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RE: Analysis Report – AFA 2012 small harvest batches were mixed to get larger batch sizes. Cell biomass in Alpha batch # 510 was analyzed for Microcystin by enzyme linked immunosorbent assay (ELISA).

Extraction. The sample was stored at 4°C prior to analysis. An aliquot of the dried material was extracted in 0.1 M acetic acid in 100% methanol at a ratio of 1 g/50 mL and sonicated for 5 minutes. The extract was centrifuged. The supernatant was dried, and fractionated by solid phase extraction (C18) using water; 20% methanol and then 100% methanol. The 100 % methanol elution was used for analysis of Microcystins. This fraction was dried and taken up in 1 mL of PBS buffer. The filtrate was stored at -20 C and used as needed for ELISA.

A. ELISA assay for the cyclic peptides microcystin and nodularin. The ELISA method is based upon the original polyclonal antibody method described by Chu *et al.* (1989, 1990) and adapted by An and Carmichael (1994) and Carmichael and An (1999). These methods have been adapted to a commercial ELISA kit (Microcystin Plate Kit, EP-022-kit batch#040307) that is produced by Envirologix, Inc. (Portland Maine). This microcystin (MCYST) Plate Kit is calibrated to measure MCYST concentrations between 1.6 and 0.16 µg/L. Samples containing MCYST in greater or lesser amounts of this range are diluted or concentrated, respectively, to bring them into this range for measurement. This range covers the WHO guideline level for MCYST in finished drinking water supplies (Chorus and Bartram 1999). Twenty microliters (20 µl) of sample, were used for the assay, providing a minimum detection level of 5 pg. Full strength and 1:5 dilution were used to run the assay in triplicate.

Microcystin Results.
Batch #510
Sample dry weight .(mg) 100
Mcyst conc. 0.25 µg/g dry weight of cells

References:
An, J-S. and Carmichael, W.W. (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon **32**: 1495-1507.

Carmichael, W.W. and An, J-S. (1999) Using an enzyme linked immunosorbent assay

(ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. Natural Toxins. **7**: 377-385.

Chu, F.S., Huang, X., and Wei, R.O. (1990). Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. J. Assoc. Off. Analyt. Chem. **73**: 451- 456.

Chu, F.S., Huang, X., Wei, R.O., and Carmichael, W.W. (1989). Production and characterization of antibodies against microcystins. Appl. Environ. Microbiol. **55**:1928-1933.

Summary: Alpha Batch #510 from the 2012 harvest season was tested by ELISA for microcystin. The microcystin content in batch #510 was 0.25 µg/g dry weight.

Signed

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