METHODS FOR MEASURING TOXINS IN FINISHED WATER [PROJECT #4020]

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

BACKGROUND:
A number of toxicity bioassays have been proposed as screening methods for detecting toxicity in water. However, one of the problems limiting the use of these assays with finished drinking water is the interference from disinfectants (chlorine). This project focused on evaluating methods to neutralize chlorine that would be compatible with the bioassays. Both standard and novel chemical quenchers were evaluated.

APPROACH:
A range of toxicity screening assays known to detect waterborne toxins such as cyanobacterial toxins and disinfection byproducts were chosen to evaluate their suitability for use with finished waters. The assays chosen included antibody (ELISA) and enzyme based assays, mammalian cell culture assays, a bacterial cell test system, and an invertebrate bioassay. The chemical quenchers sodium thiosulphate, sodium sulfite, ascorbic acid, and taurine were evaluated for neutralizing chlorine in the finished water. For each assay, the following parameters were determined: (1) effect of chlorine on the assay, (2) effect of quenchers on the assay, (3) effect of neutralized water (chlorine + quencher) on the assay, and (4) effect of neutralized source waters 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 Enviroylogix 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 $\geq 5$ 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.

RESEARCH PARTNERS:
- Drinking Water Inspectorate
- Water Quality Research Australia Limited