Measuring Total Microcystins in Fish Tissue using the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) procedure.

Toby Sanan, Jim Lazorchak, Devi Sundaravadivelu, Renata Adkins, Josh Kickish, Jennifer Jones, Raghu Venkatapathy.

There are limited methods for the analyses of multiple algal toxins in aquatic food webs, phytoplankton, zooplankton, periphyton, macroinvertebrates, forage fish, bottom feeders and top carnivore fish. Algal toxins in freshwater systems do not necessarily occur as single contaminants; mixtures of toxins may be produced from Cyanobacteria, *Prymnesium parvum (Prymnesins)*, and *Euglena sanguinea*, including microcystins, saxitoxins, cylindrospermopsin, anatoxin-a., prymnesins and euglenophycin. This can be challenging when the chemical properties of toxin variants complicate the use of a single extraction method. The objective of the first phase of this research was to spike existing fillet and whole fish homogenates with 3 congeners of microcystins (LR, LA and RR) individually and as mixtures, and to develop a method for their recovery and measurement using the MMPB derivatization technique. The second phase of the project is to field-test this method on fish collected from water bodies experiencing algal blooms and compare results with individual congener measurements. Extraction methods and analytical methods being developed for this research will be a starting point for developing extraction procedures for plankton, periphyton, and macorinvertebrates. Ten and 100 mg of fish homogenates from fish containing 1, 4 and 14% lipids were spiked with 4 and 40 ng of each of the microcystin congeners, LR, LA and RR. Various extraction techniques and conditions were tested to optimize recovery and simplify the procedure. Overall toxin recoveries were found to range from 30 to 50% based on spike concentrations, and were not significantly impacted by the lipid content. The lipid content did impact the workup/extraction procedure in ways which were accountable through the use of a surrogate standard, allowing for accurate evaluation of microcystin concentrations.