# Regulatory Guidelines for Microcystins in Drinking and Surface Waters require update

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## ABSTRACT

Cyanobacteria, also known as blue-green algae, pose an emerging issue due to their potential impacts on drinking water and recreational waters in lakes. They produce a variety of toxins including microcystins and nodularins. Microcystins are the most commonly detected cyanotoxins of major health concern in surface and drinking water. There are over 90 variants of microcystins. Many freshwater lakes around the world are troubled by cyanobacteria and microcystin concentration become one of the key parameters for the evaluation of lake management programs.

The drinking water standard in Ontario and Canada for Microcystin-LR is set at 1.5μg/L. Until 2010, the only test method licensed by the Ontario Ministry of the Environment (MOE) for analysing microcystins in drinking water was liquid chromatography (electrospray ionization) – tandem mass spectrometry (LC-MS/MS). Enzyme-linked immunosorbent assay (ELISA) (Abraxis, 2013) has been accepted by MOE as an analytical method for microcystins in both surface water and drinking water. This ELISA is now US EPA Method 546 since 2016.

ELISA cannot quantify individual variants in a water sample. Instead, it measures all variants that cross-react with the antibodies. Since ELISA is capable of measuring total microcystins inclusive of multiple variants, the Ontario Drinking-Water Quality Standards Regulation (O. Reg. 169/03) should be changed again from focusing solely on microcystin-LR to total microcystins (MOE, 2002). This change will also alleviate the regulatory reliance on the LC-MS/MS method.

Over a 3-year period, microcystins concentrations in 849 water samples (including surface water and both raw and treated drinking water) were analysed by ELISA and (LC-MS/MS) in parallel. In the ELISA-positive, LC-MS/MS-positive samples set (n=308), paired t-test found a high correlation ($p < 0.001$). However, t-test is meaningless when the sample population is not normally distributed. In this case, the Tukey-lambda Probability plot correlation coefficient plot demonstrated Cauchy distribution for both the LC-MS/MS and ELISA populations. No correlation was demonstrated between ELISA and LC-MS/MS results when the correct statistics was applied ($p < 0.0001$ Wilcoxon signed rank test), which led to the conclusion that the ELISA and LC-MS/MS results were discrepant on a population basis. This finding begged a serious

question: which one is the laboratory method of choice for the specific purpose of supporting drinking and surface water regulations? Due considerations of a list of choice criteria including user-friendliness, sensitivity, reliability, turn-around speed, costs, and limitations show a preference of ELISA over LC-MS/MS. Furthermore, ELISA empowers remote territories, developing nations and private laboratories lacking LC-MS/MS capabilities to carry out microcystins monitoring. Using ELISA as a tier-1 screen test and LC-MS/MS as a tier-2 confirmatory test is scientifically unsound because ELISA detects more microcystins than LC-MS/MS ever could. ELISA is responsive to numerous microcystins variants bearing the ADDA toxic moiety. In contrast, microcystins detection by LC-MS/MS is limited to a few variants for which standards are available. Currently, the safe drinking water standards is set for Microcystin-LR, which is but one member of the 90+ variants of this toxin family [http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/water-cyanobacteria-cyanobacterie-eau/index-eng.php]. The wordings of such regulations prohibit ELISA as a stand-alone method. Hence, an amendment is hereby proposed to remove the "microcystin-LR" specification by deleting the two alphabets "-LR". In doing so, the regulations would provide a more comprehensive protection of public, wildlife and environmental health and at the same time improve cost-effectiveness in service delivery.

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30min full oral presentations (25 min talk + 5 min discussion) preferred
Presentation topic: Harmful algal blooms, taste & odor, and toxin problems in sources water - Cyanobacterial toxins