Anatoxin-a, Cylindrospermopsin, Microcystin & Saxitoxin Report

Project: Utah Department of Agriculture and Food

Sample Identification	Sample Site	Date Collected
mcq 7/21/16 - 1	Pepper Field Ryan Schmidt	7/21/16
mcq 7/21/16 - 2	Ryan Schmidt Corn	7/21/16
mcq 7/21/16 - 3	Rasmussen Corn - Draper	7/21/16
mcq 7/21/16 - 4	Rasmussen Zucchini	7/21/16
mcq 7/21/16 - 5	Rasmussen Corn - East Jordan	7/21/16
mcq 7/21/16 - 6	Peterson Tomato	7/21/16
mcq 7/21/16 - 7	Peterson Kale	7/21/16
mcq 7/21/16 - 8	Peterson Potatoes	7/21/16
mcq 7/21/16 - 9	Wilber Watermelon	7/21/16
mcq 7/21/16 - 10	Peterson Pumpkin Flower	7/21/16
mcq 7/21/16 - 11	Olsen Onions	7/21/16

Toxins – Anatoxin-a (ANTX-A), Cylindrospermopsin (CYN), Microcystins (MC), Saxitoxin (STX)

Toxin extraction

Samples were received fresh and were stored frozen until sample preparation. Inedible biomass (e.g. corn husks, cobs, outer onion skin, etc.) was removed and samples were cut into equal size pieces. Prior to extraction, samples were homogenized using a Warring laboratory blender. Samples were diluted, as necessary, for complete homogenization.

MC, CYN & ANTX-A

Homogenized samples were extracted in 1.000 ± 0.005 gram subsets with 75% acidified methanol solution. Duplicate spiked samples (lab fortified matrices, LFMs) were prepared in the same manner with MC-LR, CYN and ANTX-A standards added for spike concentrations of 10 ng/g, 10 ng/g, and 2 ng/g, respectively. Samples were sonicated via water bath for 20 minutes and centrifuged at 3,000 RPM for 10 minutes. The supernatant was collected and the pellet was rinsed with acidified MeOH solution. The pooled supernatants were mixed and split for solid-

phase extraction (SPE) with Strata X for MCs & ANTX-A and Resprep SPE CarboPrep 90 for CYN. Samples (extracts) for ANTX-A extraction required pH adjustment (>10) prior to SPE. Post SPE eluates were blown to dryness with N_2 and heat and reconstituted at a concentration of 1.0 g/mL for MC and CYN. Subsequent dilutions were employed (0.1 g/mL) for analysis with the MC and CYN ELISAs. ANTX-A eluates were blown to dryness with N_2 and heat and reconstituted to a concentration of 0.5 g/mL in DI water prior to analysis via LC-MS/MS.

STX
Homogenized material was extracted in 1.000 ± 0.005 gram subsets with a 0.1 M acetic acid solution. Duplicate spiked samples (lab fortified matrices – LFMs) were prepared in the same manner at a spike concentration of 2 ng/g using STX. Samples were heated in a boiling water bath for 5 minutes, then cooled and centrifuged at 3,000 RPM for 10 minutes. The supernatant was collected and the pellet was rinsed with 0.1 M acetic acid. The pooled supernatants were mixed and required pH adjustment (>10) prior to SPE. Resprep SPE CarboPrep 90 cartridges were used for extraction and clean-up. The eluates were blown to dryness with N2 and heat and reconstituted at a concentration of 1.0 g/mL with ELISA diluent. A subsequent dilution was employed (0.1 g/mL) for analysis with the STX ELISA.

Analytical Methodology

MC
A microcystins/nodularins ELISA (Abraxis) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive down to a detection/quantification limit of 1.5 ng/g as determined from dilution factors and kit sensitivity (0.15 ng/mL).

CYN
A cylindrospermopsin ELISA was utilized for the determination of CYN. The current assay is sensitive down to a detection/quantification limit of 1.0 ng/g as determined from dilution factors and kit sensitivity (0.10 ng/mL).

STX
A saxitoxin ELISA was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a detection/quantification limit of 0.5 ng/g as determined from dilution factors and kit sensitivity (0.05 ng/mL).

ANTX-A
Liquid chromatography- mass spectrometry/ mass spectrometry (LC-MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (166 *m/z*) was fragmented and the product ions (56.0, 91.1, 107.0, 131.1 & 149.6 *m/z*) were monitored. The method detection limit was 0.5 ng/g for ANTX-A.

Summary of Results

Sample	MCs ELISA	CYN ELISA	STX ELISA	ANTX-A LC-MS/MS
mcq 7/21/16 - 1	ND	ND	ND	ND
mcq 7/21/16 - 2	ND	ND	ND	ND
mcq 7/21/16 - 3	ND	ND	ND	ND
mcq 7/21/16 - 4	ND	ND	ND	ND
mcq 7/21/16 - 5	ND	ND	ND	ND
mcq 7/21/16 - 6	ND	ND	ND	ND
mcq 7/21/16 - 7	ND	ND	ND	ND
mcq 7/21/16 - 8	ND	ND	ND	ND
mcq 7/21/16 - 9	ND	ND	ND	ND
mcq 7/21/16 - 10	ND	ND	ND	ND
mcq 7/21/16 - 11	ND	ND	ND	ND
MDL (ng/g)	*1.5*	*1.0*	*0.5*	*0.5*

ND = Not detected above the MDL

The following are tolerable daily intake values based on no-observed-adverse-effect-levels (NOAELs) from literature for the toxins analyzed in this work. Due to the acute nature of saxitoxin, an acute reference dose is used to make assumptions about consumption, in contrast to a TDI. These values have been used to determine guidance values for consumption of blue-green algal supplements, drinking water and other consumables by humans.

Algal Toxin	Ref. Type	Dose µg toxin/kg (b.w.) per day
Microcystin-LR	TDI (WHO)	0.04
Cylindrospermopsin	TDI	0.03
Anatoxin-a	TDI	0.098
Saxitoxin(s)	ARfD	0.5

If one were to utilize the above TDIs and ARfD to determine the safe levels of consumption of the food products submitted to GreenWater Labs for testing, it would be conservative to base the assumptions on the weight of a child, approximately 20 kg b.w. The following table illustrates safe levels of product that could be consumed by a child if the toxin levels in the products tested were at the method detection limits (MDLs).

Algal Toxin	Child Body Weight (b.w.) kg	TDI (µg)	TDI (ng)	MDLs (ng/g)	**Grams (g) of food consumed per day**
Microcystin-LR	20	0.8	800	1.5	**533**
Cylindrospermopsin	20	0.6	600	1	**600**
Anatoxin-a	20	1.96	1960	0.5	**3,920**
Saxitoxin(s)	20	10	10000	0.5	**20,000**

Based on the above assumptions, approximately 533 grams of food can be consumed per day of the above tested products before there is a risk of adverse health effects for a child, if toxins were present at the method detection limits. If this approach were applied to an adult with a weight of 60 kg, the level would be 1,600 grams per day. Since toxins were not detected in the products tested at or above the MDLs, it would be unlikely that consuming these products would result in adverse health effects from algal toxins tested.

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