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Cyanotoxins: Which detection technique for an optimum risk assessment?

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ABSTRACT

The presence of toxigenic cyanobacteria (blue-green algae) in drinking water reservoirs poses a risk to human and animal health worldwide. Guidelines and health alert levels have been issued in the Australian Drinking Water Guidelines for three major toxins, which are therefore the subject of routine monitoring: microcystin, cylindrospermopsin and saxitoxin. While it is agreed that these toxic compounds should be monitored closely, the routine surveillance of these bioactive chemicals can be done in various ways and deciding which technique to use can therefore be challenging. This study compared several assays available for the detection of these toxins and their producers in environmental samples: microscopy (for identification and enumeration of cyanobacteria), ELISA (Enzyme-Linked Immuno-Sorbant Assay), PPIA (Protein phosphatase inhibition assay), PSI (Protein synthesis inhibition), chemical analysis and PCR (Polymerase Chain Reaction). Results showed that there was generally a good correlation between the presence of potentially toxigenic cyanobacteria and the detection of the toxin by ELISA. Nevertheless data suggest that cell numbers and toxin concentrations measured in bioassays do not necessarily correlate and that enumeration of potentially toxic cyanobacteria by microscopy, while commonly used for monitoring and risk assessment, is not the best indicator of real toxin exposure. The concentrations of saxitoxins quantified by ELISA were significantly different than those measured by LC-MS, while results were comparable in both assays for microcystin and cylindrospermopsin. The evaluation of these analytical methods led to the conclusion that there is no "gold standard" technique for the detection of the aforementioned cyanotoxins but that the choice of detection assay depends on cost, practicality, reliability and comparability of results and essentially on the question to be answered, notably on toxin exposure potential.

1. Introduction

Cyanobacteria are able to produce a large number of bioactive secondary metabolites (Burja et al., 2001; Wiegand and Pflugmacher, 2005). Amongst these metabolites different toxin types have been identified, comprising neurotoxins, hepatotoxins, cytotoxins and lipopolysaccharide endotoxins all grouped under the term cyanotoxins (Humpage, 2008). Some of these toxins, such as microcystins, are more potent than cyanide and can represent a significant risk to human and animal health. While cyanotoxins and in particular microcystins have been associated with cattle deaths around the world (Mez et al., 1997; Frazier et al., 1998; Puschner et al., 1998), microcystin was identified as the cause of human fatalities on one occasion in Brazil when water used for dialysis was contaminated and several patients were affected (Azevedo et al., 2002).

In order to minimise the risk of exposure to cyanotoxins, the WHO (World Health Organization) developed a framework of recommendations for the management of algal toxins in drinking and recreational waters (Chorus and Bartram, 1999). This approach has been adopted by many countries around the world (Chorus, 2012). The Australian Drinking Water Guidelines (ADWG) detail Guideline

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Levels in drinking water supplies of 1.3 μg/L for microcystins (Fawell et al., 1999) and Health Alert values of 1 μg/L for cylindrospermopsin (Humpage and Falconer, 2003), and 3 μg/L for saxitoxin (Fitzgerald et al., 1999; ADWG, 2011). ADWG (2011) also advises adoption of a two-tiered Alert Level Framework comprising a notification level and an Alert Level (equivalent to the ADWG health guideline) based on toxin concentration, with a cyanobacteria cell number or biovolume calculated from the toxin concentration multiplied by a worst case toxin cell quota. For example, the microcystin fact sheet states: "Initial notification to health authorities could be provided when numbers of *M. aeruginosa* reach 30% of the density equivalent to the guideline value of 1.3 μg/L microcystin (2000 cells/mL; biovolume 0.2 mm^3/L), while an alert could be provided when cell numbers are equivalent to the guideline value (6500 cells/mL; biovolume 0.6 mm^3/L). For microcystin-producing species other than *M. aeruginosa*, notifications and alerts should be based on biovolumes." (ADWG, 2011).

Effectiveness of risk management depends upon accurate hazard assessment, which in turn depends upon accurate, reliable data that are representative of the real world situation. The quality of the information used for risk management depends on the quality of the analytical method and the accuracy of the assumptions that underlie the conversion of an assay result into an estimate of potential risk (see Table 1). It is this potential risk that is compared to Alert Levels, which are key in the management of toxigenic blooms.

The methods currently most frequently used by the water industry for monitoring the risk of cyanobacterial blooms require application of some assumptions. For example, standard cell toxin content (cell quota) is assumed in translating a toxin guideline value into a cell number or a biovolume that may trigger an alert level, but it is known that reported cell quotas vary considerably with cyanobacterial strain composition (Davis et al., 2014; Willis et al., 2016).

Similarly, relative mouse bioassay potency factors are used to convert saxitoxin (STX; also known as one of the known paralytic shellfish toxins (PSTs)) quantified by chromatography, into toxicity equivalents of STX, but reported values for some variants vary up to 3-fold (Humpage et al., 2010). Additionally, some cyanotoxins occur as a large variety of structural variants. For example, over 80 variants of the hepatotoxic microcystins and more than 30 variants of the neurotoxic PSTs have been described (Onodera et al., 1997; Chorus and Bartram, 1999). Given this diversity of structures and toxic endpoints, it is perhaps no wonder that a range of detection methods have been proposed for estimation of potential toxicity: (1) identification and enumeration of toxigenic cyanobacteria, (2) detection of genes that code for toxin production, (3) detection and quantification of individual toxins and (4) observation of the effects that the toxins produce in model biological systems. This has the potential to give a treatment engineer with limited knowledge of the details of analytical methods, a false sense of security when dealing with a cyanobacterial bloom.

The work reported in this paper aimed to (1) evaluate various methods for detection of cyanotoxins and (2) assess the risk associated with known cyanotoxins in samples collected in Australia from a range of water bodies. This manuscript describes the detection of toxins in these environmental samples using a variety of chemical (HPLC-MS), biochemical and molecular (PCR) methods. Where toxin levels were quantified or cell counts were obtained these were compared to Alert Levels (ADWG, 2011). Findings highlighted the relative advantages and disadvantages of each method and its efficiency in providing data to make an appropriate risk assessment.

Table 1
Assumptions, strengths and weaknesses of various assays available for determination of cyanotoxins in water.

Assay type	Assumptions	Strengths	Weaknesses
Cyanobacterial cell counts	• All important toxin producers have been identified • Toxigenic species can be reliably identified • Toxin production rates per cell are accurately determined and consistent	• Relatively cheap • Minimal capital set-up cost • Minimal recurrent costs • Available as NATA-accredited service	• Relatively low accuracy and precision • Requires well trained microscopists (an issue if staff retention is low) • Only provides an indication of potential toxicity
Chromatography (HPLC, LC-MS)	• Standards are available for all the most abundant and toxic congeners • Standards have been accurately quantified • Relative toxicities of congeners accurately determined and applicable to human exposure patterns	• High level of accuracy and precision • Available as NATA-accredited service	• Highly technical, requires highly trained staff • High capital set-up cost • High recurrent costs • Relatively expensive • May not detect all congeners
Antibody-based (ELISA, dip-stick)	• Antibodies are able to recognise all relevant congeners • Affinity of antibodies all congeners is similar and known	• Reasonable level of accuracy and precision • Standardised and relatively simple protocol for all toxins • Minimal capital set-up cost • Moderate recurrent costs • Theoretically, able to detect unknown congeners	• Assay response is not proportional to toxin quantity or toxicity • Potential for matrix interference
Toxin gene (PCR, qPCR)	• Targeted gene sequences only occur in toxin-producing cells • Gene sequences validated in one species/strain will detect the gene in other species/strains • Gene copy number reflects toxicity (qPCR)	• Relatively cheap • Minimal capital set-up cost • Minimal recurrent costs • Standardised and relatively simple protocol for all toxins • Theoretically able to detect unknown congeners • Toxin gene number generally correlates to toxin production (qPCR)	• Relatively low accuracy and precision (PCR) • Potential for matrix interference • May not detect toxigenic species with target gene sequence variations
Toxicity (cell-based, enzymatic)	• Assay response indicates potential human toxicity	• Assay response is proportional to toxic potency of congeners present in sample	• Relatively low accuracy and precision • Potential for matrix interference