EXECUTIVE SUMMARY

OBJECTIVES

The principle objective of this project was to investigate a range of biological methods that are suitable for detecting toxins in finished water. A key objective was to define methods for quenching chlorine in finished waters to avoid interference in the biological assays.

BACKGROUND

The water industry is moving towards a multi-barrier approach to ensure the security of water quality. This approach includes maintenance of source waters from potential contamination, use of effective water treatment processes and maintenance of the distribution system. To ensure public health, the integrity of these barriers must be continually monitored, including the finished product. Contaminants of concern that may be found in the finished water include those found in the source waters (e.g., atrazine, paraquat) and endocrine disrupting chemicals (e.g., 17β-estradiol, EE2). Toxic cyanobacterial blooms are also capable of producing large amounts of toxin (e.g., microcystins, cylindrospermopsins, saxitoxins, anatoxin-a) along with organic material that can severely stress a water treatment plant. There are also chemicals that are unlikely to occur normally in drinking waters but which may be of concern in the current security climate (e.g., cyanide, botulinum toxin, ricin).

As it is impossible to individually quantify every potential known or unknown contaminant chromatographically an alternative is required. A number of toxicity bioassays have been proposed as screening methods for detecting toxicity in water. The majority of these types of assays have only been validated for detecting toxicity in raw waters or effluents where levels of contaminants are considerably higher than in drinking water (i.e., well above drinking water guideline levels). These bioassays are based on a variety of organisms ranging from luminescent bacteria (e.g., CheckLight assay), yeast (e.g., YES assay), crustaceans (e.g., Thamnotox), zooplankton (e.g., Daphnia and Artemia based assays), fish (e.g., based on chemical avoidance or on biochemical changes), and mammalian cell lines (e.g., primary hepatocytes, various cell lines). These methods detect acute toxicity or cytotoxicity, but generally cannot detect chronic effects such as reproductive toxicity.

The major problems limiting the use of screening bioassays for toxicity detection in finished drinking water are:

- Interference from disinfectants (chlorine), disinfection by-products, various metals such as aluminium, iron and copper.
- Lack of sensitivity to low contaminant levels that are of concern (i.e., around drinking water guideline levels).
- Turn-around times that are too slow to allow real-time management of water supplies.

This project examined ways of overcoming these potential problems.
Table ES.1
Assays evaluated for use with finished waters

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Specific assay</th>
<th>Toxins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian cell-based</td>
<td>1. GFP-VERO assay for protein synthesis inhibitors and general cytotoxins</td>
<td>Cylindrospermopsis</td>
<td>Froscio et al. (2009)</td>
</tr>
<tr>
<td>Bacterial cell-based</td>
<td>Checklight bioluminescence test for water quality</td>
<td>2,4-Dinitrophenol</td>
<td><a href="http://www.checklight.biz/">http://www.checklight.biz/</a></td>
</tr>
<tr>
<td></td>
<td>Brine shrimp assay</td>
<td>Paraquat</td>
<td>Metcalf et al. (2002)</td>
</tr>
</tbody>
</table>

**APPRAOCH**

A range of toxicity screening assays were chosen to evaluate suitability for use with finished water. The assays were chosen to be representative of particular assay types as detailed in Table ES.1.

As disinfection was considered the most significant source of interference that would be consistently present in the finished water, evaluation of methods to neutralize chlorine that would be compatible with the toxicity assays was the major focus of the project. Both standard and novel chemical quenchers were chosen for evaluation. These included sodium thiosulphate, sodium sulphite, ascorbic acid and taurine. Ascorbic acid and taurine were chosen as these are naturally occurring compounds and were expected to be benign in biological systems.

Assays were chosen to be as sensitive as possible to detect expected contaminant levels in finished waters. For example the Envirologix ELISA can detect microcystin levels below drinking water guideline levels (1.3 μg/L in Australia). Assays with short turn-around times e.g., Checklight assay were also evaluated as these may have potential to be carried out in the field.

Chemical quenchers were first evaluated for neutralizing capacity, then applied for use with the assays. For each assay, the following steps were taken.

Determine:

- Effect of chlorine on the assay
- Effect of quenchers on the assay
- Effect of neutralized water (chlorine + quencher) on the assay
- Effect of neutralized real water on the assay performance

**RESULTS/CONCLUSIONS**

Chemical quenchers were determined to be more suitable for use with bioassays than any of the physical methods of dechlorination evaluated. Quenchers were shown to rapidly neutralize
chlorine (within seconds) while the physical methods of dechlorination tested were only capable of partially reducing chlorine levels over the 45 minute test period.

Further evaluation of chemical quenchers for use with the bioassays illustrated that any one quencher does not suit all assays. The appropriateness of the quencher, either sodium thiosulphate, sodium sulfite, ascorbic acid and taurine, was determined by the assay. For example, for testing finished waters for microcystin by Envirologix ELISA, ascorbic acid was determined to be the most suitable chemical to neutralize chlorine. Other quenchers were shown to cause an over-estimation of the microcystin quantification. In contrast, sodium thiosulphate was the most suitable quencher for use with the CheckLight Bioassay. In this case, sodium sulfite and ascorbic acid were themselves toxic to this bacterial assay, while taurine was unable to quench the chlorine present under the assay conditions.

Surprisingly, a number of the bioassays tested were found not to be adversely affected by chlorine, meaning that finished water samples can be tested in these formats without any quencher treatment. These assays included reticulocyte lysate assay for protein synthesis inhibitors, and the cell culture based assays utilizing either toxicity or genotoxicity endpoints.

In addition to the effects of chlorine and the quenchers, the natural waters tested affected some assays. Thus, validation of bioassays using the waters they are intended to be used with should be included during assay establishment.

APPLICATIONS/RECOMMENDATIONS

The research described in this report is directly applicable to the water industry, providing information on the sample preparation required for application of the various assay types to detection of toxins in finished waters. The following quencher/assay compatibilities were determined.

- Envirologix ELISA for microcystin detection—quench chlorine with ascorbic acid prior to sample analysis.
- CheckLight Tox Screen test—quench chlorine with sodium thiosulphate prior to sample analysis.
- Cell culture assays—no chemical quencher required. Assays tolerate chlorine up to 10 mg/L and chlorine does not interfere with toxin detection.
- Reticulocyte lysate assay for protein synthesis inhibitors—no chemical quencher required. The assay tolerates chlorine up to 10 mg/L and chlorine does not interfere with toxin detection.
- Brine shrimp invertebrate bioassay. Sensitive to chlorine ≥5 mg/L chlorine. Use ascorbic acid as a quencher prior to sample analysis.
- Assays should also be validated for use with the particular waters they will be employed to test.