

Alternative Bioassays for the Detection of Cyanotoxins

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Abstract

The presence of cyanobacteria, and their associated toxins, in drinking water supplies presents a public health risk. When specific cyanotoxins are suspected, analyte specific techniques such as HPLC are generally used. However, if there is a bloom of taxa not previously known as toxic, its potential risk has traditionally been determined by the mouse bioassay. Despite its long standing use, this bioassay has several constraints including a lack of precise quantification of cyanotoxins at low concentrations, insensitivity, difficulties in the interpretation of results and slow turnaround time. Additionally, animal ethics guidelines are calling for the elimination of the use of vertebrates for this application. Thus, the aim of this thesis was to investigate, optimise and validate alternative whole organism bioassays for the detection of cyanobacterial toxicity.

As this study was industry funded via the Cooperative Research Centre for Water Quality and Treatment, the potential for research uptake in water industry and other laboratories contracted to undertake routine water quality monitoring, was a guiding factor for research outcomes. The general experimental approach was based on investigating dose-response relationships for selected bioassays tested against cyanotoxins and a variety of cyanobacterial aqueous extracts.

It was found that neither the ToxScreen-II Test, a proprietary bioassay employing the bioluminescent test organism *Photobacterium leiognathi*, nor any of a comprehensive panel of bacteria tested in the disc diffusion bioassay, was sensitive to cylindrospermopsin or microcystin-LR at a maximum concentration of 800 µg/L. The bacteria species tested by disc diffusion technique were also not sensitive to a variety of aqueous cyanobacterial extracts; however, the yeast *Saccharomyces cerevisiae* indicated selective sensitivity to cylindrospermopsin and an aqueous extract *Cylindrospermopsis raciborskii*. It was recommended, that future studies of the suitability of *S. cerevisiae* for detecting cylindrospermopsin, utilise a growth inhibition bioassay utilising a liquid medium with the endpoint measured by photometric determination of turbidity.

The importance of the correct taxonomic identification of *Artemia* species used in the brine shrimp nauplii bioassay was addressed. It was found that *Artemia franciscana*,

not the commonly cited *Artemia salina*, represented the vast majority of studies in which *Artemia* was used as an experimental test organism. A validated and optimised *A. franciscana* nauplii bioassay was reported with maximum sensitivity at 72 h with LC₅₀ values of 0.58 (0.54-0.63) dw µg/mL for cylindrospermopsin and 2.0 (1.8-2.2) dw µg/mL for microcystin-LR. This species was also used to investigate the protective efficacy of the antioxidants vitamin E and Trolox against microcystin-LR. It was revealed that both of these antioxidants offered significant protection against the lethal effect of microcystin-LR. These findings support the use of *A. franciscana* as a suitable test organism for both the detection of cyanotoxins and as a model for exploring mechanisms of toxicity. It was found, however, that caution should be exercised regarding the importation and free availability of this species because of its invasive potential in Australia. Thus, the native Australian species of brine shrimp *Parartemia* spp. should be investigated for future bioassay development.

An insect bioassay was developed using the globally distributed speckled cockroach *Nauphoeta cinerea* for the selective detection of Paralytic Shellfish Poison toxicity of an *Anabaena circinalis* aqueous extract and saxitoxin. This bioassay was found to be tolerant to cylindrospermopsin and microcystin-LR at doses 10 fold greater than the mouse LD₅₀ values while being sensitive to saxitoxin. Likewise, it was found to be tolerant of toxin containing aqueous extracts of *C. raciborskii*, *Microcystis aeruginosa* and *Nodularia spumigena* while being sensitive to *A. circinalis*. Peak sensitivity of *N. cinerea* to saxitoxin was 60 min post injection with an ED₅₀ of 31.2 (27.7-35.1) ng/g body weight.

As the limits of detection for whole organism bioassays are often considerably higher than the health alert values for cyanotoxins, techniques for sample concentration were considered. The use of passive sampler technology was demonstrated for the sequestration and concentration of cylindrospermopsin and deoxy-cylindrospermopsin with the recommendation that future studies refine this technology and that its use is combined with laboratory bioassays.

Both the *A. franciscana* nauplii and *N. cinerea* bioassays were deemed as suitable complementary methods for extending the laboratory capacity in commercial laboratories, normally contracted to undertake cyanobacterial monitoring, to include toxicity testing.