

CYANOBACTERIAL TOXINS IN THE WATER ENVIRONMENT

*Authors: J S Metcalf and G A Codd,
University of Dundee*

A Review of Current Knowledge

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**Foundation for Water Research
Allen House, The Listons,
Liston Road, Marlow,
Bucks SL7 1FD, U.K.**

Tele: +44(0)1628 891589

Fax: +44(0)1628 472711

E-mail: office@fwr.org.uk

Home page: www.fwr.org

TECHNICAL ENQUIRIES

**Any enquiries related to this report
should be addressed to:-**

**Dr R G Ainsworth
Foundation for Water Research**

Front cover image: Sinclair Stammers / Science Photo Library

Light micrograph of a filamentous cyanobacterium (blue-green alga) (group name *Cyanophyta*), called *Oscillatoria sp.*

Cyanobacteria are among the most primitive organisms, dating back to pre-Cambrian times.

Magnification: x80 at 6x7cm, x40 at 35mm size.

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This review is one of a series of ‘reviews of current knowledge (**ROCKs**)’ produced by **FWR**. They focus on topics related to water supply, wastewater disposal and water environments, which may be the subject of debate and inquiry. The objective of each review is to produce concise, independent scientific and technical information on the subject to facilitate a wider understanding of the issues involved and to promote informed opinion about them.

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Review of Current Knowledge

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1 Introduction

Cyanobacteria, also known as blue-green algae, are common members of the plankton of marine, brackish and freshwaters throughout the world. They also occur on rocks and soils and in symbioses with plants and fungi. They have a simple structure at subcellular level and lack a nucleus, a characteristic feature defining them, along with bacteria, as prokaryotes (1). The cyanobacteria also possess a photosynthetic apparatus enabling them to perform photosynthesis as in algae and higher plants, but they lack the chloroplasts in which these reactions occur in the latter organisms. Unicellular and filamentous forms are commonly found amongst the cyanobacteria, with both morphotypes able to produce structures visible to the naked eye, such as pinhead or larger, spherical or irregular colonies (e.g. of *Microcystis*), and bundles of filaments (e.g. of *Aphanizomenon*), like sawdust in shape and size. Further differentiation amongst the cyanobacteria includes the ability of certain filamentous genera, such as *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Nostoc* and *Nodularia* to enzymically fix atmospheric nitrogen in specialised cells termed heterocysts. Several of the filamentous genera also produce other differentiated cells termed akinetes, (spore stages) which permit them to survive periods of adverse conditions such as cold and drought.

Cyanobacteria have the potential to produce mass populations in natural and controlled waterbodies. Such developments, leading to cyanobacterial blooms, scums and mats, is a common, but not invariable, consequence of eutrophication, the enrichment of waters with plant (and cyanobacterial) nutrients (2). These large growths and accumulations of cyanobacteria are often aesthetically undesirable since they discolour the water and cause turbidity in recreational and amenity facilities. Furthermore, cyanobacteria are well documented as being able to potentially synthesise a large number of low molecular weight compounds which cause taste and odour problems. These substances, including geosmin and methyl isoborneol often result in complaints regarding recreational

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and amenity waterbodies and the quality of raw and treated drinking water. Of particular concern are further low molecular weight compounds produced by cyanobacteria which have been shown to have high toxicity to vertebrates, including mammals. These compounds, termed cyanobacterial toxins or cyanotoxins (3-6), are largely unnoticed by users of waterbodies, when compared to the problems associated with taste and odour compounds, since the toxins are colourless and odourless. Many of the cyanobacterial toxins are of high toxicity compared with other biological toxins such as fungal, higher plant and shellfish toxins. Indeed, saxitoxins are considered as chemical weapons by the international Chemical Weapons Convention (7) and saxitoxin and microcystin are listed in the toxins core list for Export Control by the Australia Group (www.australiagroup.net) (8).

Of the cyanobacterial genera which include toxin-forming species, the ones of particular concern when mass populations occur include *Microcystis*, *Anabaena*, *Planktothrix* (formerly known as *Oscillatoria*), *Aphanizomenon*, *Cylindrospermopsis*, *Phormidium*, *Nostoc*, *Anabaenopsis* and *Nodularia*. Together these genera can produce a wide range of cyanobacterial toxins, of varying structures, toxicities and modes of action. Examples of toxic mass populations of cyanobacteria have been reported in fresh-, brackish and marine waters on a global basis (Table 1). Findings with samples of natural populations have been confirmed by the establishment of pure laboratory cultures of cyanobacteria which also produce the respective toxins. In some cases, the cyanobacterial toxins which laboratory cultures synthesise can account for up to 0.5% of cell dry weight biomass, confirming the high, but variable abundance of cyanobacterial toxins in natural populations.

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Table 1. Geographical reports of toxic cyanobacterial blooms, scums or mats.

Europe	Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Norway, Poland, Portugal, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom
Americas	Argentina, Bermuda, Brazil, Canada, Chile, Mexico, USA (at least 27 States), Venezuela
Middle East and Asia	Bangladesh, India, Israel, Japan, Jordan, Malaysia, Nepal, Peoples' Republic of China, Philippines, Saudi Arabia, Sri Lanka, South Korea, Thailand, Turkey, Vietnam
Australasia	Australia (New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia), New Caledonia, New Zealand
Africa	Botswana, Egypt, Ethiopia, Kenya, Morocco, South Africa, Zimbabwe
Marine	Baltic Sea, Caribbean Sea, Atlantic, Indian and Pacific Oceans
Antarctica	McMurdo Ice Shelf

2 What toxic compounds are produced by cyanobacteria?

Cyanobacterial toxins are generally divided into categories based on their principle modes of action in mammalian test systems (3-6). As research into the characterization of cyanobacterial products is intensified, further low molecular weight compounds are being identified, which show adverse biological activities in a range of toxicity-based systems involving aquatic animals, cells and enzymes. Understanding of the true range of cyanobacterial toxins, of their occurrence and health significance, at least to aquatic life, is therefore incomplete.

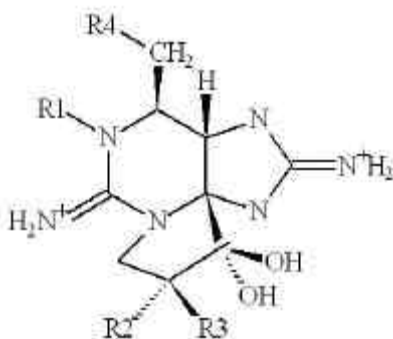
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2.1 Neurotoxins

Of the cyanobacterial neurotoxins, the most widely known are a group of carbamate toxins known as saxitoxins. These are more commonly known as products of marine red tides of dinoflagellate algae. They are known for Paralytic Shellfish Poisoning (PSP), and a group of 21 structurally-related saxitoxin variants is currently recognised (Fig. 1, Table 2).

Their major mode of action regarding toxicity in vertebrates is via the blockage of voltage-gated sodium channels, resulting in paralysis and in acute cases, death.

Figure 1 General Structure of the saxitoxin class of cyanobacterial toxins



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Table 2. Commonly occurring saxitoxin variants

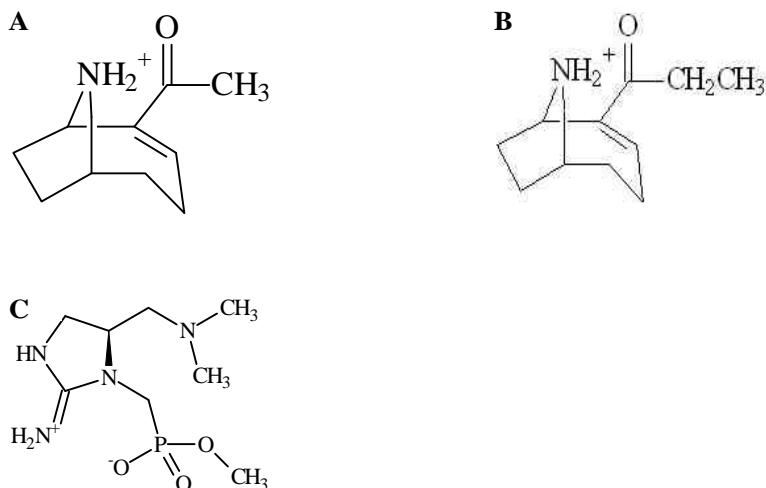
Saxitoxin		R ₁	R ₂	R ₃	R ₄
C1		H	H	OSO ₃ ⁻	CONHSO ₃ ⁻
C2		H	OSO ₃ ⁻	H	CONHSO ₃ ⁻
C3		OH	H	OSO ₃ ⁻	CONHSO ₃ ⁻
C4		OH	H	H	CONHSO ₃ ⁻
Gonyautoxin I	(GTX1)	OH	OSO ₃ ⁻	OSO ₃ ⁻	CONH ₂
Gonyautoxin II	(GTX2)	H	OSO ₃ ⁻	OSO ₃ ⁻	CONH ₂
Gonyautoxin III	(GTX3)	H	H	H	CONH ₂
Gonyautoxin IV	(GTX4)	OH	H	H	CONH ₂
Gonyautoxin V	(GTX5)	H	H	H	CONHSO ₃ ⁻
Gonyautoxin VI	(GTX6)	OH	H	H	CONHSO ₃ ⁻
Decarbomoyl GTX2	(dc-GTX2)	H	H	OSO ₃ ⁻	H
Decarbomoyl GTX3	(dc-GTX3)	H	OSO ₃ ⁻	H	H
Saxitoxin	(STX)	H	H	H	CONH ₂
Neosaxitoxin	(NEO)	OH	H	H	CONH ₂
Decarbomoyl saxitoxin	(dc-STX)	H	H	H	H

R positions in molecule are shown in Table 1

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A second group of cyanobacterial neurotoxins is named after anatoxin-a, the principal member of this neurotoxin family (Fig. 2). Anatoxin-a has only been documented as being produced by cyanobacteria, unlike the saxitoxins. This toxin is a secondary amine and its molecular mode of toxic activity is as a post-synaptic acetylcholine antagonist, resulting in paralysis, asphyxiation and death. Five naturally occurring structural variants, including homoanatoxin-a (Fig. 2), are known, with some of them thought to be degradation products of the parent toxin.

Figure 2 The cyanobacterial neurotoxins, anatoxin-a (A), homoanatoxin-a (B) and anatoxin-a(s) (C)



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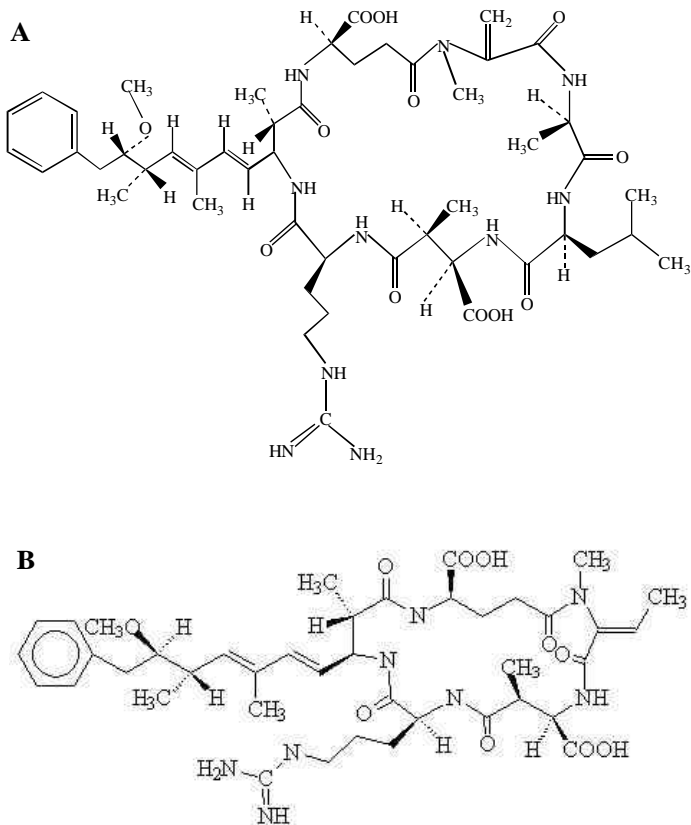
Anatoxin-a(s), although similar in name to anatoxin-a, is quite different in terms of structure and toxicity (Fig. 2). This cyanobacterial toxin is a naturally occurring organophosphate molecule. The suffix (s) indicates that, as with synthetic organophosphorus pesticides, one of the symptoms of intoxication is hypersalivation. So far, no other variants of this neurotoxin have been discovered.

2.2 *Hepatotoxins*

The cyanobacterial hepatotoxins appear to be the most widely distributed types of cyanobacterial toxins in aquatic environments. Of these, the most commonly encountered in freshwaters, and extensively studied, are the microcystins (Fig. 3). These are cyclic peptides consisting of 7 amino acids including the novel amino acid, Adda ((2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid). 71 structural variants of this toxin are currently known. The most commonly found microcystin variant is microcystin-LR. The two-letter suffix is derived from the variability of the molecule, as a result of amino acid substitutions at positions 2 and 4 of the heptapeptide ring, with L-leucine and L-arginine occupying these positions in this particular microcystin variant. Death due to acute microcystin intoxication is preceded by circulatory shock, with the liver approximately doubling in weight as a result of blood pooling within this organ in mammals. At the molecular level, microcystins are potent inhibitors of protein phosphatases, such as PP1 and PP2, key enzymes which regulate developmental, energetic and physiological processes in plants and animals, including humans. The presence of the amino acid methyldehydroalanine, although not present in all microcystins, permits tight binding of the toxin to PP1, which results in inactivation.

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Figure 3 Examples of microcystin and nodularin cyanobacterial hepatotoxins; microcystin-LR(A) and nodularin (B).



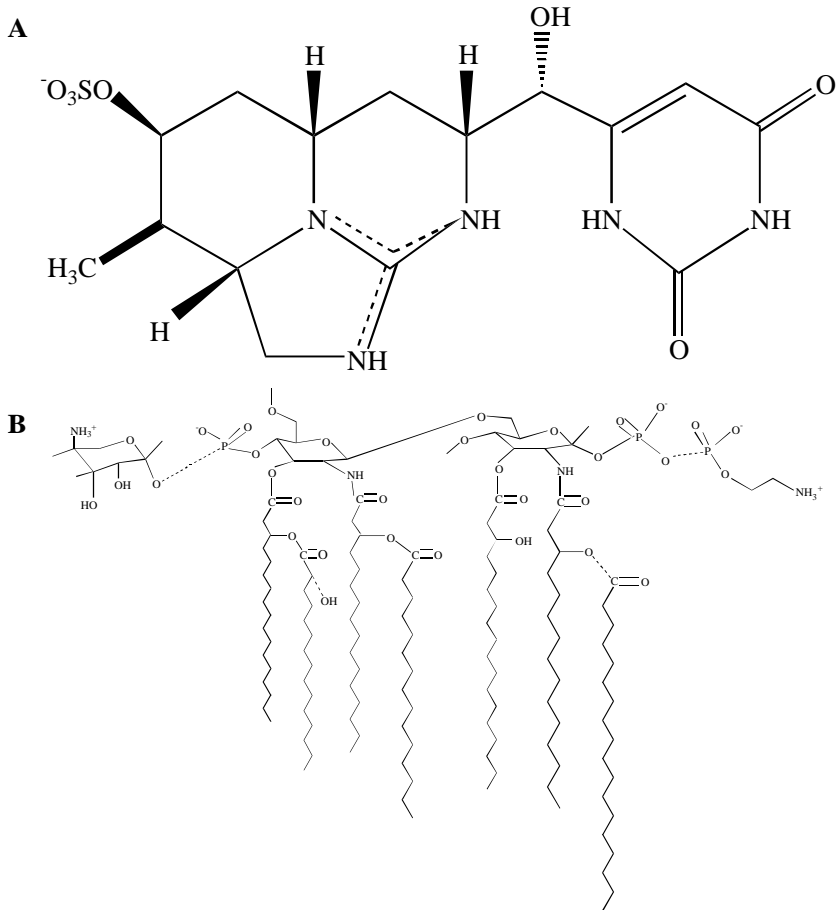
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Similar in structure to the microcystins are the nodularins (Fig. 3). These are cyclic peptides also containing Adda, but with only 5 amino acids in total, compared to 7 in microcystins. Although microcystins are produced by a number of freshwater cyanobacterial genera (e.g. *Microcystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Nostoc*, *Phormidium*), nodularins have only been documented in cyanobacterial blooms and strains of *Nodularia spumigena*, a bloom-forming cyanobacterium more typical of brackish and marine waters. The mode of action of nodularins is similar to that of microcystins with death resulting from haemorrhage into the liver. Carcinogenic activity by nodularin, although apparently not by microcystin, has been demonstrated in laboratory animals. Once tumour development has been initiated, both nodularins and microcystins can serve as potent tumour promoters, stimulating tumour growth.

Cylindrospermopsin is one of the most recently discovered cyanobacterial hepatotoxins. It was identified after a human poisoning incident at an Australian drinking water reservoir in 1979. The toxicity was attributed to a bloom of the cyanobacterium *Cylindrospermopsis raciborskii* in the drinking water supply. Cylindrospermopsins are guanidine alkaloid hepatotoxins (Fig. 4) which are produced by members of the genera *Cylindrospermopsis*, *Aphanizomenon*, *Umezakia*, *Anabaena* and *Raphidiopsis*. Although slower acting than microcystins and nodularins (days *versus* hours), this hepatotoxin affects a wide variety of body organs (e.g. kidney, intestine, lungs), although the liver is the main organ affected. Cylindrospermopsin inhibits protein synthesis and causes gene damage.

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Figure 4 The cyanobacterial hepatotoxin cylindrospermopsin (**A**) and the lipid A region of lipopolysaccharide endotoxin (**B**).



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2.3 *Lipopolysaccharide*

A standard feature of Gram negative prokaryotes, including enteric bacteria, is the potential to produce lipopolysaccharide (LPS). Cyanobacteria are no exception. LPS is considered to be produced by all cyanobacteria and is a structural constituent of the outer layers of the cell wall. LPS is heat-stable and toxic to mammals, with potency varying between different sources of bacteria and cyanobacteria. LPS molecules consist of 3 main parts: O antigens, core polysaccharides and lipid A moieties. The lipid A region (Fig. 4B) is responsible for biological responses and symptoms of LPS-exposure including fever, diarrhoea, vomiting and hypotension. Fevers are generally caused by the release of pyrogenic compounds by the host body in response to LPS ingestion, with haemodialysis water and aerosolised LPS being important potential sources of exposure.

2.4 *Other bioactive compounds*

Cyanobacteria produce a wide range of further novel products with biological activities which include from enzyme inhibition, to skin and gastrointestinal irritation. As with the cyanobacterial toxins summarised in sections 2.1-2.2, these are low molecular weight compounds and include microviridins, anabaenopeptolins, microginins, cyanobacterins, fischerellins and nostocyclamides. These are not acutely toxic to higher animals, but include products which have toxic effects on developmental and digestive functions of zooplankton and with the potential to act as grazing deterrents. Several cyanobacteria which grow as shoreline mats in tropical and subtropical waters produce irritant toxins including aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin-A, which present health hazards to swimmers and include tumour promoters.

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3. How are cyanobacterial toxins detected and quantified?

The detection and accurate quantification of cyanobacterial toxins is necessary to: (a) provide understanding of their occurrence and abundance in natural waters and potable supplies; (b) investigate their toxicities and roles in waterborne health incidents; and (c) contribute to the risk management of waterbodies affected by cyanobacterial abundance. Detection and analytical methods for the toxins require specificity and sensitivity in order to enable adequate warning to be given of impending toxin production and to measure toxin concentrations at below alert or guideline values for permissible toxin concentrations in water supplies. Analytical method development and validation are subjects of intense activity for the protection of human and animal health.

3.1 *Physicochemical methods*

A wide range of chromatographic methods is being used to detect and quantify saxitoxins, anatoxin-a and its analogues, microcystins, nodularins and cylindrospermopsins (9-11). High performance liquid chromatography (HPLC) has been the most widely used tool. As developments in HPLC technology have progressed, these have been applied for the detection of cyanobacterial toxins, as with numerous other classes of environmental analytes. For the analysis of microcystins, cylindrospermopsins and anatoxin-a with UV detection at the respective absorption maximum, photodiode array (PDA) detection has been successfully applied to detect cyanobacterial toxin variants, such as microcystins, when individual calibrated standards are not available. This is due to the fact that the PDA measures the absorption spectra of the individual compounds. These characteristic spectra can be used to detect previously unknown microcystins. The same approach is applicable for the detection of multiple nodularins, anatoxin-a and cylindrospermopsin variants. Other liquid chromatography (LC) detection systems include the use of fluorescence. This has been successfully used to detect further variants of anatoxin-a and

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saxitoxins which do not permit detection by UV absorption. In order to produce fluorescent derivatives of saxitoxins, oxidation of the parent compound is required. So far, this has been performed by two methods, namely pre- and post-column oxidation under acidic conditions by the use of periodic acid. Although the LC methods for the saxitoxins are complicated, resolution of the 21 currently known variants is possible.

Other physicochemical methods for the analysis of certain microcystin and nodularin variants include electrochemical techniques employing cyclic voltametry. However, this procedure is only suitable for those variants that contain arginine and tyrosine residues such as microcystins –LR, -RR and –YR. One drawback of such a method is that due to the nature of the system, environmental samples for analysis may require prior clean-up. In order to detect microcystins and nodularins by physicochemical methods, it is not always necessary to analyse the complete molecule. Methods have been developed to produce and detect a characteristic oxidation product of Adda. The product, erythro-2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) is then analysed by LC with fluorescence detection or by flame ionisation after gas chromatography (GC). Methods for MMPB production are being extended and include ozonolysis followed by analysis using LC and GC-MS (mass spectrometry) systems.

MS offers improved sensitivity for the analysis of anatoxin-a and derivatives compared to UV-based absorbance systems. MS systems such as fast atom bombardment mass spectrometry (FAB-MS), are increasingly being used to elucidate toxin structures and some methods offer the possibility to analyse multiple toxin classes present in the same sample. Presently, LC-MS systems and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) offer the possibility to detect cyanotoxin variants, although capital outlay for such systems is currently high. One potential advantage of MALDI-TOF, although not a quantitative method, is that it can detect a wide

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array of cyanobacterial products. This has resulted in the production of peptide toxin mass peak libraries which are proving useful in cyanobacterial toxin identification.

3.2 *Biological, biochemical and immunological methods*

Traditionally, cyanobacterial toxins have been detected and quantified according to the toxicity which they exhibit in mammalian test systems. The mouse bioassay has held an important role in the detection of cyanobacterial hepato- and neurotoxins. Indeed, the mouse bioassay is still the Association of Official Analytical Chemists (AOAC) approved method for the analysis of marine saxitoxins in contaminated shellfish, and is legislated for the same purpose under EC law. However, as the mouse bioassay is ethically undesirable, and as further knowledge of the molecular modes of action of the toxins advances, new *in vitro* methods as alternatives to mouse bioassay are being introduced (10,11). Currently, *in vitro* systems exist for the detection and quantification of saxitoxins (saxiphilin-binding assays), anatoxin-a(s) (acetylcholine esterase inhibition), cylindrospermopsins (inhibition of protein synthesis) and for the microcystins and nodularins (protein phosphatase inhibition). Most of these methods have been adapted to microtitre plate formats for ease-of-use and mass sample screening, and show good sensitivity in comparison with other methods such as LC.

Other sensitive *in vitro* systems use antibodies, generated against the parent toxin, in immunoassays (11,12). Antibodies against the microcystins and saxitoxins have been developed and results show good correlation with traditional methods of analysis such as HPLC. As the cyanobacterial toxins have molecular weights of less than about 1000 Daltons, these compounds are known as haptens, in that they are unable to invoke an immune response on their own. It is necessary to link these haptens to larger molecules to make a complex which will invoke an immune response to produce specific antibodies against these compounds and also drastically reduce their toxicity before immunisation.

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The cross-reactivity of known cyanobacterial toxins with anti-cyanobacterial toxin antibodies has been shown to vary, dependent on the toxin variant tested, with potentially wide differences in the degree of detection between variants. Antibody-based systems allow detection of microcystin-LR at concentrations of below $1\mu\text{g l}^{-1}$, the provisional Guideline Value for this toxin in drinking water, as derived by the World Health Organisation (WHO; 13). Although much of the work with immunoassays has centred on traditional antibody techniques, new approaches such as naïve phage display libraries and Molecularly Imprinted Polymers are showing promise for microcystin detection.

However, although immunoassays offer the potential of high specificity and sensitivity, and the nature of the reaction is based on structural recognition, one drawback is that they do not provide a direct indication of the toxicity of a cyanobacterial or water sample, which can be possible with *in vitro* enzyme inhibition assays. In order to obtain the advantages of both approaches, it is possible to combine these formats. For example, by combining antibodies against microcystins with protein phosphatase inhibition assays to protect the enzyme from the action of the cyanobacterial toxin, or by isolating the toxin from complex matrices using immunoaffinity before analysis by protein phosphatase inhibition assay. Other *in vitro* methods developed for microcystin analysis include the use of aptamers. These DNA-binding proteins can detect microcystins using voltage systems and by measuring the changes as the DNA binds to the toxin. One possible advantage of these over traditional antibodies is that they can be generated rapidly by the use of the Polymerase Chain Reaction (PCR).

Cyanobacterial LPS can be quantified by haemagglutination assays. The most common method is the *Limulus* amoebocyte lysate (LAL) assay (14,15). Although not specific for LPS, this works on the principle that aqueous extracts from blood cells of the Horseshoe Crab *Limulus polyphemus* will react with the lipid component of LPS, resulting in gel clotting. The method originally

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determined turbidity to measure LPS concentration, to which this is proportional. Now a range of newly developed systems, such as the use of chromogenic substrates, is allowing more accurate LPS quantification. One of the challenges to the risk management of LPS problems associated with cyanobacterial blooms is to determine the relative contributions of the cyanobacterial LPS, versus that of the co-occurring bacteria, to overall LPS toxicity.

3.3 *Concentration of cyanobacterial toxins for analysis*

When cyanobacterial blooms, scums and mats are present, cyanobacterial toxin concentrations are applicable to direct analysis by methods such as HPLC-PDA. However, when cyanobacteria-free - or cyanobacterial toxin-containing water samples require analysis, concentration procedures may be required to permit toxin detection by such methods. Solid phase extraction (SPE) procedures have been successfully applied to meet these needs. For microcystins, nodularins and anatoxin-a, the use of C18 SPE materials has allowed successful retention of these cyanotoxins from water matrices. An analytical method for the analysis of multiple microcystins in particulate material and in potable water, using SPE and HPLC-PDA has been successfully validated and adopted as the British “Blue Book” method in the series “Methods for the examination of waters and associated materials” (16). The International Organisation for Standardisation (ISO) is currently assessing SPE and HPLC-UV methods for the analysis of microcystins and nodularins. Recently, HPLC methods with prior SPE have been applied for cylindrospermopsin analysis in blooms and laboratory strains of *Cylindrospermopsis raciborskii* (17), indicating that high concentrations of this toxin, potentially up to 60% of the total pool, can occur in dissolved form in the cell-free phase. Graphite carbon SPE cartridges are being used to recover cylindrospermopsin from solution, as C18 sorbents are unsuitable for the concentration of this toxin, in contrast to microcystins and nodularins. Although SPE sorbents can concentrate cyanobacterial toxins from water matrices, these materials also retain other small molecules such as pesticides. In order to

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improve the specificity of solid phase sorbents for the specific retention of cyanobacterial toxins, the SPE solid phase, such as C18-bonded silica particles, can be replaced with particles attached to cyanobacterial toxin antibodies to provide an immunoaffinity approach (12). This can give further specificity for the toxins of interest and aid in the further removal of compounds that might interfere with the subsequent analytical method. Recent immunoaffinity methods have shown that 15 purified microcystins and nodularin can be successfully retained from water samples with >80% recovery (18). This use of immunological reagents against the cyanobacterial toxins could have wide and straightforward application for the recovery of the toxins from water for analysis.

3.4 *Determination of the potential for cyanobacterial toxin production*

Increasing research is elucidating the mechanisms of cyanobacterial toxin synthesis. Although the microcystins are peptides, these (and other cyanobacterial toxins) are synthesised by enzymic mechanisms, rather than via the classical biological mechanism of peptide and protein synthesis involving ribosomal RNA (19). By analogy with other peptides and alkaloids produced enzymically by micro-organisms, genetic methods such as PCR are increasingly being applied to detect the presence of genes for the production of microcystins, saxitoxins and cylindrospermopsin by cyanobacteria. However, although genes responsible for the production of the cyanobacterial toxins are known and methods for their analysis are available, only the potential for toxin production can be thereby assessed, and depending on the information required by for example end-users and regulatory bodies, toxin analysis by more traditional methods may still be necessary.

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4 Cyanobacterial toxins as hazards to health

4.1 Human and animal poisoning episodes

Associations between cyanobacterial scums and blooms, and the deaths of wild and domestic animals and human illnesses at waterbodies, were made by several societies in Australia, North America and Europe in the pre-industrial era and before the application of scientific method (20). The occurrence of cyanobacterial toxins was inferred from the deaths of sheep which had drunk water from Lake Alexandrina, South Australia in the 1870's. External and internal signs of poisoning, and survival times leading to sheep deaths after drinking *Nodularia* scum, were reproduced by experimental oral-dosing with this material. It is now well-established that cyanobacterial toxins present hazards to animal and human health. Mortalities of sheep, cows, horses, pigs, dogs, poultry and fish have occurred as a result of the ingestion of cyanobacterial scum, mat and/or bloom material (Plate 1). Microcystins, anatoxin-a, saxitoxins, nodularin, cylindrospermopsin and anatoxin-a(s) have been identified as causative agents, either alone or in combinations.



Plate 1: **a**, microcystin-containing scum of *Anabaena* spp. at a recreational freshwater (former reservoir); **b**, shoreline accumulation of brown mat-forming cyanobacteria (*Phormidium/Oscillatoria*), containing anatoxin-a, at freshwater site of associated dog deaths.

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Although not as commonly reported as animal poisonings, human populations have been adversely affected by the ingestion of water containing cyanobacterial cells and their toxins and by recreational and occupational skin contact (4, 5, 20-22). The most recent and serious known human poisoning episode attributed to cyanobacterial toxins occurred at Caruaru, Brazil in 1996. Water from a drinking water reservoir, which had recently experienced cyanobacterial blooms was tankered to a haemodialysis clinic where it was ineffectively treated and then administered to haemodialysis patients. As a result, 126 patients were severely affected and 60 patients eventually died over a number of months. Of the patients, 86% experienced toxic symptoms, including tender hepatomegaly and biochemical evidence of liver injury. Severely affected patients also showed a range of neurological impairments. Other cyanobacterial poisoning episodes, although not resulting in human mortalities have resulted in the hospitalisation of people. In the UK, these have included soldiers who had been “barrel rolling” and swimming during canoeing and training exercises in water containing *Microcystis* scums. Symptoms included gastrointestinal illness and mucosal membrane blistering, with severe atypical pneumonia and indicators of liver damage requiring hospitalisation.

Poisoning episodes from the ingestion of reservoir water supporting cyanobacterial blooms have also been reported. In 1979, on Palm Island, Australia, indigenous people were hospitalised after drinking water from a source which contained a bloom of *Cylindrospermopsis raciborskii*. Over a 21-day period, 139 children and 10 adults were affected, with approximately 70% receiving intravenous therapy. The syndrome included vomiting, headache, abdominal pain and diarrhoea with injury to the liver, kidneys, lungs, adrenals and intestines. Cyanobacterial strains isolated from bloom material contained cylindrospermopsin, which remains as the primary suspected cause.

Other episodes of human illness associated with cyanobacteria included a statistically significant correlation between the drinking of water from a

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reservoir in Armidale, Australia, with a bloom of hepatotoxic *Microcystis aeruginosa* and indications of liver damage. Eutrophication of waterbodies followed by the rapid development of cyanobacterial blooms can occur due to catchment disturbance. The construction of the Itaparica Dam Reservoir in Brazil resulted in increased eutrophication and the development of blooms of *Microcystis* and *Anabaena*. The human population which drank from this reservoir reported cases of gastrointestinal upset and 88 deaths were recorded over a 42-day period, with cyanobacterial toxins suspected as causatory compounds.

Within the past 20 years, areas of South East China have shown an above average incidence of human primary liver cancer. Microcystins, via their actions as tumour promoters, are thought to have played a key role. Although fungal toxin (carcinogenic aflatoxin) in food and endemic hepatitis B, both known to result in tumour production, were common in these areas, comparison of the microcystin content of different water sources and the incidence of primary liver cancer indicated that people who drank from water sources with concentrations of microcystins were more likely to develop primary liver cancer, although the findings were not statistically significant.

Reports on the effects of human exposure to cyanobacterial LPS are limited, although two exposure routes, via inhalation and haemodialysis, have been inferred to be significant. Concerning haemodialysis, an endotoxaemia incident was reported in 1974 among patients in a Washington DC clinic, coincident with the detection of LPS in the local drinking water and a cyanobacterial bloom in the supply reservoir. The toxins of marine cyanobacteria, the inflammatory and tumour-promoting dermatotoxins, aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin have been reported in Florida USA, Okinawa Japan, and Queensland Australia, particularly in the summers between 1996 and 1998 (20). This dermal route of cyanobacterial intoxication is responsible for severe contact dermatitis conditions such as “swimmers itch” and can occur when people swim

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in the sea in contact with cyanobacteria. These allergic and irritative effects, although not confined to marine environments, have been attributed to several cyanobacterial genera such as *Anabaena*, *Aphanizomenon*, *Nodularia*, *Planktothrix* and *Gloeotrichia*. The irritative effects caused by these and other genera are often exacerbated by swim suits and wet suits which trap the cells next to the skin. The abrasive nature and disruption caused by movement in such suits may help to break down cyanobacterial cells, releasing the toxins, whilst retaining the material next to the skin.

Other risk activities include showering in ineffectively-treated water and during work practices where dermal and respiratory exposure to cyanobacterial scums, blooms and toxins may occur.

4.2 *Animal-dosing studies and risk assessment for the protection of human health*

Much of the data concerning the uptake, toxicity and persistence of cyanobacterial toxins has been obtained by laboratory animal-dosing studies (Table 3; 13). Lethal Dose (LD) concentrations, including those which kill half of a test population (LD_{50}) have been derived for most of the major cyanobacterial toxins. Of further value for risk assessment are the concentrations of toxin which cause the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL). Oral toxicity determinations and lifetime-exposure studies are ideally required to minimise uncertainties. These have only been determined for microcystin-LR and, regarding oral toxicity, recently for cylindrospermopsin (23).

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Table 3. Toxicity of known cyanobacterial toxins by various administration routes

Cyanobacterial toxin	LD ₅₀ (µg per kg)					
	i.v.	i.p.	i.n.	Oral	LOAEL	NOAEL
Microcystins		25-150	36-122	5000-10900	100	40
Nodularin		50				
Anatoxin-a	<100	375	2000	>5000		100
Homoanatoxin-a		250				
Anatoxin-a(s)		20				
Saxitoxins	3.2-3.6	7.6-10.5		251-267		
Cylindrospermopsin		200-2000		4400-6900		30

i.v., intravenous; i.p., intraperitoneal; i.n., intranasal; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level.

The information from these studies permits the estimation of Tolerable Daily Intakes (TDI) for the individual toxins. From these, Guideline Values (GV's) for cyanobacterial toxins in drinking and recreational waters can be derived. For example, the WHO has produced a GV for microcystin-LR in drinking water of 1µg per litre (13). There are grounds for the same GV for cylindrospermopsin. Such GVs take into account uncertainty factors and are designed to afford health protection during potential lifetime exposure via drinking water, and versus occasional recreational exposure. Data from poisoning cases, the dynamics of cyanobacterial populations in waterbodies and, drinking water GVs, can be used to derive guideline levels (GL, or warning thresholds) for cyanobacterial cell and toxin concentrations in recreational waters. Further quantitative toxicity data via oral and lifetime exposure are needed to derive GVs and GLs for the remaining known cyanobacterial toxins.

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Although there is a large amount of information on the toxicity of individual, purified cyanobacterial toxins in laboratory tests, little is known about the effects of multiple dosing, simultaneous or sequential exposure to cyanobacterial toxins of different classes, or to exposure to cyanobacterial toxins plus other toxicants, e.g. pesticides or metals. For example, intranasal exposure of mice to microcystin-LR was found to have a toxicity 10 times greater than that of oral administration of the toxin by gavage. Examination of the nasal cavities revealed extensive necrosis of the olfactory and respiratory zone epithelium. In the same study, anatoxin-a was also administered with microcystin-LR intranasally and synergistic effects were noted (24).

5 Effects of cyanobacterial toxins on wild animals and plants

Cyanobacterial mass populations can have adverse effects on wildlife in addition to humans and domestic livestock (3-6). A wide range of wild animals (mammals, amphibians, fish, invertebrates) and birds have been affected, with consequences ranging from non-fatal (inhibition of invertebrate feeding, delayed fish egg-hatching) to fatal (mass mortalities). Wild animal poisonings can occur after incidental ingestion of cyanobacterial biomass and toxins during drinking or feeding. In the case of fish, it is possible that additional exposure can occur via the gill surfaces. Little information is known about the transfer of cyanobacterial toxins along food chains, although there is evidence for this. The consumption of waterfleas (*Daphnia*), which had previously taken up microcystin, by roach results in the accumulation of microcystin-derived carbon in the fish. Deaths of muskrat have been ascribed to feeding on mussels which had accumulated microcystins. Certainly, cyanobacterial blooms containing μg to mg per litre concentrations of cyanobacterial toxins pose health risks to adults and juveniles of aquatic vertebrates and invertebrates. Aquatic plants (submerged and emergent pondweeds, reeds) can take up microcystin at environmentally-encountered concentrations. The inhibition of whole leaf photosynthesis in French bean plants and in the pondweed *Ceratophyllum* by

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microcystin suggests that cyanobacterial toxins may contribute to the decline in plant diversity in waterbodies dominated by toxic cyanobacterial blooms. Not all wildlife cyanobacterial poisoning episodes are as a result of accidental ingestion of cyanobacteria. Periodic mass mortalities of many thousands of Lesser Flamingos have occurred over recent decades at the Rift Valley lakes of Kenya. These phytoplanktivorous birds are known to feed on cyanobacteria as a major, or sole food-source. Their pink plumage is a result of the ingestion of cyanobacterial pigments. Analyses of dead flamingos have shown significant concentrations of microcystins and anatoxin-a in their livers, stomach, intestines and faecal pellets. These findings, plus signs of poisoning of intoxicated birds and the presence of the hepato- and neurotoxins in environmental cyanobacterial samples indicate that cyanobacterial toxins in the diet, alongside additional toxicants and stressors, are significant contributors to the bird mortalities. Microcystins have also been identified recently as the most likely causes of mass deaths of Greater and Chilean Flamingos in a national park wetland and a zoo pond (25).

6 Multiple fates of cyanobacterial toxins

Cyanobacterial toxins undergo multiple fates after biosynthesis (6). These are not only of biological interest, but of potential for the management of cyanobacterial toxin problems. When cyanobacterial cells are actively growing and healthy, the microcystin pools are mostly retained within the producer-cells. However, with cylindrospermopsin for example, even during growth, a large proportion of the total pool may occur in the external water. However, extracellular release of cyanobacterial toxins is accelerated during cell lysis, such as during natural bloom decay and in water treatment processes if these disrupt the cyanobacterial cells by physical or chemical action.

Even when cyanobacterial toxins have been released, they can potentially persist for long time periods. These small molecules are non-volatile and relatively

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stable. Microcystins can withstand boiling and extremes of pH. However, in natural environments, they are subject to photodegradation by UV and visible light, and to biodegradation by a range of naturally-occurring, harmless bacteria. The adsorption of microcystins to mineral matter and binding to metals have been demonstrated. In addition to the uptake of microcystins and nodularin by aquatic biota, these toxins can be detoxified after uptake by common, naturally-occurring, multispecific enzymes including glutathione-S-transferases (26).

7 What is being done about cyanobacterial toxins?

Raising awareness of the properties and production of cyanobacteria and their toxins is a necessary part of the risk management of cyanobacterial toxin problems in the health, recreation, amenity, agriculture, aquaculture and drinking water supply sectors (27). This is being achieved by workshops, seminars, handbooks for health, environmental and water industry professionals and current European Community (e.g. EC TOXIC, www.cyanobacteria-platform.com; EC MICRORISK, www.microrisk.com projects) and other international agency programmes (e.g. American Waterworks Association Research Foundation, www.awwarf.org). These actions are also needed in developing countries to help to avoid the health problems with cyanobacterial toxins encountered in e.g. Europe, Australia and Brazil over recent years.

The risk management of cyanobacterial toxins currently includes reactive and proactive measures (27-29). Regarding reactive actions, the effectiveness of traditional and advanced drinking water treatment processes for the removal/destruction of cyanobacterial toxins is receiving attention, with encouraging results for microcystins, anatoxin-a and cylindrospermopsin. The removal of cyanobacterial LPS (15) has been demonstrated with a 59 to 97% reduction caused by conventional treatments such as coagulation, settling and sand filtration. Reactive measures also include the formulation of decision-making systems, including emergency measures for access to, and use of

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waterbodies in the event of cyanobacterial populations and toxins having already developed to unacceptable concentrations (27-30).

Basic and applied research on the production, properties and control of cyanobacterial toxins is now at a relatively high international level of activity after decades of neglect. However, numerous uncertainties remain. Research is constrained by the limited availability of purified, quantitative analytical standards for the cyanobacterial toxins and of certified reference materials (11).

Proactive measures need to include increasing basic knowledge of cyanobacterial toxin toxicity, production and persistence. This is necessary to permit the further, confident derivation of drinking water GVs for additional toxins.

Current recommendations exist concerning GL thresholds for acceptable concentrations of cyanobacterial cells and respective chlorophyll *a* and equivalent microcystin concentrations (13). These concentrations, corresponding to risks of high-, medium- and low-severity adverse health outcomes refer to cyanobacterial scums (high risk), followed by about 100,000 cells per ml and 20,000 cells per ml, respectively. At the lowest level, 20,000 cells per ml, the concentration of cyanobacteria is equivalent to about 10µg per litre chlorophyll *a* and 1µg per litre microcystin-LR, the WHO provisional GV for microcystin-LR in drinking water. Guidelines for acceptable concentrations of cyanobacterial cells and microcystin are being introduced at national and local level for potable and recreational waters in several countries. The guidelines, ideally being integrated into decision-making systems and action plans to permit better risk management of water resources which are sensitive to the occurrence of cyanobacterial toxins, are aimed to help to ensure the provision of safe drinking waters and the protection of healthy working- and aesthetically pleasing recreational, aquatic environments. Multidisciplinary working groups have been established at national level to specifically assess the occurrence of

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cyanobacterial blooms and toxins and the potential of waterbodies to support cyanobacterial mass development. Those established in the UK and Australia (27, 28) over the past 15 years have served as a model. Further national working groups have continued with this work in e.g. the USA, Norway, South Africa, the Netherlands, Germany, Poland and France. These groups have and continue to formulate and implement policies for cyanobacterial bloom and toxin risk management. The procedures include exposure and risk assessments, taking into account the internationally-derived GVs and GLs, alongside the national experience.

The WHO GVs and GLs for cyanobacterial toxins and cells are not specifically named in EC legislation. However, with national modification in some cases, they are being used as tools in policy design and decision-making in several countries. In some cases, legislation is being established to govern acceptable concentrations of microcystin and cyanobacterial cells in drinking and recreational waters, e.g. in Poland, Spain, France and Canada.

Since policy development for cyanobacterial toxin risk management follows closely behind the primary research and developing awareness of the health significance and impacts of the toxins, it is necessary for the effectiveness and suitability of action plans to be reviewed periodically, and if necessary modified. This rolling approach is presently being taken by e.g. the Environment Agency for England and Wales and the Scottish Executive (30).

Finally, cyanobacterial toxins and their undesirable effects are being increasingly seen as part of the consequences of eutrophication (2). Measures to reduce the latter by e.g. restricting the excessive enrichment of water resources due to agricultural runoff and inadequately-treated sewage, now being addressed from local to catchment level, are seen as important longer-term actions which will contribute to cyanobacterial toxin risk management.

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8 Summary

Cyanobacteria (blue-green algae) are natural inhabitants of fresh-, brackish and marine waters and are of worldwide distribution. They produce a diverse range of small molecules (cyanobacterial toxins) which are hazardous to human and animal health. The sources and properties of these toxins are briefly reviewed. Their harmful effects range from mild to serious, and include gastrointestinal upsets, skin irritations, liver and neurological damage. Examples of the adverse effects on human health, domestic animals and wildlife are given. Risk assessments for health protection against some of the most common and potent cyanobacterial toxins have been made and included in emerging schemes for the risk management of cyanobacterial toxin problems which can occur in potable and recreational waters. Reactive and proactive measures and further needs in this context are presented. The reduction of cyanobacterial toxin problems in natural and controlled waters as a potential benefit of eutrophication control is also discussed.

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9 References

1. Fogg, G. E., Stewart, W. D. P., Fay, P. and Walsby, A. E. (1973). *The Blue-Green Algae*. Academic Press, London, 459pp.
2. FWR: Eutrophication of Freshwaters. Review of Current Knowledge, FR/R0002. Foundation for Water Research, Marlow, Buckinghamshire, 19 pp. (2000).
3. Codd, G. A. (1995). Cyanobacterial toxins: occurrence, properties and biological significance. *Wat. Sci. Tech.* **32**: 149-156.
4. Carmichael, W. W. (1997). The cyanotoxins. In, J. A. Callow (ed.), *Advances in Botanical Research*, vol. 27, Academic Press, pp. 211-256.
5. Falconer, I. R. (1998). Algal toxins and human health. In, J. Hrubec (ed.), *The Handbook of Environmental Chemistry 5 part C*, Springer-Verlag, Berlin, pp. 53-82.
6. Sivonen, K. and Jones, G. (1999). Cyanobacterial toxins. In, I. Chorus and J. Bartram (eds.), *Toxic Cyanobacteria in Water*, E and F.N. Spon, London, pp. 41-111.
7. Organisation for the Prohibition of Chemical Weapons (2000). *Convention on the prohibition of the development, production, stockpiling and use of chemical weapons and on their destruction*, The Hague, pp. 183.
8. *The Scientific Response to Terrorism* (2003). House of Commons Science and Technology Committee, volume 1, The Stationery Office, London, pp.95.

Review of Current Knowledge

9. Meriluoto, J. (1997). Chromatography of microcystins. *Anal. Chim. Acta* **352**: 227-298.
 10. Harada, K-I., Kondo, F. and Lawton, L. (1999). Laboratory analysis of cyanotoxins. In, see Ref. 6, pp. 369-405.
 11. Codd, G. A., Metcalf, J. S., Ward, C. J., Beattie, K. A., Bell, S. G., Kaya, K. and Poon, G. K. (2001). Analysis of cyanobacterial toxins by physicochemical and biochemical methods. *J. AOAC Int.* **84**: 1626-1635.
 12. Metcalf, J. S. and Codd, G. A. (2003). Analysis of cyanobacterial toxins by immunological methods. *Chem. Res. Toxicol.* **16**: 103-112.
 13. Kuiper-Goodman, T., Falconer, I. and Fitzgerald, J. (1999). Human health aspects. In, see Ref. 6, pp. 113-153.
 14. Anderson, W. B., Slawson, R. M. and Mayfield, C. I. (2002). A review of drinking-water-associated endotoxin, including potential routes of human exposure. *Can. J. Microbiol.* **48**: 567-587.
 15. Rapala, J., Lahti, K., Rasanen, L. A., Esala, A-L., Niemela, S. I. and Sivonen, K. (2002). Endotoxins associated with cyanobacteria and their removal during drinking water treatment. *Wat. Res.* **36**: 2627-2635.
 16. Environment Agency (1998). The determination of microcystin algal toxins in raw and treated waters by high performance liquid chromatography (1998). Methods for the examination of waters and associated materials. Environment Agency, Bristol, pp. 13.
 17. Griffiths, D. J. and Saker, M. L. (2003). The Palm Island Mystery Disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Env. Toxicol.* **18**: 78-93.
-

Review of Current Knowledge

18. Arando-Rodriguez, R., Kubwabo, C. and Benoit, F. M. (2003). Extraction of 15 microcystins and nodularin using immunoaffinity columns. *Toxicon* **42**: 587-599.
 19. Kaebernick, M. and Neilan, B. A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.* **35**: 1-9.
 20. Codd, G. A., Bell, S. G., Kaya, K., Ward, C. J., Beattie, K. A. and Metcalf, J. S. (1999). Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* **34**: 405-415.
 21. Duy, T. N., Lam, P. K. S., Shaw, G. R. and Connell, D. W. (2000). Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Rev. Env. Contam. Toxicol.* **163**: 113-186.
 22. WHO (2003). Guidelines for safe recreational water environments. Vol. 1, Coastal and fresh waters, World Health Organisation, Geneva, pp. 219.
 23. 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. *Env. Toxicol.* **18**: 94-103.
 24. Fitzgeorge, R. B., Clark, S. A. and Keevil, C. W. (1994). Routes of intoxication. In, G. A. Codd, T. M. Jefferies, C. W. Keevil and E. Potter (eds.), *Detection methods for cyanobacterial toxins*. The Royal Society of Chemistry, Cambridge, pp. 69-74.
 25. Codd, G. A., Metcalf, J. S., Morrison, L. F., Krienitz, L., Ballot, A., Pflugmacher, S., Wiegand, C. and Kotut, K. (2003). Susceptibility of flamingos to cyanobacterial toxins via feeding. *Vet. Rec.* **152**: 722-723.
-

Review of Current Knowledge

26. Pflugmacher, S., Ame, V., Wiegand, C. and Steinberg, C. (2001). Cyanobacterial toxins and endotoxins- their origin and their ecophysiological effects in aquatic organisms. *Wasser Boden* **53/4**: 15-20.
27. NRA (1990). Toxic blue-green algae. Water Quality series 2, National Rivers Authority, London, pp. 125.
28. NSWBGATF (1992). Final report of the New South Wales blue-green algae task force. Department of Water Resources, Parramatta, Australia, pp. 159.
29. Yoo, R. S., Carmichael, W. W., Hoehn, R. C. and Hrudey, S. E. (1995). Cyanobacterial (blue-green algal) toxins: A resource guide. American Waterworks Association Research Foundation, Boulder, Colorado, pp. 229.
30. Scottish Executive (2002). Blue-green algae (Cyanobacteria) in inland waters: Assessment and control of risks to public health. Scottish Executive Health Department, Edinburgh, pp. 44.