often observed in the phytoplankton are *Anabaena* and *Aphanizomenon*. Dense *Microcystis* blooms are not only found in the smaller regional waters, but also in the major lakes like the IJsselmeer (1136 km²) and Volkerak Zoommeer (61.5 km²), and surface scums of *Microcystis* may cover tens to hundreds of square km in these lakes. Another string of large lakes (Randmeren), including lakes Veluwe and Wolderwijd used to have year round blooms of *Planktothrix agardhii*, but here a combination of restoration measures (reduction of P-loading, flushing, removal of bream) have resulted in a regime shift. *Planktothrix* has completely disappeared and *Chara* meadows have been restored to their former glory.

In summary it is clear that the potential for recreational exposure to (toxic) cyanobacteria in the Netherlands is rather large. We have numerous lakes that are eutrophic and support dense blooms of cyanobacteria in a crowded country with a high demand for water related recreational activities. For many years the authorities did not seem to take the risk of cyanobacterial blooms too seriously, but this has changed somewhat over the last few years, at

least in parts of the country. The general public is relatively well informed about the health risks of swimming in lakes. There are special hotlines where information about the waterquality of specific waterbodies can be obtained ('Zwemwater telefoon') and there are dedicated websites (e.g. www.waterland.net – link 'Zwemwaterkwaliteit') with clickable maps of the provinces, showing whether swimming is unsafe in particular waterbodies. Nowadays most warnings and even closure of lakes are related to blooms of toxic cyanobacteria. In the next two paragraphs we will discuss regulations with respect to cyanobacteria and their toxins that have been developed in the Netherlands and we will briefly present data from surveys about health complaints and microcystin concentrations in Dutch lakes to put the relevance of the guidelines into perspective. In the discussion special attention will be given to the accumulation of toxic cyanobacteria in surface scums, which pose the largest threat to swimmers. One paragraph will be dedicated to cyanobacterial toxins in drinking water.

Guidelines for microcystin in recreational waters

In 2002 the Health Council of the Netherlands published the report: "Microbial risks of recreational waters" (link: <http://www.gr.nl/pdf.php?ID=92>). The report gives advice to the government on epidemiological research, control measures (the 'safety chain'), legislation etc. The focus is not just on cyanobacteria but also on other agents in surface water that may transmit a disease, e.g. *Leptospira*, *Clostridium botulinum*, *Naegleria fowleri* or *Acanthamoeba*. Since in the Netherlands microcystin (MC) has by far the most widespread occurrence of all the cyanobacterial toxins, the report only gives an exposure limit for this hepatotoxin, and not for neurotoxins. Based upon the tolerable daily intake in food (MC-LR < 0.04 µg per kg bodyweight), from which the provisional WHO guideline for drinking water (MC-LR < 1 µg L⁻¹) was derived, and assuming that a swimmer ingests 100 mL of water (and bathes 365 days per year – more likely this would be less than 35 days) an exposure limit of 20 µg MC-LR per litre of bathing water is derived. Note that this value is just a guideline, there are no legal requirements to monitor microcystin levels or to act upon levels exceeding 20 µg MC-LR L⁻¹ (the only legal requirement with respect to microbial risks in recreational waters is for faecal contamination).

At an earlier stage a working group of cyanobacterial experts had advised the Committee on Integrated Watermanagement (CIW – now LBOW) on a guideline about the risks of toxic cyanobacteria for recreation. Such a guideline, issued by CIW advises the different authorities (waterboards, provinces etc) on management of specific waterborne problems. The guideline on cyanobacteria aimed to achieve that - nationwide - authorities would use the same criteria for microcystin in recreational waters. This ambition had some success, most provinces use the guideline. The following values are used in the guideline:

- MC-LR > 10 µg L⁻¹: issue warning
- MC-LR > 20 µg L⁻¹: issue warning and continued monitoring; if levels are persistently high close bathing facility
- Presence of scums: at least a warning and continued monitoring

Figure 1 shows which actions should be taken by the responsible authorities on basis of the CIW guideline. In the top row of the figure an attempt is made to focus the monitoring efforts on relevant waterbodies only. There are simply too many lakes with a recreational function to monitor MC in all. Criteria that are used to select lakes are based upon historical (reputation of a waterbody, i.e. earlier problems with cyanobacteria) and recent (e.g. health complaints or strong discoloration of the water) information. Based upon the level of MC one of the actions outlined above is required: i.e. no further action; issue warning, or in the most severe cases ban swimming. Presence of surface scums in a lake should always result in either a warning or closure of the lake for recreation. Personnel in many cases has been instructed to take samples for microcystin analysis especially where cyanobacteria are concentrated in a surface bloom. Obviously the chances that MC will exceed 20 µg L⁻¹ and that swimming will be banned are much increased by sampling the scums.

This was for instance the case in May 2004 in Lake Zwemlust. Microcystin concentrations rose to $390 \mu\text{g L}^{-1}$ in an ephemeral surface bloom which had accumulated near the shore. The lake was closed for recreation by the province of Utrecht. Elsewhere in the lake (outside the scum) MC did not exceed $2 \mu\text{g L}^{-1}$, and was usually much less than $1 \mu\text{g L}^{-1}$. The water was clear and the bottom was visible in the lake. The problem for Zwemlust was not that the water quality was all that bad, the problem was that the cyanobacteria had accumulated (both vertically and horizontally) at a position in the lake where the risk of exposure to the scum was reasonably large. Because there was plenty of water of good quality available in the lake, a solution was found in mixing the lake artificially (using Flygt mixers). Microcystin levels immediately decreased to acceptable levels and the risk of scum formation was much reduced. The lake was re-opened for recreation. This is obviously an exceptional case and this type of solution was only made possible because Zwemlust is a small lake (1.5 ha) and has constant supervision, but the Zwemlust example does emphasize the crucial role of buoyancy and scum formation in the risks of toxic cyanobacteria for swimmers.

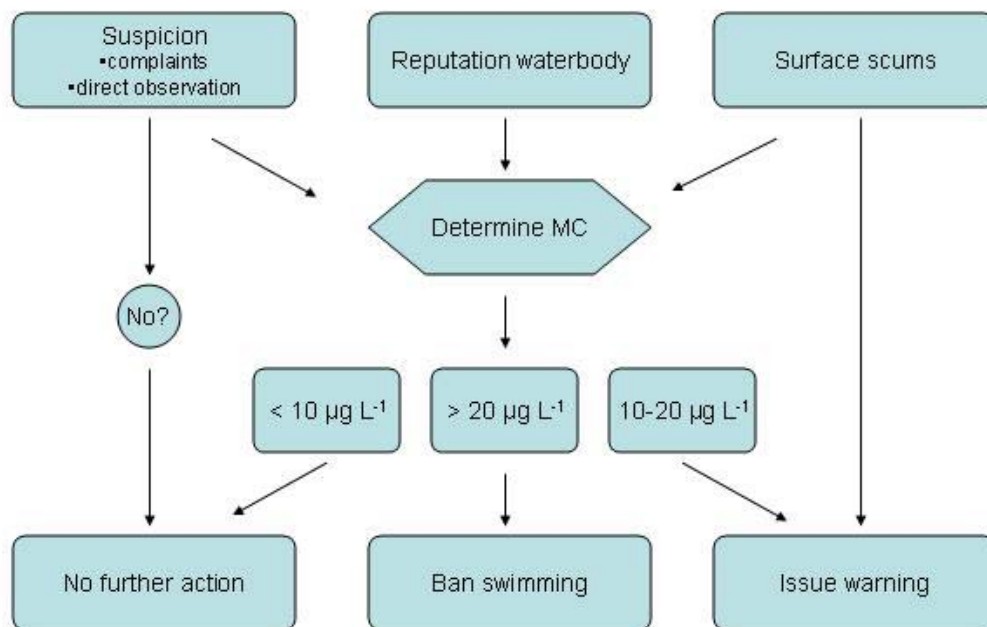


Fig. 1. Guidelines for watermanagement, developed to minimize the risk of exposure to microcystin in recreational waters in the Netherlands

Survey of health complaints related to recreation in surface waters

An inventory of health complaints related to recreation in surface waters was conducted by a survey among Municipal Health Services and provinces. In the summer of 1999 approximately 43% of the respondents reported such complaints. Identified were gastro-intestinal complaints (15 incidents with 80 patients) and skin complaints (22 incidents with 120 patients). Some complaints made a direct reference to the presence of cyanobacteria (22 incidents). The incidents usually concerned small numbers of patients, isolated in time and place. The microbiological quality of the water met the current legal water quality standards. A few notifications (4 incidents) related to health problems of dogs were made (Leenen, 2000). The specific contribution of exposure to toxic cyanobacteria to all these health problems remained unknown.

Survey of MC-LR values in Dutch lakes

Two surveys of the presence of (toxic) cyanobacteria have been carried out in the last 6 years: 1998 and 2003. In 1998 48 recreational waters with a presence of cyanobacteria were sampled by AquaSense consultancy, and in the great majority of cases (83 %) potentially toxic cyanobacteria were found (*Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Microcystis* and *Planktothrix*) in concentrations between 500 – 147.000 particles mL⁻¹ (Stowa, 2000). The lower WHO guidance level of 20.000 cells mL⁻¹ was exceeded in 35% of the cases; the higher guidance level of 100.000 cells mL⁻¹ was exceeded at 10% of the sampled locations. Microcystin (total of all microcystins, expressed as MC-LR equivalents and analyzed on HPLC-DAD) was nearly always found, in concentrations varying 0.15 – 147 µg L⁻¹. Neurotoxins were not found. The CIW guidance level of 20 µg MC-LR L⁻¹ was exceeded in 21 % of the cases. This was found even at relatively low chlorophyll-a levels (starting from 22 µg L⁻¹).

Data on microcystin level in lakes in the summer of 2003 were gathered from the monitoring activity by various waterboards. A quickscan was published by Krot & Visser (2003). Some waterboards by 2003 had started microcystin analyses, but the extend to which different authorities treat toxic cyanobacterial blooms as a serious problem varies greatly. Some waterboards take no samples at all, others only when a problem is obvious (e.g. presence of scums), or even only when health complaints have been reported. In a few cases MC analyses are carried out by the waterboard for all their swimming locations but only 1 to 5 times a year. Some waterboards state that they do not monitor cyanobacteria since bathing water quality is the responsibility of the provinces.

A disadvantage of the 2003 survey is that different techniques were used by the different waterboards for analysis of MC (ELISA, HPLC and LC-MS). Moreover (and potentially more damaging for the interpretation of the results) different extraction protocols were used, and in some cases (e.g. freezing and thawing only once) it is very unlikely that all microcystins would have been extracted. Like in 1998, also in 2003 all different microcystins were converted to MC-LR equivalents. Microcystin concentrations in the great majority of cases were relatively low (326 samples), in a small number of samples (13) MC-LR varied between 10-20 µg L⁻¹, and in a somewhat larger number of samples MC-LR exceeded 20 µg L⁻¹ (33). No relationship with chlorophyll-a levels was found. The latter group of locations with a high microcystin level consisted of 13 different lakes under control of 5 different waterboards. It is not clear in all cases which actions were taken in response to MC levels > 20 µg L⁻¹. Lakes were closed for recreation by at least two different provinces (Utrecht and Zeeland). Warnings were issued more often and the province of Zuid Holland advised against bathing in all recreational waters on 7 August 2003, because cyanobacteria were abundantly present.

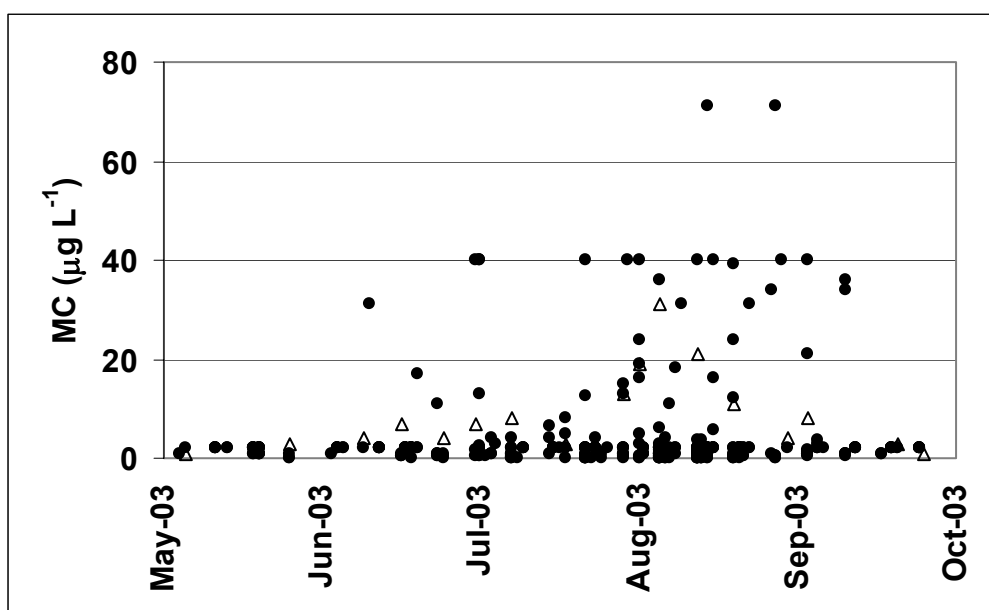


Fig. 2. Concentration of MC-LR equivalents (closed symbols) from samples taken from a number of different Dutch lakes during the summer of 2003. The open symbols represent the number of reports – per week - on scum formation in these lakes.

Discussion

The situation with respect to (monitoring) toxic cyanobacteria in the Netherlands is problematic and potentially puts the public at risk. There are a number of arguments for this: i) recreation (swimming, surfing, sailing) is intense; ii) many lakes support high densities of potentially toxic cyanobacteria; iii) microcystin levels in two surveys were shown to reach relatively high levels in different lakes and at various moments in the summer; levels on several occasions exceeded $20 \mu\text{g L}^{-1}$; iv) there is no legal requirement to monitor the presence of toxic cyanobacteria or their toxins; v) hence there are only (voluntary) guidelines, set by CIW and the Health Council (it is possible that the CIW guidelines will obtain a legal status in the coming years); vi) different provinces and waterboards have different approaches to monitoring cyanobacteria (varying from no monitoring at all to several times per year); vii) sometimes it is unclear who has the responsibility to manage the risks of cyanobacteria; viii) the methodology for measuring microcystin is inadequate in some cases.

At this point I would like to emphasize that although cyanobacteria were inadequately monitored in all waterbodies, reliable monitoring of toxic cyanobacteria and their health risks is not a trivial task. To start with the latter, the National Institute for Public Health and the Environment (RIVM) has developed a standard protocol (PLONZ) that should facilitate the collection of health complaints from the Municipal Health Authorities. In case of a multitude of complaints for one location RIVM can instigate a more specific investigation. The lack of good epidemiological data is probably one of the reasons why the risks of toxic cyanobacteria are not always taken seriously. Monitoring the actual presence of cyanobacteria in a lake is equally hard. Not only does the number of cyanobacteria in the water vary at relatively short intervals through growth and loss processes, the formation of surface scums of floating cyanobacteria is even much more dynamic, and varies at a time scale of hours. Monitoring these dynamics with routine means is impossible; it requires an innovative approach. Arguably, scum formation is what we should be really worrying about. Whereas normally one would have to drink one to several buckets of lake water to ingest a lethal dose of toxin, once concentrated in a surface scum a small sip may be lethal. It may perhaps be unlikely that people enter (dense) scums, the presence of extremely high concentrations of a potent toxin is cer-

tainly a reason for concern (especially with little children playing at the lake shore where the chances of getting into contact with concentrates of cyanobacteria are higher). Which other toxin in watersystems has the capacity to concentrate 1000 fold or more in a time scale of just a few hours? How to keep an eye on this potential threat to the health of swimmers and surfers?

Ibelings et al (2003) published a fuzzy logic model that predicts the formation of surface scums on basis of i) the biomass of cyanobacteria, suspended in the watercolumn, and ii) the long term weather forecast, giving relevant information to derive buoyancy of the cyanobacteria and water column stability. Surface scums will develop whenever a population of buoyant cyanobacteria is present in a lake with a stable water column. Biomass of cyanobacteria may be obtained from traditional models or from routine optical monitoring (see e.g. Gons, 1999). The model was validated on basis of a long term data set of NOAA satellite images and proved to be reliable. This approach could relatively easy be made into an online warning system, where the authorities receive an automatic warning when there is a risk of scum formation in the next three to five days in one or more of their waterbodies.

Finally a paragraph about the potential exposure to microcystin via drinking water in the Netherlands. An growing proportion of drinking water in the country is obtained directly from surface water (now approaching one third of the total volume of drinking water produced yearly – Leltz personal communication), with the IJsselmeer as the main freshwater reservoir. Blooms of toxic cyanobacteria are present every year in the IJsselmeer, and Ibelings et al (in press) report microcystin concentrations (MC-LR equivalents) of up to $68 \mu\text{g L}^{-1}$. In one drinking water reservoir 'De Gijster' an extreme microcystin concentration of $52000 \mu\text{g L}^{-1}$ was measured in surface scums (Hoogenboezem et al, 2004). Drinking water companies take the risk of microcystin increasingly seriously. A theoretical worst case scenario demonstrated that the provisional WHO guideline for drinking water ($\text{MC-LR} < 1 \mu\text{g L}^{-1}$) could be exceeded in the Netherlands, with values reaching $2.9 \mu\text{g L}^{-1}$ in the purified water (Carpentier et al, 1999). The authors concluded however that exposure to microcystin levels exceeding $1 \mu\text{g L}^{-1}$ was no more than a theoretical risk, resulting from various assumptions in the worst case scenario. Hoogenboezem et al (2004) however argue that some of the assumptions regarding the concentrations of microcystin in the (raw) intake water were quite realistic, backed up by measurements of microcystin in the intake water of several drinking water companies. Hoogenboezem et al (2004) hence conclude that "for evaluation of possible threats for drinking water production in some purification plants a special analysis is needed to make sure that toxin removal is adequate".

Concluding, although much progress has been made in managing the risks of toxic cyanobacteria for recreation and the production of drinking water, there remains work to be done in the Netherlands.

Acknowledgements

Figure 2 was kindly provided by Petra Visser of the University of Amsterdam

References

- Carpentier CJ, Hoogenboezem W & Ketelaars HAM (1999). Cyanobacterietoxines en drinkwater bereid uit oppervlaktewater. Een worst case benadering
- Gons, HJ (1999). Optical teledetection of chlorophyll a in turbid inland waters. *Env. Sci. Technol.* 33, 1127-1132
- Hoogenboezem W, Wagenvoort AJ & Blaauboer K (2004). The occurrence of toxic cyanobacteria. In some Dutch surface waters used for the production of drinking water. RIWA report.
- Ibelings BW, Vonk M, Los HFJ, van der Molen DT, Mooij WM (2003). Fuzzy modeling of cyanobacterial surface waterblooms: Validation with NOAA-AVHRR satellite images. *Ecol. Appl.* 13, 1456-1472
- Ibelings BW, Bruning K, De Jonge J, Wolfstein, K, Dionisio Pires LM, Postma, J & Burger T (2005). Distribution of microcystin in a lake foodweb: no evidence for biomagnification. *Microbial Ecology* (in press)
- Krott, B & Visser, PM (2003). Inventarisatie naar de concentraties van cyanotoxins in Nederlandse meren gedurende de zomer van 2003 en naar eventuele hiermee samenhangende incidenten, een Quick Scan. Report University of Amsterdam
- Leenen, EJTM (2000). Gezondheidsklachten in verband met recreatie in oppervlaktewater in de zomer van 1999. *Infectieziekten Bulletin* 11, 133-135
- Stowa (2000). Toxische blauwalgen in binnenwateren. Stowa report 2000-20

NEW ZEALAND:

Risk assessment, management and regulatory approach for cyanobacteria and cyanotoxins in drinking-water

Alexander KOUZMINOV

Senior Advisor, Communicable Disease & Environmental Health Policy Public Health Directorate, Ministry of Health, PO Box 5013, Wellington, New Zealand, E-Mail: alexander.kouzminov@moh.govt.nz

Cyanobacterial blooms occur throughout New Zealand in fresh, estuarine and coastal waters, including those used for drinking-water supplies, recreation and stock-watering. Eutrophication of water bodies in New Zealand has led to an increase of their incidence. Cyanobacteria species in New Zealand are known to produce the cyanotoxins; microcystins, nodularin, cylindrospermopsin, anatoxin-a and saxitoxins. These cyanotoxins have been found in numerous waterways across New Zealand. Microcystins are the most common cyanotoxin. At times these cyanotoxins can reach levels that are hazardous to human and animal health. Each summer in New Zealand health warnings are placed at a number of recreational lakes and there have been several incidences involving cyanotoxins and drinking water supplies. It seems likely that the frequency, intensity, duration and geographic spread of cyanobacterial blooms will continue to increase in New Zealand as both land modification and the resulting eutrophication intensify.

In New Zealand, 73% (in terms of total population) of our drinking water comes from surface water supplies (Table 1). It is therefore likely that cyanobacterial blooms will have significant economic impacts due to an increase in water supply treatment costs or the need to use an alternative drinking-water source. In addition, there are social impacts from the disruption of recreational use of water bodies.

Table 1: Water supplies using surface water as one or more contributing sources,
WINZ, 2004 data

Unit	Total	Distribution zones where one or more contributing sources are surface water
Registered drinking-water zones	2,244	685 (30%)
Population	3 664 307	2 665 826 (73%)

The New Zealand Ministry of Health has developed national criteria for assessing the risk of toxic cyanobacteria in drinking-water supplies. These criteria are part of an integrated management system for drinking-water. The various components of this system complement and mutually reinforce each other. The main objective of this system is an improvement of the public health management of drinking-water supplies.

Management of cyanotoxin risk is addressed in the Drinking-Water Public Health Risk Management Plans (PHRMPs), developed by the Ministry of Health. These cover cyanobacteria and cyanotoxins in a drinking-water source. Each drinking-water supplier should include validation of control measures, which are most effective against cyanotoxins in an individual water safety plans (PHRMPs). The Ministry of Health has revised the *Drinking-Water Standards*

for New Zealand 2000 (DWSNZ:2000). In addition, the Ministry of Health has developed a new section 'Cyanobacteria' in the amended (revised) *Guidelines for Drinking-Water Quality Management for New Zealand 2005* (Guidelines 2005). The health alert levels for cyanobacteria and their toxins, including provisional maximum acceptable values (PMAVs) for cyanotoxins are among the topics considered by this review. This activity is carried out in a framework of the first of two years of transition during which the foundations for the implementation of the Health (Drinking-Water Supplies) Amendment Act will be laid.

The principal emphasis of the health risk management system for cyanobacteria and cyanotoxins is to use a comprehensive multi-barrier process control approach to promote quality assurance. This is complemented by a monitoring programme used as a final quality control which provides a trigger for remedial action where this is necessary. The aim of this management system is to provide a high degree of confidence in the safety of the water for all drinking-water supplies, whether they are large or small (see Table 2).

Table 2: Current and proposed categories of drinking-water supplies in New Zealand

Category of drinking-water supply	Population served	
	Current structure	Proposed structure
Large	More than 10 000	More than 10 000
Medium	5 000-10 000	5 000-10 000
Minor	501-5 000	501-5 000
Small	500 or less	25-500
Very small		Less than 25

Drinking-Water Standards

The current DWSNZ:2000 prescribe:

- Provisional Maximum Acceptable Values for cyanobacterial cells and cyanotoxins
- Referee and some alternative analytical methods for cyanobacteria and cyanotoxins.

The new (revised) *Drinking-Water Standards for New Zealand 2005* (DWSNZ:2005) prescribe:

- new (revised) Provisional Maximum Acceptable Values for cyanobacteria and cyanotoxins
- minimum frequencies of monitoring for source water inspections (linked to different population bands)

Several risk assessment and management activities are specified in the DWSNZ:2005, including recommended actions in response to the cyanotoxins' thresholds being exceeded.

More details

NZDWS:2005 define four priority classes of determinands. The priority classes are ranked according to the potential impact of the determinand on public health if present in excess of its maximum acceptable value (MAV) in drinking-water and quantity of the determinand present in the water supply. For example, Priority 1 determinands are determinands whose presence can lead to major outbreaks of illness, and include *E. coli*, *Giardia* and *Cryptosporidium*. Priority 1 determinands apply to all community drinking-water supplies in New

Zealand and must be monitored in all supplies because they constitute a major public health risk.

Determinands specified to be Priority 2 determinands for the drinking-water supply under consideration are required to be monitored to establish compliance with NZDWS:2005. The Ministry of Health carries out investigations on water supplies from time to time to identify the presence of P2 determinands, until this process is adequately covered by water supply risk assessment procedures carried out by the drinking-water suppliers.

Cyanotoxins, when present at concentrations more than 50% of their MAVs in a distribution zone, are assigned as Priority 2 determinands.

The Medical Officer of Health, on the basis of data collected by the water supplier, has responsibility for determining when cyanotoxins should be assigned as a Priority 2 determinand to a supply distribution zone. The procedure for doing this and the monitoring requirements for Priority 2 cyanotoxins are set out in the DWSNZ:2005.

Cell counts should also be made in the event of animal or human health incidents that may have resulted from exposure to cyanobacteria or cyanotoxins.

The results of cell counts are to be provided to the Medical Officer of Health. Sampling for cells counts may cease once the signs of algal growth disappear, but regular inspection of the source water is to continue.

Guidelines for Drinking-Water Quality Management

The current *Guidelines for Drinking-Water Quality Management for New Zealand 1995* provide details on the enumeration of cyanobacteria and the characterisation of cyanotoxins in general form.

To assist water supply authorities in minimising the risk of public exposure to cyanotoxins the Ministry of Health has developed a new section 'Cyanobacteria' for the new (revised) *Guidelines for Drinking-Water Quality Management for New Zealand 2005* (Guidelines 2005). Some of this information has also been used in the PHRMPs dealing with source waters. The new *Guidelines 2005* provide advice to water supply authorities on how to monitor and manage cyanobacteria and cyanotoxins in drinking-water supplies and how to prevent potentially toxic cyanobacteria blooms.

The *Guidelines 2005* prescribe:

- cyanobacterial cell count and cyanotoxin concentration thresholds and associated compliance conditions
- recommended actions in response to the thresholds being exceeded
- new (revised) sampling requirements (linked to population bands)
- reference list for cyanotoxin testing methods.

The *Guidelines 2005* provide multi-barrier and trigger level-based risk assessment and management approach for routine and in cyanobacterial bloom formation conditions.

The aim of this approach is to achieve an acceptable level of certainty about the water quality through the use of risk management principles to ensure that the barriers to cyanotoxins are working properly. This is done by:

- identification of the causes of blooms formation
- public information on health significance of cyanotoxins
- use of preventive measures to reduce the risk associated with cyanotoxins
- monitoring of water quality to assess the effectiveness of the preventive measures
- responses to failures in preventive measures shown by monitoring results or routine operational checks.

Where river, stream, lake or reservoir water is used as the source of a drinking-water supply, the new *Guidelines 2005* require routine monitoring of cyanobacteria. The *Guidelines 2005* specify minimum frequency for routine monitoring. Samples for algal cell counts, and identification of the organisms, are to be taken fortnightly, if inspection of the source water shows:

- the development of scum on the surface or
- the development of algal growths just below the surface or
- any other evidence of algal growth

Risk management issues include the following main activities:

- barriers for water protection against toxic cyanobacteria and their toxins (Table 3)
- assessment of risk in a drinking water source, including a preparation of a Risk Management Plan for each individual drinking-water supply (see below)
- alert level framework – a matrix-based monitoring and management action sequence approach that water treatment plant operators and managers can use to provide a graduated response to the onset and progress of a cyanobacterial bloom event (based on cell counts in drinking-water source and/or drinking-water toxicity determination) and actions
- preventive and remedial measures
- management of the source water or reservoir
- treatment options, including information on removal of cyanobacterial cells and cyanotoxins

Table 3: Maximum Acceptable Values (MAVs) for cyanotoxins

Name	MAV	Unit	Remarks ¹
Anatoxin	0.006	mg/L	PMAV
Anatoxin-a(S)	0.001	mg/L	PMAV
Cylindrospermopsin	0.001	mg/L	PMAV
Homoanatoxin-a	0.002	mg/L	PMAV
Microcystin-LR toxicity equivalents	0.001	mg/L	PMAV
Nodularin	0.001	mg/L	PMAV
Saxitoxins (as STX eq)	0.003	mg/L	PMAV

The *Guidelines 2005* also provide advice to water supply authorities on how:

- to monitor and manage cyanobacteria in drinking water supplies
- to prevent potentially toxic cyanobacteria blooms
- to minimise the risk of public exposure to cyanotoxins
- to minimise the risk of public exposure to cyanotoxins via drinking water supplies

Public Health Risk Management Plans (PHRMP's) for Water Supplies

PHRMPs for drinking-water supplies, which are an essential part of the Ministry of Health process-control approach in case of potential contamination of water supplies by cyanotoxins, provide the additional benefit of reducing the likelihood of cyanobacterial cells and cyanotoxins entering supplies in the first place. PHRMPs encourage the use of risk-

¹ PMAV – Provisional Maximum Acceptable Values

management principles during treatment and distribution so that monitoring of compliance with drinking-water standards is not the only water quality management technique used further reduce the risk of cyanotoxins.

A matrix-based approach is used in PHRMPs which provides a set of modules for each individual stage of a generalised drinking-water supply system, including barriers, preventive measures and corrective actions against water contamination by cyanotoxins.

The key steps for developing a PHRMP for individual water supply include:

a) Stage - Identification of the barriers to cyanotoxin contamination (Checklist of barriers present)

A supplier should identify the barriers to cyanobacterial contamination of the supply. Water quality is best protected by having several barriers to the entry of cyanotoxins. These barriers may include the following examples, e.g., stop contamination of raw water with eutrophication nutrients; removal of cyanobacterial cells by drinking-water treatment; destruction of cyanotoxins. The barriers need to:

- prevent cyanobacterial cells and cyanotoxins from entering the raw water
- remove cyanobacterial cells and cyanotoxins from the water
- destroy cyanotoxins in the water
- maintain the quality of the water during distribution.

The supplier by using a 'Barriers to Contamination' module (guide) should decide which of the four types of barrier noted above are in place. The guide helps in recognising which barriers are in place, and also provides instruction on what actions or supply elements contribute to these barriers.

b) Stage – Estimating Risk (Risk Information Table)

This module includes guides which can help to identify (a) possible causes of each event, (b) preventive measures to avoid each event, and (c) corrective actions to use if preventive measures fail. A matrix for estimating risk is based on five categories of likelihood (i.e. rare, unlikely, possible, likely, almost certain) and five of consequence (i.e. insignificant, minor, moderate, major, catastrophic), including their detailed descriptions.

c) Stage – Preparing Contingency Plans (Set of Contingency Plans for each supply element)

A supplier should look through the *Contingency Plans* provided in the guide for each *supply element*, and decide which may be useful in his situation. The *Contingency Plans* help to identify the reasons for the failure of the system.

d) Stage – Performance Assessment (Set of instructions for review of the performance of the Plan)

The *PHRMP Performance Assessment* section of the guides can be used as a basis for preparing instructions for reviewing the operation of supplier's overall Public Health Risk Management Plan.

e) Stage – Decision on communication policy and needs

A supplier should identify and record to whom reports concerning the management of public health risk for the supply need to be made, what information they are to receive and how often.

The above-mentioned planning reorganisation of the public health management of drinking-water in New Zealand is aimed at further improvement of drinking-water quality for New Zealand.

landers. This approach will also provide public confidence regarding strict control drinking-water safety in respect to cyanobacteria and cyanotoxins in New Zealand.

Acknowledgements:

Dr Michael Taylor, New Zealand Ministry of Health, Wellington, New Zealand; Dr. Susie Wood, Research Scientist, Cawthron, New Zealand; Anna Crowe, Freshwater Biologist, Cawthron, New Zealand.

References

Drinking-Water Standards for New Zealand 2000, Ministry of Health, Wellington, New Zealand, August 2000. Available: <http://www.moh.govt.nz/water>

Guidelines for Drinking-Water Quality Management for New Zealand 1995, Ministry of Health, Wellington, New Zealand

Kouzminov, A. 'Blooming good' Guidelines for Drinking Water, Public Health Perspective, March 2004, 7(3), Ministry of Health, Wellington, New Zealand. Available: <http://www.moh.govt.nz>

New Zealand Ministry of Health, '*How to Prepare and Develop Public Health Risk Management Plans for Drinking-Water Supplies*,' Ministry of Health, Wellington, New Zealand, 2001, 12pgs. Available: <http://www.moh.govt.nz/water>

Section 'Cyanobacteria', *Drinking-Water Standards for New Zealand 2005* (draft), Ministry of Health, Wellington, New Zealand

Section 'Cyanobacteria', *Guidelines for Drinking-Water Quality Management for New Zealand 2005* (draft), Ministry of Health, Wellington, New Zealand

Water Information System New Zealand (WINZ), 2004. Available: <http://www.moh.govt.nz/water>

POLAND:

Regulation on Cyanotoxins in Legislation

Joanna MANKIEWICZ^{1*}, Tomasz JURCZAK², Katarzyna IZYDORCZYK¹,
Maciej ZALEWSKI^{1,2}

¹ International Centre for Ecology, PAS, 3 Tylna, 90-364 Lodz, Poland, E-mail: mankiew@biol.uni.lodz.pl

² Department of Applied Ecology, University of Lodz, 12/16 Banacha, 90-237 Lodz, Poland

INTRODUCTION AND SURVEY RESULTS

As in many countries of temperate climate, *Cyanobacteria* and in their consequence cyanotoxins appear in Polish freshwater ecosystems. Cyanobacteria are a serious problem for many waterworks and supply systems and for recreation. The lakes of North Poland, situated in picturesque forested landscape, have a high recreational value. Considering socio-economic importance of tourism in this region, good ecological status and quality of water have to be acknowledged as fundamental factors of sustainable development. In 2002, pilot studies of species composition, biomass and toxins production were performed to assess the risk of cyanobacterial blooms in selected Northern lakes of Pomeranian, Olsztynian and Mazurian Districts (Fig.1) (Mankiewicz et al. 2003, 2004). The highest concentration of total microcystins (dissolved and cell-bound) found in surface water in this research amounted to 9.4 µg/L. Following WHO Guidance, the cyanobacteria toxicity levels found in the investigated lakes are in the range for the first alert level for recreation (Falconer et al., 1999), as health impacts cannot be excluded if exposure is intensive.

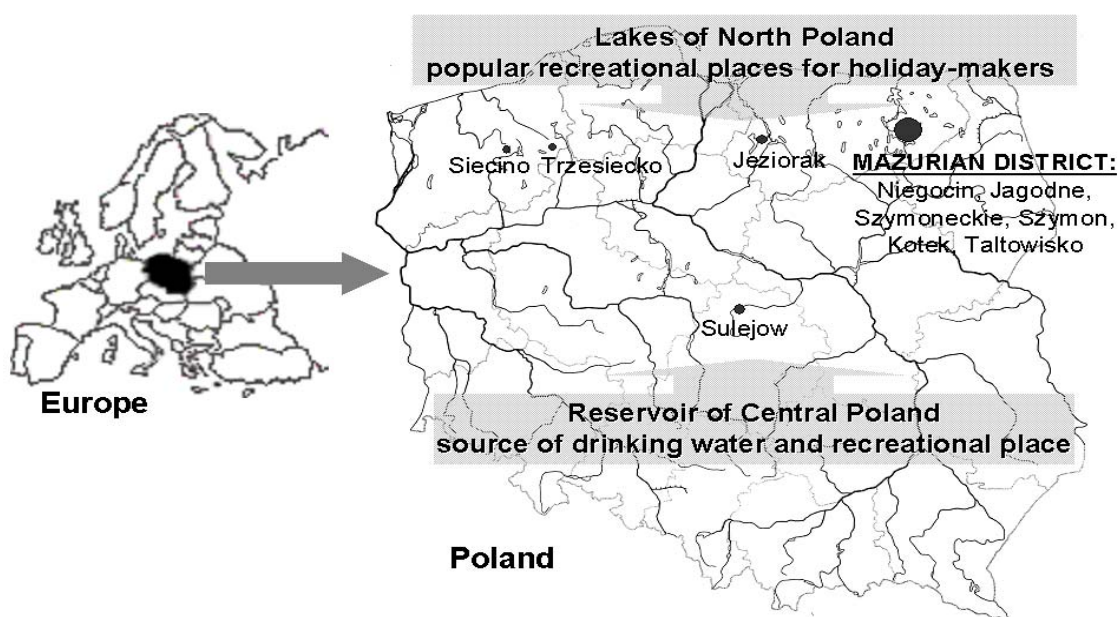


Fig 1. Location of Polish waterbodies with seasonal cyanobacterial blooms included in the survey.

The composition of cyanobacterial blooms in Sulejow Reservoir, a waterbody of Central Poland (Fig. 1) used as municipal drinking water source for the cities of Lodz and Tomaszow Mazowiecki, was typical for Central Europe, i.e. with dominance of the microcystin-producing genus *Microcystis* (Tarczynska et al., 2001). Continued monitoring of Sulejow Reservoir during the summer season from 1996 to 2001 indicated concentrations of microcystins in bloom material ranging from 49 to 1687 µg/g dry mass (Zalewski et al., 2000; Tarczynska et al., 2001; Jurczak et al., 2004) and hygienically relevant levels of microcystins: the highest total microcystins concentration (dissolved and cell-bound) in the raw water from Sulejow Reservoir amounted to 6.7 µg/L and was detected in August 2002. However, microcystin has been shown to break through into finished drinking-water, and in September 1999 a maximum microcystin concentration of 0.8 µg/L was determined in treated water from Sulejow Reservoir (Tarczynska et al., 2001).

The main microcystin (MC) variants detected in the blooms from Polish waterbodies were MC-LR, MC-RR and MC-YR (Jurczak et al., 2004). Additionally, desmethyl microcystin-RR (dm-MC-RR) was determined as a characteristic hepatotoxin for the Mazurian District in North Poland.

MANAGEMENT

Sulejow is a lowland reservoir situated in the middle course of the Pilica River and is very vulnerable for eutrophication, and the scientific background for its restoration has been elaborated by the Department of Applied Ecology, University of Lodz and the International Centre for Ecology, Polish Academy of Science in the framework of the International Hydrological Programme under the auspices of UNESCO and UNEP (Zalewski, 2002; Zalewski and Wagner, 2004). The ecohydrology concept has already been implemented as demonstration project in the Pilica river catchments (<http://www.biol.uni.lodz.pl/demosite/pilica/>). The major goal of the project has been to validate the application of ecohydrology and phytotechnology for converting nutrients from point and non-point sources of pollution into biomass and bioenergy. Research carried out in Sulejow Reservoir also shows that regulation of reservoir hydrodynamics can be successfully applied for control of planktivorous fish to maintain a stable high level of zooplanktonic biofiltration. Large cladocerans by effective filtration are able to control algal and cyanobacterial abundance (Frankiewicz, 2004).

POLISH LEGISLATION

Regulations in Poland for drinking and recreational waterbody use specifically address exposure to Microcystin-LR (drinking water) and exposure to cyanobacteria (bathing water).

For drinking water, the Guideline value recommended by WHO of 1 µg/L of Microcystin-LR has been included in Polish legislation by the Ministry of Health in 2002 (Ministry of Health regulation, 2002a). However, the capacity of proper equipment and laboratories with qualified staff able to determine Microcystin-LR in drinking water is still limited. The use of analytical methods such as high performance liquid chromatography (HPLC) coupled with photodiode-array UV detection (DAD) is needed. Moreover, determination only of Microcystin-LR in drinking water is insufficient because the other toxic variants of microcystin such as MC-RR or MC-YR occur quite frequently in the Polish waterbodies studied (Jurczak et al., 2004). Therefore the Polish regulation should be improved to include the sum of all variants of microcystins, using concentration equivalents to Microcystin-LR for quantification. In this case ELISA (enzyme-linked immunosorbent assay), a rapid (2 hours - treatment time) and sensitive screening test for detection of the sum of all variants of microcystins, could be applied as a useful and cheaper method.

In 2002, the Polish Ministry of Health added the assessment of cyanobacteria to requirements for monitoring bathing water quality. Appearance of cyanobacterial blooms should be controlled by observation of water colour, turbidity and/or odour (Ministry of Health regulation, 2002b). Monitoring should be conducted from April till the end of September every two weeks. Required analysis do not include a determination of total phosphorus and nitrogen as parameters which determine growth of cyanobacteria, or chlorophyll a concentration as quantitative measure. More precise and structured Bathing water regulations, including the determination of cyanobacterial biomass and toxicity, would be desirable.

References:

- Falconer IR, Bartram J, Chorus I, Kuiper-Goodman T, Utkilen H, Codd G. 1999. Safe levels and practices. In: Chorus I, Bartram J (eds.). Toxic cyanobacteria in water: a guide to their public health consequence, monitoring and management. Published on the behalf of WHO by E & FN Spon, London, pp. 155-178.
- Frankiewicz P. How to manage biotic structure in a reservoir. In: M. Zalewski and I. Wagner-Lotkowska [eds.]. Integrated Watershed Management – Ecohydrology and Phytotechnology - Manual. UNESCO, 2004, 194-196 pp.
- Jurczak T, Tarczynska M, Karlsson K, Meriluoto J. 2004. Characterization and Diversity of Cyanobacterial Hepatotoxins (Microcystins) in Blooms from Polish Freshwaters Identified by Liquid Chromatography-Electrospray Ionisation Mass Spectrometry. *Chromatographia* 59: 571-578.
- Mankiewicz J, Izydorczyk K, Jurczak T, †Tarczynska M, Zalewski M. MIDI-CHIP-TOX - “Linking cyanobacterial diversity and cyanotoxins” European Commission, Contract No. EVK2-2002-00546
- Mankiewicz J, Izydorczyk K, Jurczak T, Komarkova J, †Tarczynska M, Zalewski M. 2004. Special distribution of various types of the toxic cyanobacterial blooms in Polish water bodies. 6TH International Conference on Toxic Cyanobacteria. Bergen, Norway, 21-27 June 2004.
- Ministry of Health regulation. 2002a. Regulation of the Minister of Health of 19/11/02 on the requirements concerning the quality of drinking water. *Journal of Laws* № 203/2002, item 1718.
- Ministry of Health regulation. 2002b. Regulation of the Minister of Health of 16/10/02 on the requirements concerning the quality of bathing water. *Journal of Laws* № 183/2002, item 1530.
- Tarczynska M, Romanowska-Duda Z, Jurczak T, Zalewski M. 2001. Toxic cyanobacterial blooms in a drinking water reservoirs – causes, consequences and management strategy. *Water Science and Technology* 1: 237-246.
- Zalewski M, Wagner-Lotkowska I, Tarczynska M. 2000. Ecohydrological approaches to the elimination of toxic algal blooms in a lowland reservoir. *Verh. Internat. Verein. Limnol.* 27: 3176-3183.
- Zalewski M. Guidelines for the Integrated Management of the Watershed. Phytology and Ecohydrology. Newsletter and Technical Publications. Freshwater Management Series No. 5. UNEP, 2002.
- M. Zalewski and I. Wagner-Lotkowska [eds.]. Integrated Watershed Management – Ecohydrology and Phytotechnology - Manual. UNESCO, 2004

SOUTH AFRICA:

Regulatory Approaches to Cyanobacteria

William R. HARDING¹ and Phillip KEMPSTER²

¹ DH Environmental Consulting, P O Box 5429, Helderberg, 7135, South Africa. E-mail: info@dhec.co.za

² Resource Quality Services, Department of Water Affairs and Forestry, P Bag X313, Pretoria 0001.
E-mail: ebp@dwaf.gov.za

Background

South Africa is a water scarce country, mostly devoid of natural fresh water lakes and largely dependent on impounded runoff as a bulk source of raw potable, livestock watering and irrigation water. South African dams have a combined water storage capacity equivalent to 50% of the Mean Annual Runoff, and capture virtually all the available runoff from the interior regions. Several dams are eutrophic and present potential health risks to water consumers and recreational users, as well as impairment of the reservoir ecosystems (DWAF 2001).

The supply of water in South Africa and the southern African region is exacerbated by, *inter alia*:

- the semi-arid climate and the resultant general lack and poor distribution of existing water resources;
- the highly-variable nature of river flows, and extended periods of up to 10 years in which less than average flows are experienced;
- the deteriorating quality of the available water resources, especially in the inland areas of the country where the runoff from developed catchments has impacted several dams to the extent that some water quality safety limits for human and ecosystem health have been exceeded.

Cyanobacteria and cyanotoxins in South Africa

South Africa has a long and proud history of cyanobacterial and cyanotoxin research – with several pioneering advances in this field accredited to South African researchers (see review by Harding and Paxton, 2001). As is the global experience, and as will be evident from this brief synthesis, eutrophication constitutes a major threat to the supply of water free from cyanobacteria and/or cyanobacterial metabolites and toxins in South Africa. Cyanotoxins produced by genera of *Microcystis* and *Anabaena* have been commonplace in South Africa for many decades, while more recent and sustained appearances of *Oscillatoria* (*Planktothrix*) and *Cylindrospermopsis* spp have been regularly recorded (e.g. Van Ginkel and Conradie, 2001).

Regulation and management

After a period of inactivity that spanned much of the 1990s, South Africa is again devoting attention to the related problems of eutrophication and cyanobacteria. Various initiatives have been completed, or are presently underway, *inter alia*:

- an assessment of eutrophication policy and research needs (Walmsley 2000, 2003);
- a review of cyanobacterial research in South Africa (Harding and Paxton, 2001);
- development of a eutrophication-aligned national monitoring protocol (DWAF, 2002);
- an assessment of the trophic status of South African dams (DWAF, 2001);

- development of a guide and modelling tools for assessing eutrophication;(Rossouw and Harding, 2005).
- formulation of an Alert Levels Response Framework for cyanobacteria in relation to the treatment of raw potable waters (in preparation);
- development of a Methods Manual for monitoring phytoplankton, including cyanobacteria (in preparation);
- various continuing studies on cyanobacterial physiology, toxin production and genomics;
- formulation of a strategic research protocol for cyanobacteria and their toxins (in preparation);
- participation in the international, UNESCO-supported CYANONET initiative www.dhec.co.za/cyanonet.

Cyanobacterial toxins: Regulatory guidelines and standards

Guidelines

Following the early 1990s global attention to the public health problem posed by cyanobacterial toxins (e.g. Falconer, 1993), the South African Department of Water Affairs and Forestry incorporated the generic, microcystin-LR-based World Health Organization (WHO, 1999) guidelines for cyanobacterial toxins in drinking (potable) waters into the national water quality guidelines. Here a Target Water Quality Range (TWQR) of 0 --0.8 $\mu\text{g l}^{-1}$ is deemed desirable and concentrations in excess of 1 $\mu\text{g l}^{-1}$ as posing a possible risk of an acute risk of hepatotoxicosis. These values are variously supported by guidelines for chlorophyll-*a* and cyanobacterial cell counts (DWAF, 1999). It is likely that the recently-published Australian recreational guidelines (NHMRC, 2004), such as they pertain to cyanobacteria, will serve as a surrogate in South Africa.

Standards

The issuance and management of standards in South Africa is the responsibility of the South African National Standards Bureau (SANS), formerly the South African Bureau of Standards (SABS). Standards pertaining to water are defined in terms of SANS 241 Drinking Water Standard. A recent (2004) review of SANS 241 concluded that it would be premature to include a formal standard for algal toxins due to the cost implications that this would impose on the smaller water providers.

References

- DWAF (Department of Water Affairs) (1986) Management of the Water Resources of the Republic of South Africa.
- DWAF (Department of Water Affairs and Forestry) (1999) South African Water Quality Guidelines (Second Edition).
- DWAF (Department of Water Affairs and Forestry) (2001) Assessment of the Trophic Status Project N/0000/00/DEQ/1799.
- DWAF (Department of Water Affairs and Forestry) (2002) National Eutrophication Monitoring Programme: Implementation Manual.
- Falconer, IR (ed.) (1993) Algal Toxins in Seafood and Drinking Water. Academic Press.
- Harding, WR and BR Paxton (2001) Cyanobacteria in South Africa: A Review. Water Research Commission TT153/01.

- NHMRC (National Health and Medical Research Council [Australia]) (2004) Guide to Managing Risks in Recreational Water. NHMRC Draft Document.
- Rossouw, N and WR Harding (2005) A Guide to Conduct Eutrophication Assessments for Rivers, Lakes, Dams and Lacustrine Wetlands. Water Research Commission K5/1343/1.
- Van Ginkel, CE and B Conradie (2001) Potential toxic algal incident in the Orange River, Northern Cape, 2000. Department of Water Affairs and Forestry Report N/D801/12/DEQ/0800.
- Walmsley, RD (2000) Perspectives on eutrophication of surface waters: Policy/Research needs in South Africa. Water Research Commission KV129/00.
- Walmsley, RD (2003) Development of a Strategy to Control Eutrophication in South Africa: Phase 1. A Review and Discussion Document. Department of Water Affairs and Forestry.
- WHO (World Health Organization) (1999) Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. Chorus, I and J Bartram (eds.). E & FN Spon Publishers

SPAIN:

Legislation regarding Cyanotoxins

Antonio QUESADA

Dpt. Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain, e-Mail: antonio.quesada@uam.es

Introduction

Drinking water in Spain is mostly stored in reservoirs. Lakes are not abundant and rivers are mostly dammed to store water for the summer months when rain is very scarce or virtually absent in some regions. On the other hand there are more than 1000 reservoirs throughout the country and drinking water shortages only take place sporadically, during extreme drought events. Storing the water in reservoirs, together with the high temperatures and in some cases high levels of eutrophication, means that cyanobacterial blooms can be an important problem. However, very little scientific literature has been published regarding this problem in Spain. Recently, Quesada et al. (2004) working on databases from diverse sources showed that the problem may be widespread, affecting at least 25% of Spanish reservoirs. Until very recently we had no regulations or legislation concerning cyanotoxins in Spanish drinking water supplies although the largest water companies have been doing microcystin analyses for several years.

Legislation

The present legislation in Spain, at a national level, regarding cyanotoxins and cyanobacteria is the *Real Decreto* 140/2003 (7 February 2003). This is a general law establishing the sanitary criteria for water quality for human consumption. This law represents the incorporation of the European Directive 98/83/CE into Spanish law. In general terms, this legislation includes the WHO recommendations for safe drinking water.

The *Real Decreto* makes reference exclusively to drinking water at the site where the water is available for the consumer, and does not include any concept relative to raw waters. Moreover, recognizing that drinking water may become scarce in some regions of the country, the law allows some flexibility in quality when the water distribution cannot be assured from any other source, but only when there is no potential risk for human health.

This law creates an Official Information System (<http://sinac.msc.es>), which collects all the data from the different water companies and municipalities from all the national territory and produces the Official Public Report about the drinking water quality, and delivers it to the European Commission.

In terms of cyanotoxins the law does not allow the final treated water to be used for human consumption if there is more than 1 µg/l Microcystin. However, microcystin concentration is required to be measured only when eutrophication is evident in the water sources. This legal limit for cyanotoxin is routinely revised every 5 years.

The water quality control establishes three different levels:

a) Company control, the responsibility of the water company covers all the chemical and physical analyses related to the water quality. The minimum sampling frequency depends upon the size of the distribution network in terms of the population served. For example for a

large city of 4 million people, this would be at least 1 full analysis per month, depending on the number of water sources (reservoirs, wells, etc) to produce the final treated water.

b) Sanitary surveillance, the responsibility of the sanitary authority to organize periodic samplings and analyses. The sampling frequency will depend upon the circumstances, and it is aimed to complement company analyses.

c) Tap control, the responsibility of the municipality to organize simple analyses except when there is possibility of a particular pollutant, then that pollutant is also to be analyzed. The minimum sampling frequency for a large city of about 4 million people is about 200 samples per year.

Responsibility

The ultimate responsibility for water quality lies with the sanitary authority which assures the quality of the drinking water to consumers. This responsibility of the authorities is conveyed to the water companies and municipalities who are requested to submit periodically the quality data of the water for human consumption. Moreover, the water companies and the municipalities have to publish the information about the quality of water that they are distributing, and to make those reports public.

The law does not consider the creation of a water quality commissariat with the power to sanction companies or municipalities who have failed to meet these sanitary requirements. It does, however, prohibit water distribution when the quality does not fulfil the quality criteria established in this law.

Discussion

This new Spanish legislation includes the guideline value recommended by the WHO of 1 µg/l microcystin-LR, and sets this as the legal limit for drinking water. As for any law intended to improve public health, it is a positive step towards protecting the consumer. However, there is room for improvement and the following concerns will need to be addressed in the future.

Probably the concept that is most lacking in this law is the idea that raw waters are biologically active and dynamic systems. This is a technical law that considers drinking water as a final product and does not consider the treatment process or the water origin. From my point of view considering the water source as an ecosystem would allow the cyanobacterial problem to be treated more effectively as a whole and not just as microcystin producing 'units'. This new law is also somewhat out of date by focusing on microcystins, given the other potential hazards from cyanobacterial massive growths, that are now known such as anatoxins, saxitoxins or cylindrospermopsin. These later problems are also likely to occur in some of the freshwaters of Spain as in other countries.

Focusing on microcystins, there is a problem in the terms used in the law. Specifically, the law uses the term '*microcistina*' (microcystin, i.e. as singular, not as plural), which is not defined. Apparently, it refers to microcystin-LR, as it is based on the WHO guideline value, however, such as it is written, it is not possible to be measured since there are many microcystins without any commercial standard, which hampers determination of their concentrations. This problem is also compounded considering that the law does not recommend or establish any methodology for the measurement of '*microcistina*'. This apparently minor problem can become quite important. For instance, assume the likely case that a water company performs its periodic measurements by HPLC and at a given moment measures, for example, 0.5 µg/l of microcystin LR, and 0.4 µg/l of microcystin RR, plus an important peak, with the highest absorbance, of an unidentified compound, probably an unidentified microcystin. The laboratory could certify this water as safe drinking water, because the compound is not

properly identified as a microcystin, or even if the spectrum of the unidentified peak is used to determine it as a microcystin, it could be argued that the concentration cannot be determined with sufficient accuracy in face of the lack of a quantitative standard. In this case the water would be 'drinkable' from the legal point of view but most probably would be toxic.

This problem would have been solved easily if the legislation would have included the concept of microcystin LR-equivalents, or recommended a series of methodologies to evaluate the risk associated to cyanobacteria in drinking waters. Water authorities in Spain should work together towards establishing these protocols and guidelines.

Summary

The Spanish legislation for safe drinking water adopts the WHO guideline of 1 µg/l microcystin as the legal limit to accept the water for human consumption. This law only considers the water at the point where it is distributed to the consumer, and does not include any regulation of raw waters. The law establishes a series of quality control analyses and responsibilities, but in the case of microcystin does not define the term, nor recommend methodology for its analysis.

Acknowledgements

I am very grateful to Prof. Warwick F. Vincent who helped in the preparation of this manuscript and suggested interesting points that improved the text.

References

Quesada, A., D. Sanchis and D. Carrasco (2004). Cyanobacteria in Spanish reservoirs. How frequently are they toxic?. *Limnetica* 23: 111-120

UNITED STATES OF AMERICA: CYANOBACTERIA AND THE STATUS OF REGULATORY AP- PROACHES

John W. Burns

PBS&J, Suite 1201, 701 San Marco Blvd. Jacksonville, Florida (USA) E-mail: jwburns@pbsj.com

Introduction

Cyanobacteria represent an important ecological component of freshwater, estuarine, and marine systems in the United States of America (USA). They also form toxic blooms in surface waters that are recognized as important recreational and drinking water resources. Cyanobacteria bloom conditions can persist year-round in the more southern geographic regions of the country (e.g., Florida) where sub-tropical climate, increased eutrophication (e.g., nutrient enrichment) of lakes, rivers, and estuaries, significant hydrologic modifications, and continued urbanization of sensitive watersheds exist (SJRWMD 2001). Ecological degradation of surface water systems in the USA have been documented where cyanobacterial blooms are common (Paerl et al. 2003; Paerl et al. 2002; Pickney et al. 1999), and bloom conditions have caused the posting of public warning announcements and closures of recreational water bodies and beaches (Turgeon et al. 1998; Northwest Center For Public Health Practice 1999; Occupational & Environmental Medicine for Clinicians & Public Health Professionals 2003), and the expansion of surface water treatment practices where cyanobacterial blooms are common (AwwaRF 2002). Cyanobacteria toxins have been detected in USA surface waters and in finished drinking water (SJRWMD 2001). Animal mortality and human illness, following exposure to cyanobacterial toxins, have also been well documented in the USA (Yoo et al. 1995).

No federal regulatory guidelines for cyanobacteria or their toxins exist at this time in the USA, but various state and local guidelines have been implemented (e.g. the Oregon Health Division and Oregon Department of Agriculture established a regulatory limit of $1 \mu\text{g L}^{-1}$ of microcystin-LR in dietary supplements containing cyanobacteria; see Gilroy et al. 2000) and research is ongoing.

Impaired Surface Waters

Water quality standards in the USA are set by States, Territories, and Tribes. They identify the uses for each waterbody (e.g., drinking water supply, contact recreation, aquatic life support), and the scientific criteria to support that use. However, over 40% of USA assessed waters do not meet water quality standards. This amounts to over 20,000 individual river segments, lakes, and estuaries. Impaired waters include approximately 300,000 miles of rivers and shorelines and approximately 5 million acres of lakes, polluted mostly by sediments, excess nutrients, and harmful microorganisms. Cyanobacteria blooms are often considered an ecological indicator of impaired waters and are often addressed by setting nutrient and chlorophyll-a reduction targets. An overwhelming majority of the USA population live within 10 miles of impaired waters (USEPA 2005a).

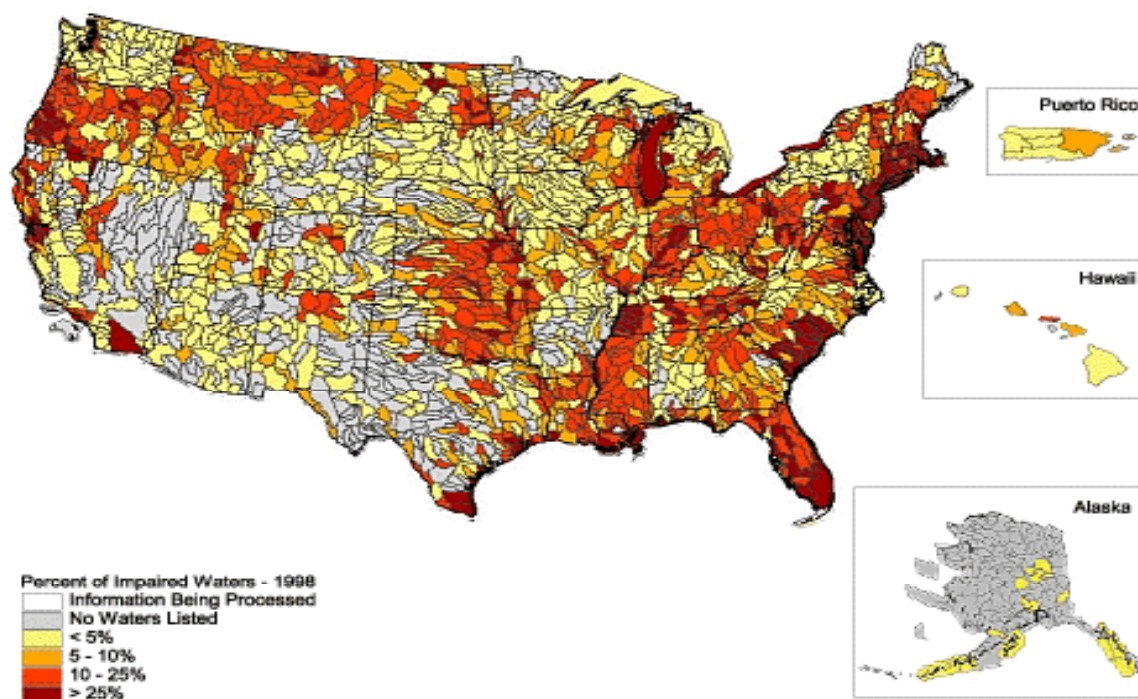


Figure 1: Percentage of water-bodies classified as “impaired” in 1998 in regions of the USA

In a recent effort to speed progress towards meeting water quality standards, the Clean Water Act, section 303, has been implemented to establish water quality standards and TMDL (Total Maximum Daily Load) programs. A TMDL is a calculation of the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards. The regulations that currently apply were issued in 1985 and amended in 1992 (40 CFR Part 130, section 130.7). These regulations mandate that States, Territories, and authorized Tribes list impaired and threatened waters and develop TMDLs (USEPA 2005b). Cyanobacterial abundance and bloom frequency are often recognized and addressed through the TMDL process for impaired water bodies.

Occurrence Data

Carmichael (2005) recently reviewed the history of cyanobacteria occurrence in the USA and found:

“the earliest documented investigation in the USA into the poisonous potential of blue-green algae was recorded in *The Bulletin of the Minnesota Academy of Science* (Arthur, 1883), though the first written description of an actual outbreak did not come until 1925 when a farmer lost 127 hogs and 4 cows after they drank from Big Stone Lake in South Dakota. He had the lake water analyzed and the livestock deaths were attributed to algae poisoning according to the *Wilmot Enterprise* (1925) of September 24th and October 1st. A few years later, *Cornell Veterinarian* published a description of five Minnesota cases of algal poisoning (Fitch, Bishop and Boyd, 1934). A massive *Microcystis* bloom in the Ohio and Potomac Rivers caused intestinal illness in an estimated 5,000 to 8,000 people. According to the article, though the drinking water taken from these rivers was treated by precipitation, filtration and chlorination, these treatments were not sufficient to remove the toxins.”

Today, cyanobacteria are common in surface waters throughout the USA and occurrence studies that investigated toxin levels include, but are not limited to, the following states and water bodies: Florida, Great Lakes, Indiana, Nebraska, Missouri, New York, and Wisconsin. Cyanobacteria blooms are often most pronounced when prolonged drought conditions exist in the USA and the hydrologic condition of lakes and reservoirs are most severely affected. Moreover, the increased need for alternative water supplies in the USA has forced some States to turn to surface waters of poor water quality due to the depletion of existing groundwater resources (e.g., Florida). During June 1996 to January 1998, twenty-four public water systems in the USA and Canada were surveyed for microcystins (AwwaRF 2001). Using ELISA and protein phosphatase assays, 80% of the 677 samples tested positive. Of the positive samples, 4% had microcystin levels greater than the provisional World Health Organization (WHO) drinking water guideline for Microcystin-LR of $1 \mu\text{g L}^{-1}$. A study of Lake Champlain in New York reported microcystin in 18% of all samples analyzed and anatoxin-a in 4% of all samples taken in the summer of 2000 (Boyer 2002). In Wisconsin, microcystin was detected above $1 \mu\text{g L}^{-1}$ in raw water, but not in finished drinking water (Standridge 1999). The Florida investigation (SJRWMD 2001) reported microcystin concentrations ranging from $0.1 \mu\text{g L}^{-1}$ to $12 \mu\text{g L}^{-1}$, cylindrospermopsin as high as $90 \mu\text{g L}^{-1}$, and anatoxin-a as high as $8 \mu\text{g L}^{-1}$ in finished drinking water (SJRWMD 2001). Saxitoxins and other novel bioactive compounds have also been detected in freshwater cyanobacteria (e.g., *Lyngbya*) samples collected from Florida (Berry et al. 2002), New Hampshire (Onodera et al. 1997), New Mexico (Albuquerque Journal 2002), and Alabama (Carmichael et al. 1997).

Ecological & Human Health

Historically, toxic cyanobacterial blooms in the USA have been linked to the death of wild and domestic animals and human illness (Yoo et al. 1995). The only death attributed to cyanobacterial poisoning in the USA occurred recently in Wisconsin. A Dane County coroner attributed the sudden death of a 17 year old male to algal toxin poisoning following exposure to a golf course pond that was dominated by cyanobacteria (Dane County Coroners Office; Madison, Wisconsin, 2002). However, subsequent analytical interpretation of the initial characterization of anatoxin-a, detected in samples from the victim, has been questioned. Suspected cases of cyanobacterial poisoning of humans in the USA have included one or more of the following symptoms: skin irritation, desquamation, eye irritation, gastroenteritis, diarrhea, and respiratory distress. One of the earliest records of gastroenteritis associated with exposure to cyanobacteria in USA drinking water was in the population of a series of towns along the Ohio River (Tisdale, 1931). Following treatment of an algal bloom with copper sulfate in another U.S. drinking water reservoir (containing *Schizothrix calcola*, *Plectonema*, *Phormidium*, and *Lyngbya*), approximately 62% of the population connected to the water supply developed symptoms of gastroenteritis within 5 days (Lippy and Erb 1976).

Increased Awareness & Cyanobacteria Related Events

Awareness of toxic cyanobacterial blooms in USA waters has increased over the past few years given the following events:

- Listing of cyanobacteria and their toxins on the USEPA Contaminant Candidate List (USEPA 1998; see also below)
- Discovery of cyanobacterial toxins in Florida drinking water (SJRWMD report to Florida HAB Task Force 2001)
- The first report of the toxin cylindrospermopsin in North American waters (SJRWMD 2001)

- *Cylindrospermopsis* blooms reported in Indiana (Indiana Division of Fish and Wildlife 2001) and North Carolina (Glasgow 2004) water supplies, as well as other surface waters throughout the USA
- Declaration of a disaster area (Marion Reservoir) by the Governor of Kansas as a precautionary measure to allow emergency response assistance to those who needed sanitary water following the discovery of a cyanotoxic bloom in a water supply reservoir (The Hutchinson News 2003)
- Recurrence of toxic *Microcystis* blooms in the Great Lakes (NOAA 2002)
- Approximately 50 people reported illness following exposure to toxic cyanobacteria blooms at Nebraska lakes and reservoir (Nebraska Health and Human Services System 2004)
- Outbreaks of freshwater *Lyngbya* blooms, in Florida springs (Stevenson et al. 2004) and reservoirs in New Mexico (Albuquerque Journal 2002) and North Carolina (<http://www.marine.unc.edu/Paerllab/personnel/jennifer.html>); identification of novel *Lyngbya* toxins in the Florida Everglades (Berry et al. 2002; marine *Lyngbya* blooms reported off the east and west coast of Florida (http://floridamarine.org/features/view_article.asp?id=2462), causing damage to coral reefs and offensive conditions on recreational beaches.

Drinking Water

There are approximately 160,000 public drinking water systems in the USA (Table 1). Most people in the USA (~268 million) receive their water from a community water supply system (54,000 community systems), but only 7% of those systems serve 81% of the population. Most water supply systems have ground water sources; however, more people drink from systems that are supplied by surface water (USEPA 2003).

In several regions of the USA, water supply is a growing concern. For example, Florida's public supply demand is expected to double by 2020. Alternative water supply sources (e.g., existing natural surface water, construction of large surface reservoirs, brackish ground water, aquifer storage and recovery) are currently under development given the Floridan aquifer can only meet a portion of the demand without ensuing environmental harm. An increased reliance on surface waters for drinking water supply may increase the probability of cyanotoxin exposure to USA consumers where source water quality is poor and water treatment is not focused on cyanotoxin removal or conversion.

Cyanotoxins in drinking water represent a significant concern in the USA due to the lack of coordinated monitoring and treatment programs for cyanotoxins in source and treated water. Although a number of countries have now developed or incorporated recommended WHO guidelines for microcystin-LR in drinking water, no current guidelines or regulatory requirements for cyanotoxins in USA drinking water exist. However, cyanobacteria, other freshwater algae, and their toxins have been listed on the U.S. EPA Contaminant Candidate List (CCL) for further research.

The USA Safe Drinking Water Act (SDWA) was originally passed by Congress in 1974 to protect public health by regulating the nation's public drinking water supply. The law was amended in 1986 and 1996 and requires many actions to protect drinking water and its sources: rivers, lakes, reservoirs, springs, and ground water wells. SDWA authorizes the USEPA to set national health-based standards for drinking water to protect against both naturally occurring and man-made contaminants that may be found in drinking water. SDWA applies to every public water system in the USA.

<u>Type</u>	<u>Attributes</u>	<u>Groundwater</u>	<u>Surface Water</u>	<u>Totals</u>
CWS	No. of Systems	41 499	11 864	53 363
	Population Served	86 348 074	186 981 326	273 329 400
	% of Systems	78%	22%	100%
	% of Population	32%	68%	100%
NTNCWS	No. of Systems	18 908	778	19 686
	Population Served	5 568 192	730 150	6 298 342
	% of Systems	96%	4%	100%
	% of Population	88%	12%	100%
TNCWS	No. of Systems	86 061	2 091	88 152
	Population Served	10 527 089	12 808 136	23 335 225
	% of Systems	98%	2%	100%
	% of Population	45%	55%	100%

Table 1: Drinking Water and Ground Water Statistics (USEPA 2001).

CWS = Community Water System: A public water system that supplies water to the same population year-round.

NTNCWS = Non-Transient Non-Community Water System: A public water system that regularly supplies water to at least 25 of the same people at least six months per year, but not year-round. Examples include schools, factories, office buildings, and hospitals that have their own water systems.

TNCWS = Transient Non-Community: A public water system that provides water in a place such as a gas station or campground where people do not remain for extended periods of time.

On February 23, 2005, the USEPA announced the second Contaminant Candidate List (CCL) (<http://www.epa.gov/safewater/ccl/index.html>). The drinking water CCL identifies priority contaminants for which the USA conducts research to make decisions about whether regulations are needed. The contaminants on the list are known or anticipated to occur in public water systems; however, they are currently unregulated by existing national primary drinking water regulations. Cyanobacteria and their toxins are currently listed on the microbial contaminant portion of the current CCL.

Environmental Health Research

The National Institute of Environmental Health Sciences (NIEHS) - National Toxicology Program, and the Center for Disease Control and Prevention (CDC) - National Center for Environmental Health, have embarked on cyanotoxin research to help provide information necessary to protect human health. The nomination of the cyanobacterial toxin cylindrospermopsin by the National Toxicology program occurred in December 2000 due to the reported presence of the toxin in USA surface waters that are used for drinking water supplies for humans and domestic animals. The CDC has been working with health agencies, universities, and federal partners to investigate how cyanobacteria affect public health. To date, technical and financial assistance have been provided to state health and environmental agencies (e.g., Florida, Vermont, North Carolina) to evaluate water treatment practices, public health impacts of cyanotoxins in drinking water, and recreational exposure to cyanotoxic blooms.

American Water Works Association Research Foundation (AwwaRF)

The water utility industry in the USA is also supporting research on cyanobacteria and their toxins through the American Water Works Association Research Foundation (AwwaRF). The AwwaRF is a member supported, international, non-profit organization that sponsors research to enable water utilities, public health agencies, and other professionals to provide safe and affordable drinking water to consumers. Currently, this organization is investigating the extent of algal toxins in drinking water and advances in new treatment methods for protecting drinking water consumers from this threat. Cyanobacteria toxin research has been ongoing since 1993, with 17 projects related to cyanobacteria and their toxins (see <http://www.awwarf.org/research/TopicsAndProjects/projects.aspx?Topic=Toxins>).

References

- AwwaRF: University of Alberta, University of Wisconsin, USEPA, Metropolitan Water District 2001. Assessment of Blue-Green Algal Toxins in Raw and Finished Drinking Water #256. Available at: <http://www.awwarf.org/research/TopicsAndProjects/projectSnapshot.aspx?pn=256>
- AwwaRF: University of Central Florida, St. Johns River Water Management District, CH2M Hill, City of Cocoa, City of Melbourne Utilities Administration 2002. Treatability of Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies #2839, available at: <http://www.awwarf.org/research/TopicsAndProjects/projectSnapshot.aspx?pn=2839>
- Albuquerque Journal. 2002. *Warning Issued for Dogs at Lake, Elephant Butte Algae May Kill*.
- Berry, J.P., Gantar, M., Gawley, R.E. and Rein, K.S. 2003. Bioactive Metabolites from a *Lyngbya* Species Isolated from Periphyton of the Florida Everglades. Proceedings of the 10th International Conf. Of Harmful Algae. St Pete Beach, Florida, Oct. 21-25, 2002.
- Boyer, G. L., M. F. Satchwell, E. A. Patchett, and X. Yang. 2002. Toxic cyanobacteria in New York state waters; Results from two years of field studies. ASLO Summer Meeting, Victoria, B. C., Canada.
- Carmichael, C. C., W R Evans, Q Q Yin, P Bell, and E Moczydlowski. 1997. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. *Appl Environ Microbiol.* 1997 August; 63(8): 3104–3110.
- Clinton, J. D. and B. C. Cowell. 1984. *Algal Studies of Eutrophic Florida Lakes: Determination of Factors Responsible for Nuisance Blooms of Planktonic Algae in Central Florida*. Final Report to Department of Biology, University of South Florida, Tampa, Florida.
- Cowell, B. C. and P. S. Botts. 1994. Factors influencing the distribution, abundance and growth of *Lyngbya wollei* in central Florida. *Aq. Bot.* 49: 1-17.
- Florida Fish and Wildlife Conservation Commission. 2005. *Blue-green Algal Blooms in Coastal Florida – 1999, 2000, and 2002*. Available at: http://floridamarine.org/features/view_article.asp?id=2462
- Gilroy, D. J., K. W. Kauffman, R. A. Hall, X. Huang, and F. S. Chu. 2000. Assessing potential health risks from Microcystin toxins in blue-green algae dietary supplements. *Environmental Health Perspectives* 108(5):1-7.
- Glasgow, H. B. 2004. Toxic cyanobacteria blooms in Piedmont reservoirs, and early detection by real-time remote monitoring systems. ASLO Summer 2004 Meeting, Savannah, GA. Available at: <http://www.sgmeet.com/aslo/savannah2004/viewabstract2.asp?AbstractID=386&SessionID=CS25>
- Indiana Division of Fish and Wildlife. 2001. Frequently Asked Questions On *Cylindrospermopsis* and Other Potential Toxin-Producing Blue-Green Algae in Indiana Waters. Available at: <http://www.in.gov/dnr/fishwild/fish/cylind.htm>
- Lippy, E. C. and J. Erb. 1976. Gastrointestinal illness at Sewickley, PA. *J. Am. Water Works Assoc.* 68:606-610.
- Nebraska Health and Human Services System. 2004. *Toxins Found In Algae In Several Nebraska Lakes, State Officials Advise Caution*. Available at: <http://www.hhs.state.ne.us/new/0504nr/algae.htm>

- NOAA. 2002. *Microcystis Blooms Return*. Available at:
<http://www.glerl.noaa.gov/pubs/brochures/mcystisflyer/mcystis.html>
- Northwest Center for Public Health Practice. 1999. *Blue-green Algae Closes Green Lake*. Available at http://www.healthlinks.washington.edu/nwcphp/news_archive/8-26-99.html
- Occupational & Environmental Medicine for Clinicians & Public Health Professionals (Occ-Env-Med-L@MC.Duke.EDU) 2003. *Toxic Algae – USA (Nebraska): Alert*. Available at <http://list.mc.duke.edu/cgi-bin/wa?A2=ind0407&L=occ-env-med-1&F=&S=P=1832>.
- Onodera, H., M. Satake, Y. Oshima, T. Yasumoto, and W. W. Carmichael. 1997. New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. *Natural Toxins*, 5:146-151.
- Paerl, H. W., J. Dyble, P. H. Moisander, R. T. Noble, M. F. Piehler, J. L. Pinckey, T. F. Steppe, L. Twomey, L. M. Valdes. 2003. Microbial Indicators of Aquatic Ecosystems Change: Current Applications to Eutrophication Studies. *FEMS Microbiology Ecology* 43:233-246.
- Paerl, H. W., M. F. Piehler, J. Dyble, P. H. Moisander, J. Leonard, A. Waggener, and W. W. Carmichael. 2002. *Phytoplankton and Zooplankton in the St. Johns River System: Factors Affecting Community Structure and Function*. Report to the St. Johns River Water Management District, Palatka, Florida.
- Pinckney, J. L., H. W. Paerl, and M. B. Harrington. 1999. Responses of the Phytoplankton Community Growth Rate to Nutrient Pulses in Variable Estuarine Environments. *Journal of Phycology* 35:1435-1463.
- SJRWMD 2001. *Assessment of Cyanotoxins in Florida's Lakes, Reservoirs, and Rivers*. Report to the Florida Harmful Algal Bloom Task Force, Florida Fish and Wildlife Conservation Commission, St. Petersburg, Florida.
- Standridge, J. H. 1999. Detection of Microcystin Algal Toxins in Raw and Finished Drinking Water. American Water Works Association - Emerging Pathogens symposium, August, 1999; Milwaukee Wisconsin
- The Hutchinson News. 2003. Blue-green algae pollutes Marion Reservoir water. Available at: www.hutchnews.com/past/06-13-2003/region/region2.html
- Tisdale, E. 1931. Epidemic of intestinal disorders in Charleston, WVA, occurring simultaneously with unprecedented water supply conditions. *Am. J. Public Health* 21:198-200.
- Turgeon, D. D., K. G. Sellner, D. Scavia, and D. M. Anderson. 1998. Status of U. S. Harmful Algal Blooms: Progress Towards a National Plan. NOAA.
- USEPA 1998. Drinking water contaminate candidate list. Available at: <http://www.epa.gov/OGWDW/ccl/index.html>
- USEPA 2001. Factoids: Drinking Water and Groundwater for 2000. Available at: <http://www.epa.gov/safewater/pws/factoids.html>
- USEPA 2005a. *TMDL National Overview*. Available at: <http://www.epa.gov/owow/tmdl/status.html>
- USEPA 2005b. Overview of current total maximum daily load – TMDL program and regulations. Available at: <http://www.epa.gov/owow/tmdl/overviewfs.html>