INVESTIGATION INTO THE FORMATION OF TRIHALOMETHANES, CHLOROPHENOLS AND DIOXINS AFTER CHLORINATING WATER CONTAINING THE CYANOBACTERIAL TOXIN CYLINDROSPERMOPSIN

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ABSTRACT

Cyanobacterial toxins such as cylindrospermopsin and microcystins are potent toxins, but recent studies have demonstrated that these compounds can be effectively degraded using chlorine treatment, providing that the residual chlorine levels are sufficiently high. However, chlorine treatment is associated with formation of a wide variety of disinfection by-products that are associated with a series of health effects including carcinogenesis. The aim of this study was to assess formation of disinfection by-products during chlorine treatment of water, which was spiked with cell free extract (CFE) material of toxic cyanobacteria. In this study the formation of trichloromethane and chlorophenols was associated with chlorination of CFE of *C. raciborskii*, while the formation of other trihalomethanes, chlorinated benzenes or polychlorinated dibenzodioxins and dibenzofurans was not detectable. With respect to THM formation, data from this study indicate that the increase in trichloromethane concentration was primarily the result of chlorination of organic matter other than cyanotoxins. Furthermore the levels of trichloromethane formation associated with degradation of high levels of cyanobacterial material is relatively low when compared to the levels which are associated with current drinking water in Brisbane and with the Australian drinking water guidelines. In summary the results from this study did not identify any significant risk associated with the chlorine treatment of water containing the cyanobacterial material containing the cyanotoxin, cylindrospermopsin.

INTRODUCTION

Toxic cyanobacteria such as *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et S. Raju, and *Microcystis aeruginosa* (Kutzing) are common and have been associated with human health problems [1,2]. Dissolved cyanotoxins are not readily removable from water using general drinking water treatment methods (flocculation, sedimentation and filtration) [3]. Cylindrospermopsin is more often in the dissolved fraction than microcystins, hence subsequent treatment steps such as use of activated carbon may be needed to reduce dissolved cyanotoxin concentration [4,5]. Chemical oxidants such as chlorine have also been shown to be effective for degradation of microcystin-LR [6] and cylindrospermopsin [7]. Chlorination can cause the formation of potentially toxic disinfection by-products (DBBs) such as the volatile trihalomethanes (THMs). DBBs are formed from precursors such as humic substances and phytoplankton metabolites [8]. Epidemiological studies have found evidence of a causal link between consumption of chlorinated drinking water and in particular THM levels, and elevated cancer occurrence (i.e. bladder and colorectal) [9]. Other potentially harmful DBBs include chlorinated phenols which have been associated with embryotoxicity and tumor promotion [10,11] and an elevated risks of non-Hodgkin's syndrome and soft tissue sarcoma [12]. In addition to traditional DBBs, formation of polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs, commonly referred to as dioxins), which include some of the most toxic compounds known, has been associated with chlorine oxidation for example in paper bleaching.

The aim of this study was to assess formation of THM's, chlorophenols and dioxins as a result of chlorination of toxin containing cyanobacterial extracts. This is essential to assess risk management steps related to the degradation of cyanotoxins. At the time of experimentation, there was very little cyanobacterial activity in reservoirs used in this study.

MATERIALS AND METHODS

Cyanobacterial material. Cultures of *C. raciborskii* were harvested and prepared for experimental work as set out in Senogles *et al.*, [7]. Microcystin was obtained from a natural occurring *M. aeruginosa* bloom in Brisbane. Microcystin-LR was the dominant toxin present, with few other microcystins present at relatively low levels.

Chlorination. Sodium hypochlorite was used as the chlorine source for this work. Chlorine residuals were determined by DPD calorimetric analysis [13], where a 25 mL sample was collected and analysed on a HACH DR-2000 spectrophotometer.

Organic carbon quantification. A standard Dissolved Organic Carbon (DOC) working solution of 50 mg/L potassium hydrogen phthalate in water was used as the source of OC in experimental samples. Total organic carbon was determined by acidifying samples to a pH ≤ 2 upon collection. A Shimadzu TOC-5000 organic carbon analyser was used for DOC quantification.

Harmful Algal Blooms 2000
Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J. and Lewis, R.J. (eds)
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Solution pH. The effects of solution pH were not measured in this study. The pH was unbuffered and varied with the addition of chlorine, pH 6.7±0.5.

Protocol

Experiments 1-2: Trihalomethane formation in low volume water samples. In Experiment 1, 250 mL of reverse osmosis (RO) water was spiked with *C. raciborskii* CFE material, and then chlorinated with a chlorine dose of 40 mg/L which is more than 10 times in excess of the dose required to degrade cylindrospermopsin (CYN) [7]. Blanks consisting of RO water, RO water containing no CFE, but which was chlorinated with 40 mg/L, RO water containing CFE but no chlorine and water from the tap were included.

In Experiment 2 the study was altered to include dissolved organic carbon (4 mg/L which is similar to the DOC in the cyanobacterial samples). Furthermore the chlorine dose was reduced to relevant doses of 4 and 8 mg/L of chlorine. Controls included water from the tap and water containing the CFE or OC, but which were not chlorinated. Expt's 1 and 2 were carried out in triplicates and subsamples were analysed for THMs.

For analysis of THMs in the samples, subsamples (10 mL) were transferred into calibrated centrifuge tubes and after the addition of 2 mL of pentane shaken for 3 min in an automated shaker. The supernatant phase was then carefully transferred into vials and analysed using GC-ECD. Quantification was performed using external calibration.

Experiment 3 Dioxins and chlorophenols. Experiment 3 focused on the more potent toxicants such as PCDD/Fs and also potentially unknown DBBs specifically related to cyanotoxin degradation, 40 - 80 L of water was spiked with CFE of *C. raciborskii* or *M. aeruginosa*. After chlorination (30 min contact time) compounds of interest were collected using a filter/sorbent system which was designed to enrich lipophilic organic chemicals from water [14], where compounds associated with suspended particles are collected on glass fibre filters and dissolved phase compounds are enriched on XAD-2 resin. For chlorophenol sampling the water was acidified to a pH < 2 prior to sampling.

For chlorophenol analysis (Expt. 3) filters and resin were spiked with a cocktail of deuterated 3-5 ring PAHs. The filters were extracted in an ultrasonic bath using first acetone (50 mL) and dichloromethane (2X). XAD-2 cartridges were rinsed on the outside with acetone and then transferred into soxhlet. Then about 200 mL of acetone and 200 mL of dichloromethane were carefully added onto the cartridge, and the samples were soxhlet extracted for at least 10 hours. Both, the extracts from the filters and the extracts from the XAD-2 were then concentrated to about 50 mL and subject to liquid/liquid partitioning adding n-hexane. The water phase was washed 2 more times with n-hexane and the combined solvent phase was filtered through anhydrous sodium sulfate and then concentrated to about 1 mL. The samples were analysed on a GC-MS operated in full ion scan. Chromatograms were qualitatively examined with respect to the occurrence of specific known compounds such as chlorobenzenes and chlorophenols as well as unspecified compounds with a mass-fragmentation typical for chlorine substitution.

For analysis of PCDD/Fs filter and resin extracts were combined from the individual treatments and the samples were sent to ERGO-Forschungslabor in Germany. At ERGO the samples were transferred into n-hexane spiked with a known quantity of 12 carbon labeled tetra- to octachlorinated PCDD/Fs. Samples were purified on an acid/base activated silica column followed by a fractionation on a column filled with basic aluminium oxide. Samples were then concentrated almost to dryness, transferred into microvials and taken up into 20 µL of toluene containing known amounts of 1,2,3,4-TCDD as a recovery standard. Samples were analysed on a GC coupled to VG-Autospec at a resolution of approximately 10,000.

RESULTS AND DISCUSSION

Reproducibility

Reproducibility of the experimental set-up was tested for experiments 1 and 2 where samples were analysed in triplicates. Trichloromethane was the key analyte which was detectable in all samples, and which varied significantly between different treatments. The mean coefficient of variation (CV) of trichloromethane was 11 %. In 85 % of the treatments the CV was smaller than 10 %. This demonstrates that the methods used were appropriate considering that the reproducibility includes variations in sample set-up, extraction and the analytical method. Experiment 3 which required relatively large quantities of the cyanobacterial material as well as other resources was not replicated.

Experiment 1 and 2

The formation of trihalomethanes at chlorination levels of 40 mg/L which is at least 10 times greater than that required to remove CYN [7] was analysed. Trichloromethane was elevated with 140 (± 4.0) µg/L in samples with chlorinated CFE compared to the concentrations in the non-chlorinated treatments which were 11 and 9.6 (± 0.3) µg/L for the controls (i.e. RO water only and RO water with cylindrospermopsin, respectively) (Fig. 1).

A minor increase in dichlorobromomethane from about 6.2 (±0.16) µg/L (RO water) and 5.7 (±0.11) µg/L (RO water with only CFE) to 7.5 (±0.17) µg/L in the chlorine treated solution which contained *C. raciborskii* material was observed. Chlorodibromomethane and tribromomethane were below the detection limit in all

treatments, however, chlorodibromomethane concentrations in tap water was 12.2 (±0.32) µg/L, at least a factor of 6 greater than those in the chlorine treated *C. raciborskii* sample or any of the control samples (detection limit 2 µg/L).

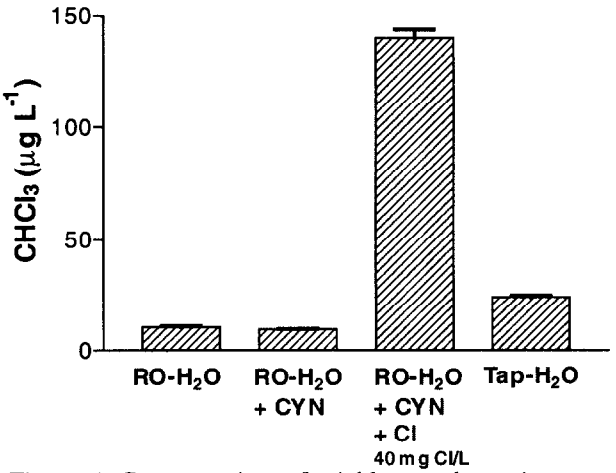

Figure 1 Concentration of trichloromethane (mean and standard deviation) in control samples (RO water and RO water with CYN as CFE) compared to water with CYN as CFE and chlorine treatment and tap water.

To test whether significant formation of THMs is observable in water containing CFE material from toxic *C. raciborskii*, more realistic doses of chlorine (4 or 8 mg/L) were examined. In addition comparisons of THM formation between the chlorination of CYN and a standard OC solution were examined. In Expt. 2, trichloromethane was the only trihalomethane which was detectable in all treatments while dichlorobromomethane, chlorodibromomethane and tribromomethane were only detectable in the tap water. Trichloromethane concentrations in Expt. 2 were lower than in Expt. 1. Mean concentration of trichloromethane in all non-chlorinated treatments (i.e. RO water only, RO plus CFE of *C. raciborskii* and RO plus OC) were 1.3 to 1.7 µg/L. Addition of 4 and 8 mg/L of chlorine resulted in a successive increase in the trichloromethane concentration for all treatments as may be expected (Fig. 2). However THM concentrations in the chlorinated controls (only RO water, open bars) were similar or even higher compared to the chlorinated *C. raciborskii* samples (grey bars). This indicates that increased THM formation (as opposed to non-chlorinated samples) is not a result of the chlorination of cyanobacterial material specifically. The OC present will lead to THM formation as seen with samples containing a standard source of OC. Hence THM formation is a result of chlorinating OC not the transformation of cyanotoxins. It is further noteworthy that the trichloromethane concentration in tap water was between 25 and 35 µg/L and thus significantly higher than those observed in all treatments from Expt. 2. This suggests that the THM related risk associated with degradation of toxic CFE material was low compared to the existing THM risk.

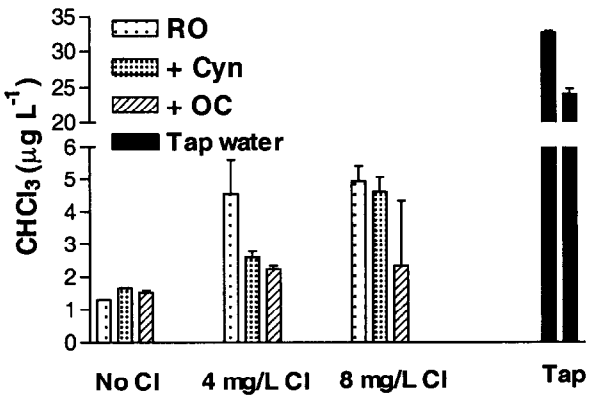

Figure 2 Concentration (mean and standard deviation of n=3) of trichloromethane in various treatments (i.e. with CYN as CFE or a commercial organic carbon source (OC) and at various chlorination levels) and water at the tap in Brisbane.

Experiment 3

In experiment 3 formation of more potent toxicants which require more sensitive analytical methods were examined. The initial focus was to analyses the samples using GC-MS (full ion scan) to identify compounds with mass-fragmentations typical of chlorine substituted compounds. No lipophilic chlorinated compounds such as chlorinated benzenes could be detected. Chlorophenols, and in particular dichloro- and trichlorophenols, were the only compounds of interest which could be identified, and which were elevated in the chlorinated *C. raciborskii* CFE samples compared to the non-chlorinated control samples. An increase in chlorophenols was also observed in chlorinated RO water compared to the control samples. The chlorophenol data from Expt. 3 should be seen mainly as qualitative data since to date these experiment have not been replicated and the extraction efficiency could not be quantified with certainty. It is thus not clear, whether the observed difference in, for example trichlorophenol concentration is significant in the treatments which received extra chlorine (i.e. with *C. raciborskii* or just RO water) (Fig. 3).

The water extracts from Expt. 3 were also analysed for 2,3,7,8-chlorine substituted tetra- to octachlorinated dibenzodioxins and dibenzofurans (the most toxic PCDD/Fs). Lower chlorinated 2,3,7,8-substituted PCDD/Fs including 2,3,7,8-tetrachlorodibenzodioxin, the most toxic of all PCDD/Fs were below the detection limit of 0.1 pg/L in all samples.

Heptachlorinated PCDDs and octachlorodibenzofuran, but in particular octachlorodibenzodioxin were detectable in the samples at low levels. However the concentration in samples which contained *C. raciborskii* or *M. aeruginosa* CFE and which were consequently treated with chlorine were not found to be elevated in comparison

to the control sample (no cyanobacterial material or chlorine added). Interestingly the highest PCDD/F concentrations (i.e. OCDD) were found for the sample of RO water which contained no cyanobacterial material but which was chlorinated (Fig. 4).

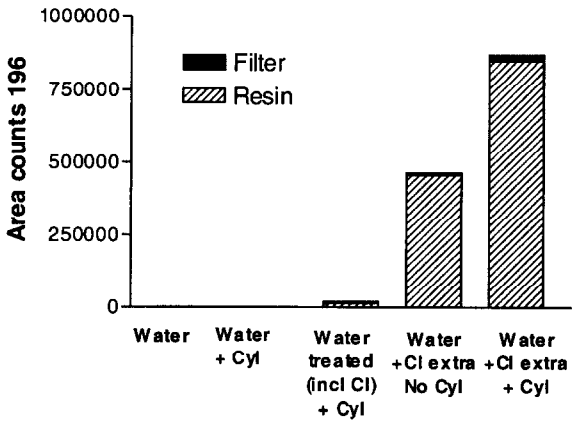

Figure 3 Response of the main ion of trichlorophenol in various treatments of water. Here the water used had background chlorine residual, and extra chlorine represents the addition of more chlorine.

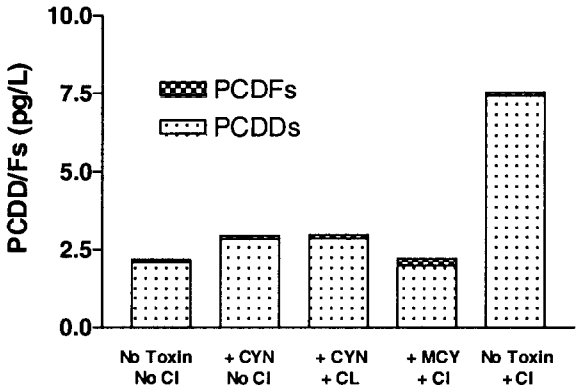

Figure 4 Concentrations of the 2,3,7,8-chlorine substituted dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) in water spiked with CYN as CFE or MCY as *C. raciborskii* or *M. aeruginosa* and/or treated with chlorine. No toxin samples have a background natural OC level of ~ 3mg/L.

In summary the results of this study found formation of relatively low levels of trichloromethane and chlorophenols during treatment of water containing toxic cyanobacterial material. Formation was mainly associated with organic compounds other than the cyanotoxins, and the levels of THMs and chlorophenols formed due to chlorination were low when compared to the drinking water guidelines or even normal drinking water collected from the tap in Brisbane. The levels found in Brisbane tap water are below guideline values. No increased risk which is associated with chlorine treatment of water containing the cyanobacterial material was detected compared to Brisbane drinking water regulartions.

REFERENCES

[1] A. T. C. Bourke, R. B. Hawes, A. Neilson, and N. D. Stallman, Toxicon (Suppl.) 3, 45-48 (1983).

[2] S. Pouria, A. de Andrade, J. Barbosa, R. L. Cavalcanti, V. T. S. Barreto, C. J. Ward, W. Presiser, G. K. Poon, G. H. Neild, and G. A. Codd, Lancet 352, 21-26 (1998).

[3] K. Himberg, A.-M. Keijola, L. Hiisvirta, H. Pyysalo, and K. Sivonen, Water Res. 23, 979-984 (1989).

[4] A. Keijola, K. Himberg, A. Esala, K. Sivonen, and L. Hiisvirta, Tox. Assessm. Int. J. 3, 643-656 (1988).

[5] T. Lambert, C. Holmes, and S. Hrudey, Water Res. 30, 1411-1422 (1996).

[6] B. Nicholson, J. Rositano, and M. Burch, Water Res. 28, 1297-1303 (1994).

[7] P-J. Senogles, G. Shaw, M. Smith, R. Norris, R. Chiswell, J. Mueller, R. Sadler, and G. Eaglesham, Toxicon 38(9), 1203-1213 (2000).

[8] R. L. Jolley, Water Chlorination Chemistry, Environmental Impact and Health Effects: Volume 5. (eds.) R. L. Jolley, R. J. Bull, W. P. Davis, S. Katz, M. H. Roberts Jr, and V. A. Jacobs pg 19-37 (1985).

[9] C-Y. Yang, H-F. Chiu, M-F. Cheng, and S-S. Tsai, Enviro. Res. Sec. A. 78, 1-6 (1998).

[10] J. H. Exon and L. D. Koller, Water Chlorination Chemistry, Environmental Impact and Health Effects: Volume 5. (eds.) R. L. Jolley, R. J. Bull, W. P. Davis, S. Katz, M. H. Roberts Jr, and V. A. Jacobs pg 307- 329 (1985).

[11] J. F. Borzelleca, L. W. Condie, and J. R. Hays, Water Chlorination Chemistry, Environmental Impact and Health Effects: Volume 5. (eds.) R. L. Jolley, R. J. Bull, W. P. Davis, S. Katz, M. H. Roberts Jr, and V. A. Jacobs pg 331-343 (1985).

[12] P. Lampi, T. Hakulinen, T. Luostarinen, E. Pukkala, and L. Teppol, Arch. Enviro. Health 47 (3), 167-175 (1992).

[13] HACH. DR/2000 Spectrophotometer Procedures Manual. HACH Company (1993).

[14] J. Müller, G. Shaw, M. Mortimer, D. Connell and R. Sadler, Report S2R2 #24 South East Queensland Water Quality Strategy. Avail. from Brisbane City Council GPO Box 1434, Brisbane QLD 4001 (1999).