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Effect of chlorination by-products on the quantitation of microcystins in finished drinking water

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ABSTRACT

Microcystins are toxic peptides that can be produced by cyanobacteria in harmful algal blooms (HABs). Various analytical techniques have been developed to quantify microcystins in drinking water, including liquid chromatography tandem mass spectrometry (LC/MS/MS), enzyme linked immunosorbent assay (ELISA), and oxidative cleavage to produce 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) with detection by LC/MS/MS, the “MMPB method”. Both the ELISA and MMPB methods quantify microcystins by detecting a portion of the molecule common to most microcystins. However, there is little research evaluating the effect of microcystin chlorination by-products potentially produced during drinking water treatment on analytical results. To evaluate this potential, chlorinated drinking water samples were fortified with various microcystin congeners in bench-scale studies. The samples were allowed to react, followed by a comparison of microcystin concentrations measured using the three methods. The congener-specific LC/MS/MS method selectively quantified microcystins and was not affected by the presence of chlorination by-products. The ELISA results were similar to those obtained by LC/MS/MS for most microcystin congeners, but results deviated for a particular microcystin containing a variable amino acid susceptible to oxidation. The concentrations measured by the MMPB method were at least five-fold higher than the concentrations of microcystin measured by the other methods and demonstrate that detection of MMPB does not necessarily correlate to intact microcystin toxins in finished drinking water.

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1. Introduction

Microcystins are a class of toxic peptides that can be synthesized by cyanobacteria, also known as “blue-green algae”, during harmful algal blooms (HABs). Microcystin toxicity is attributed to inhibition of protein phosphatases 1 and 2A, which leads to alteration of cell function (U.S. Environmental Protection Agency, 2015). These toxins have been widely reported in both the popular media (Henry, 2015; Zimmer, 2014) and scientific reports (U.S. Environmental Protection Agency, 2009) and can occur due to the presence of HABs in surface waters. The World Health Organization set a drinking water provisional guidance value of 1 μg/L for microcystin-LR. (Chorus and Bartram, 1999; WHO (World Health Organization), 2003). Several countries including Brazil, China, Czech Republic, Denmark, Finland, France, Germany, Italy, Japan, Korea, Netherlands, Norway, New Zealand, Poland, South Africa, and Spain based their drinking water guidance values on the WHO provisional guidance (U.S. Environmental Protection Agency, 2015). Other countries such as Australia (1.3 μg/L microcystin-LR expressed as toxicity equivalents) (NHMRC and NRMMC, 2011) and Canada (1.5 μg/L microcystin-LR) (Health Canada, 2017) have set their own drinking water guidance values. Currently, there are no United States federal regulations for cyanotoxins in drinking water, but the U.S. EPA has published a Drinking Water Health Advisory (HA) for microcystins with a 10-day exposure level of 0.3 μg/L for young children and 1.6 μg/L for school-age children through adults (U.S. Environmental Protection Agency, 2015). Additionally, states have implemented programs monitoring the presence of cyanotoxins in both ambient and finished drinking waters (U.S. Environmental Protection Agency, 2017). Therefore, there is a need to ensure the validity of analytical methods used to quantify these cyanotoxins.

Microcystins are cyclic heptapeptides consisting of five conserved and two variable amino acids. Fig. 1 shows the molecular structure of microcystin indicating the conserved and variable amino acids. Microcystin nomenclature is based on the one-letter

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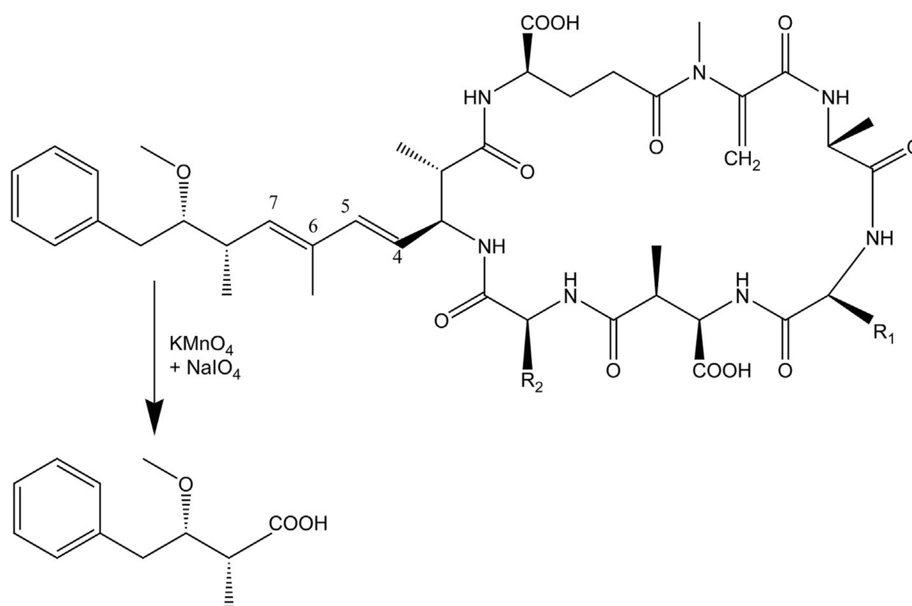

Fig. 1. Oxidative cleavage of a microcystin to form MMPB. R_1 and R_2 denote variable amino acids. Selected carbons of the Adda side chain are numbered to facilitate discussion of the sites of reaction. MMPB is formed by the cleavage of the bond between carbons 6 and 7.

codes for the variable amino acids. For example, microcystin-LR has leucine in the position designated R_1 and arginine in position R_2. Approximately 100 microcystin congeners have been identified based on the possible combinations of amino acids and the presence or absence of methyl groups attached to the peptide backbone. However, commercial standards are only available for less than 20 congeners. All identified microcystins contain the nonstandard amino acid (4*E*,6*E*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), or an acetylated or demethylated analogue of Adda. The presence of this unique amino acid has allowed the development of analytical methods based on detection of the Adda side chain. These methods provide a total microcystin concentration without requiring analytical standards for each congener.

Microcystins are included in the Fourth Unregulated Contaminant Monitoring Rule (UCMR 4) published on December 20, 2016 (U.S. Environmental Protection Agency, 2016). This monitoring of treated drinking water will serve as one of the primary sources of information on occurrence and exposure and could support risk management decisions for emerging contaminants in the public drinking water supply. The UCMR 4 specifies two methods for the determination of microcystins in drinking water: EPA Methods 544 and 546. EPA Method 544 uses solid phase extraction followed by liquid chromatography tandem mass spectrometry (LC/MS/MS) to detect six individual microcystin congeners and nodularin-R (Shoemaker et al., 2015). Due to the lack of commercial standards, EPA Method 544 does not provide a measure of the total microcystin concentration. EPA Method 546 (Zaffiro et al., 2016) is based on an indirect competitive enzyme linked immunosorbent assay (ELISA) using a primary antibody raised against an Adda hapten (Fischer et al., 2001; Zaffiro et al., 2016), and the method provides a measure of the total microcystin concentration. Both methods underwent rigorous EPA validation in multiple drinking water matrixes during method development, including system background evaluations, detection limitations, precision and accuracy measurements, and storage stability studies.

As an additional total microcystin measurement, which would provide a confirmation of ELISA results, a third analytical technique was evaluated for drinking water and is based on detection of a twelve carbon fragment formed by oxidative cleavage of the Adda side chain. The oxidation of an olefin in an aqueous solution of permanganate and periodate to yield two carboxylic acids was first reported by Lemieux and von Rudloff (Lemieux and Rudloff, 1955). As shown in Fig. 1, oxidative cleavage of the double bond between carbons 6 and 7 of Adda yields 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB). Oxidative cleavage of microcystins followed by determination of MMPB by LC with detection by either photodiode array (Wu et al., 2009) or tandem mass spectrometry (Foss and Aubel, 2015) has been previously used to measure microcystin concentrations in water samples. Roy-Lachapelle et al. (2014) determined microcystin concentrations in environmental water samples by formation of the MMPB fragment followed by detection using thermal desorption-atmospheric pressure chemical ionization tandem mass spectrometry. Other researchers have determined microcystin concentrations by formation of MMPB followed by derivatization to create products amenable to GC/MS (Kaya and Sano, 1999; Xu et al., 2013) or LC with fluorescence detection (Wang et al., 2015). In addition to application of the method to surface water samples, researchers have used the detection of MMPB to determine microcystins in animal tissue (Neffling et al., 2010; Ott and Carmichael, 2006) and sediment (Wu et al., 2012). More recently, detection of MMPB has been proposed as a method for the determination of total microcystins in finished drinking water (Foss and Aubel, 2015; Roy-Lachapelle et al., 2014; Zhang et al., 2016).

The ELISA and MMPB methods rely on recognition of the Adda side chain, which is unique to microcystins and nodularins. As a result of this selectivity, false positives from compounds unrelated to microcystins or nodularins are unlikely. However, free chlorine oxidation, which is the most commonly used drinking water disinfection process in the United States, does not necessarily lead to the complete destruction of the cyclic peptide structure. Microcystin chlorination by-products have been previously reported (Merel et al., 2009; Zong et al., 2015). The double bonds of the Adda side chain are a primary site of modification with the addition of chlorines and hydroxyl groups (Zong et al., 2013a). Other sites of modification are also possible, including functional groups on the variable amino acids. Chlorination by-products resulting from

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