



Cyanotoxins below the HAL: Validating higher sensitivity ELISA for drinking water in Oregon

Kale Clauson

3/17/2023

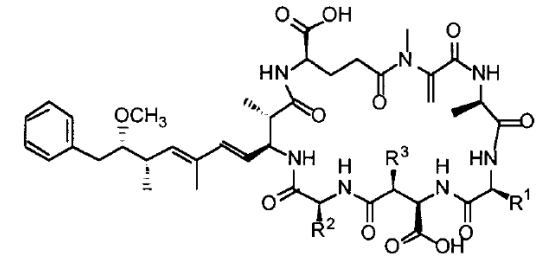
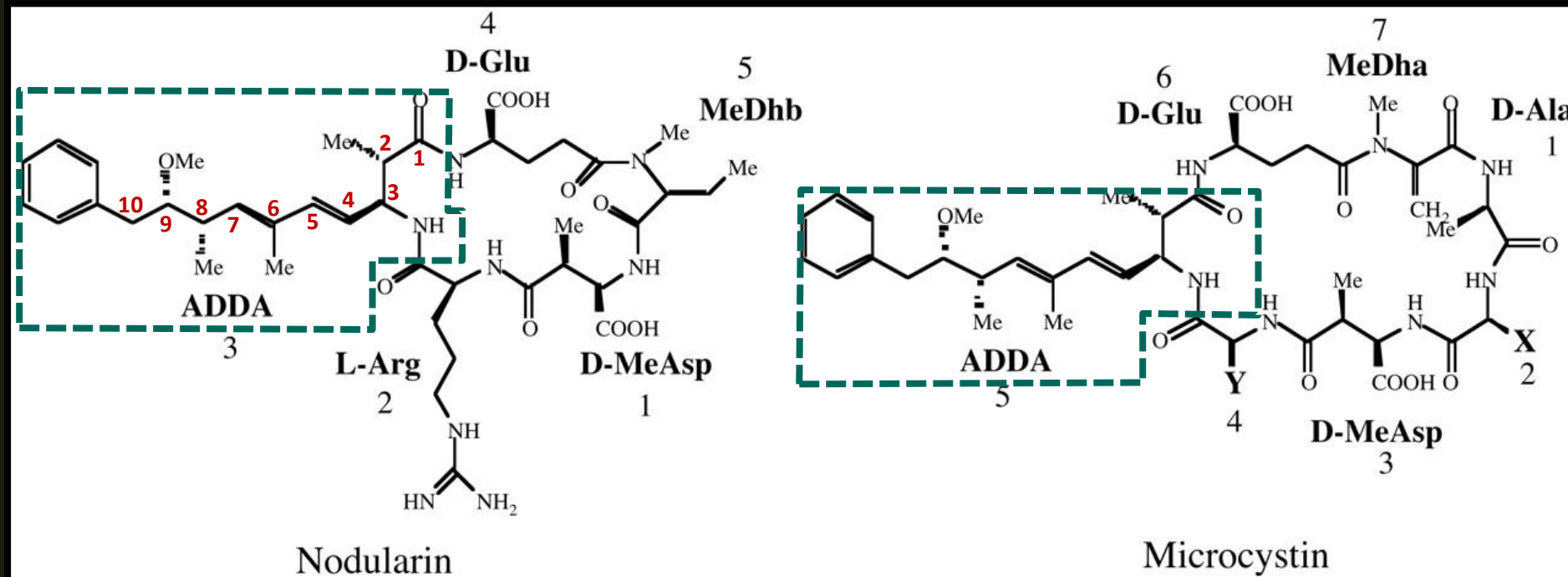
OREGON CYANOBACTERIAL HARMFUL ALGAE BLOOM STAKEHOLDER MEETING


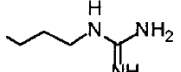
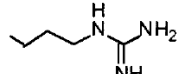
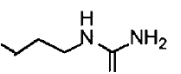

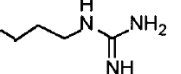

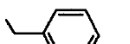

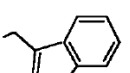

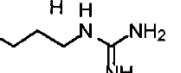

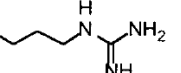
Acronyms and Definitions

- CYN – Cylindrospermopsin
- ELISA – Enzyme-Linked Immunosorbent Assay
- HAL – “Health Advisory Level”
 - Concentration under which EPA does not expect adverse health effects to occur if water consumed for 10 days.
- LCMRL – Lowest Concentration MRL
- MCT – Microcystins
- MDL – “Method Detection Limit” (LOD – Limit of Detection)
- MRL – Method Reporting Limit (LOQ – Limit of Quantitation)
 - Apply to the entire analytical method performance

Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay

3*S*-amino-9*S*-methoxy-2*S*,6,8*S*-trimethyl-10-phenyldeca-4*E*,6*E*-dienoic acid



	R ¹	R ²	R ³
MC-LR			CH ₃
MC-RR			CH ₃
MC-YR			CH ₃
MC-LF			CH ₃
MC-LW			CH ₃
dmMC-LR			H
dmMC-RR			H

>200 congeners of Microcystin identified

ELISA Kits – Abraxis/Eurofins/GSD

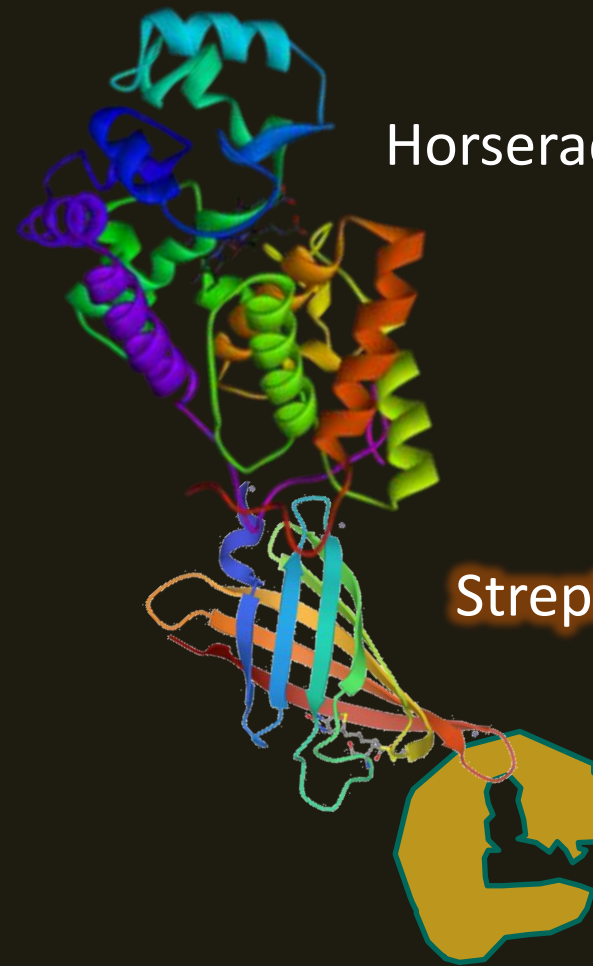
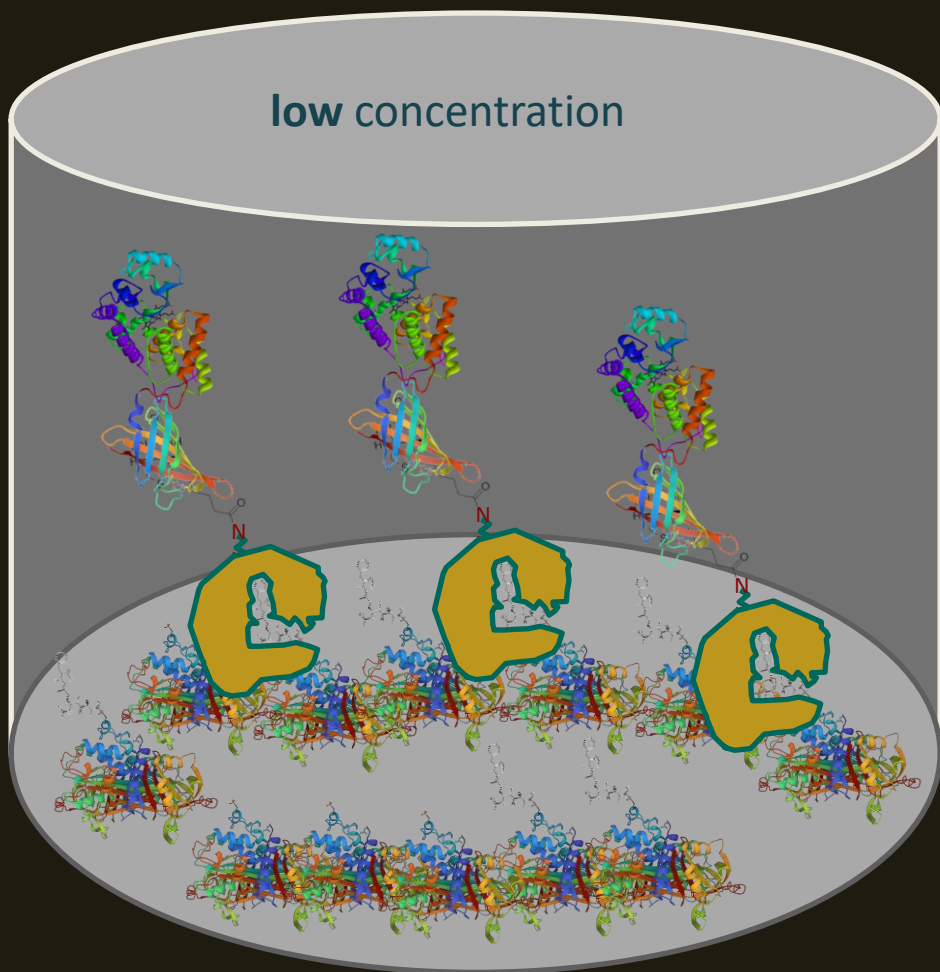
PN 520011

- Indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins
- “Specific Antibodies”
- HRP Conjugate
- TMB ‘Color Solution’
- Manufacturer DL: 0.1 µg/L
- Cal Range 0.15-5.0 µg/L

PN 520011SAES

- Indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins
- “Specific biotinylated antibodies”
- Streptavidin-HRP Conjugate
- TMB ‘Color Solution’
- Manufacturer DL: 0.016 µg/L
- Cal Range 0.05-5.0 µg/L

Simplified explanation of why the SAES kit is more sensitive



Horseradish peroxidase - Reporter

Streptavidin – Signal Enhancer

Antibody – Binds to ADDA



Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator

EPA 815-R-11-001 (12/2010)

- MRL – “estimate of the lowest concentration of a compound that can be quantitatively measured by members of a group of experienced drinking water laboratories.”
 - Accuracy and precision with 99% confidence (50-150%)
- MDL – “threshold for detection in low-level samples.”
 - Presence with 99% confidence ($x > 0$)

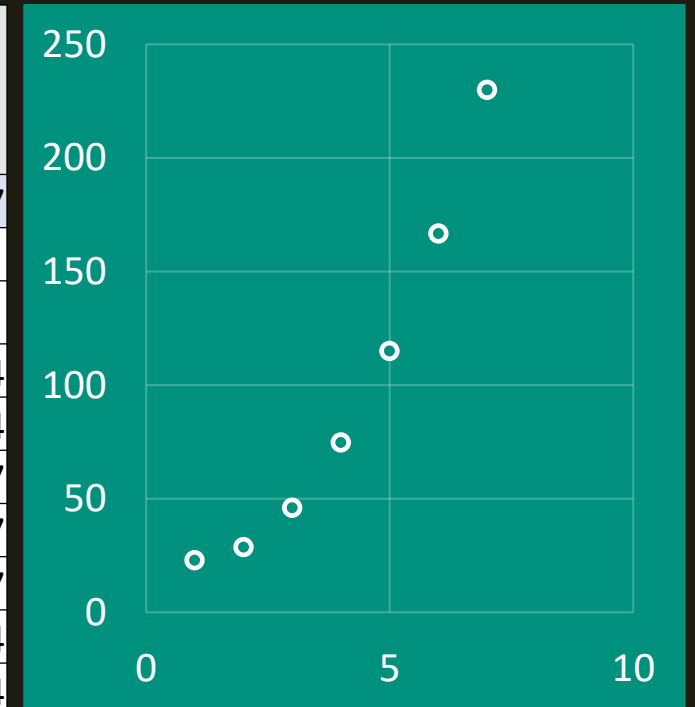
Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator

Methodology

- Regression-based model design with replicate spiking at multiple levels.
 - Method blanks required
 - Max to Min range should be ~1:10
 - At least 4 reps per level
 - More spiking levels > More reps
 - 7 levels recommended (>4)
- Software estimates LCMRL via Bayesian bootstrap resampling

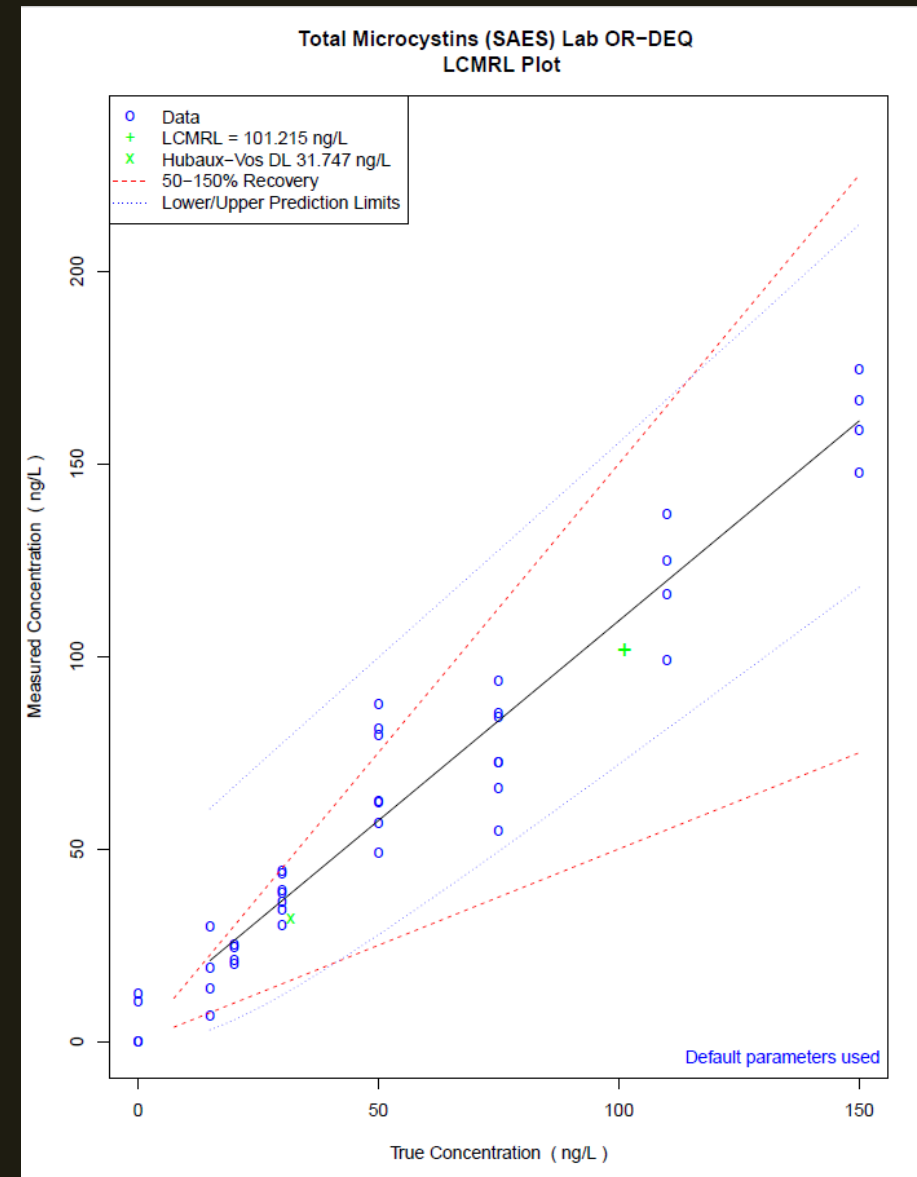
Parabolic Spiking Levels

CONSTANTS		
MIN	MAX	STEPS
X_1	X_m	m
23	230	7
STEP	STEP.CONC	REPS
i	X_i	≥ 4
7	230	4
6	110	4
5	75	7
4	50	7
3	30	7
2	20	4
1	15	4



LCMRL Results - DEQ Single Laboratory

- **LCMRL: 0.101 $\mu\text{g/L}$**
- **MDL: 0.032 $\mu\text{g/L}$**
- Confirmed LCMRL at 0.115 $\mu\text{g/L}$
with 72-116% recovery of 7 spiked
blanks.
- Mean Recovery 94% (6% RSD)



LCMRL Results - DEQ

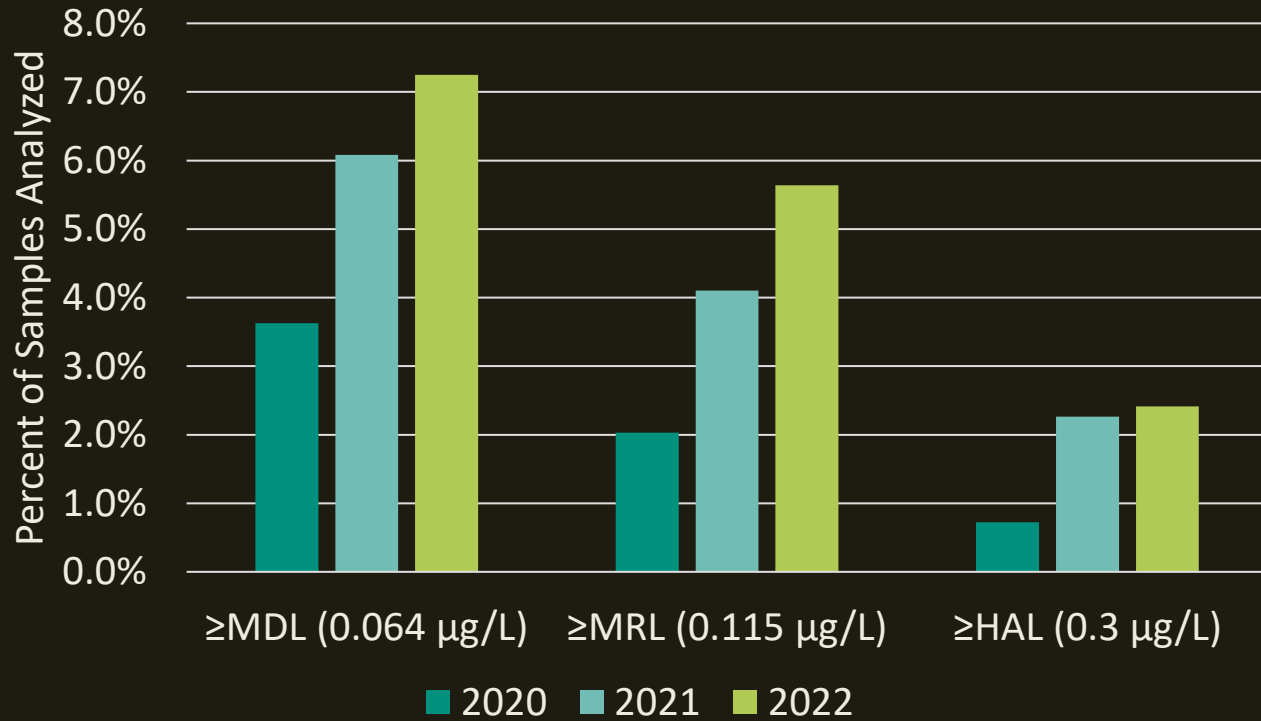
Long Term – Continuing Confirmation

- 2020 LowCV: 0.1 $\mu\text{g/L}$
 - MDL: 0.029 $\mu\text{g/L}$ (n=52)
- 2021 MRL (1 days): 0.1 $\mu\text{g/L}$
 - MDL: 0.064 $\mu\text{g/L}$ (n=7)
- 2022 MRL (4 days): 0.1 $\mu\text{g/L}$
 - MDL: 0.054 $\mu\text{g/L}$ (n=8)



Powers Pond, 9 October 2022

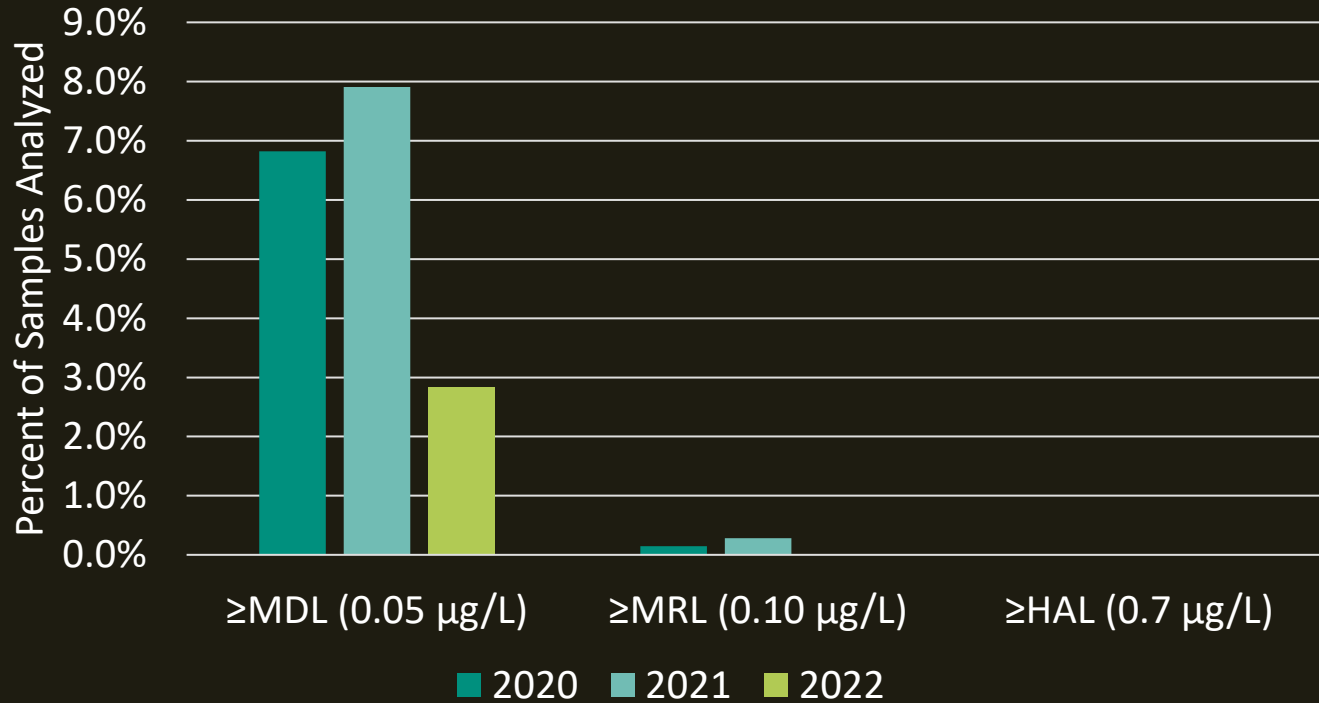
Microcystins in Oregon Source Water 2020-2022



Willamette River, 15 August 2022

	2020	2021	2022	Total
\geq MDL (0.064 $\mu\text{g/L}$)	25	43	54	122
\geq MRL (0.115 $\mu\text{g/L}$)	14	29	42	85
\geq HAL (0.3 $\mu\text{g/L}$)	5	16	18	39
Total Samples	689	707	745	2141

Cylindrospermopsin in Oregon Source Water 2020-2022

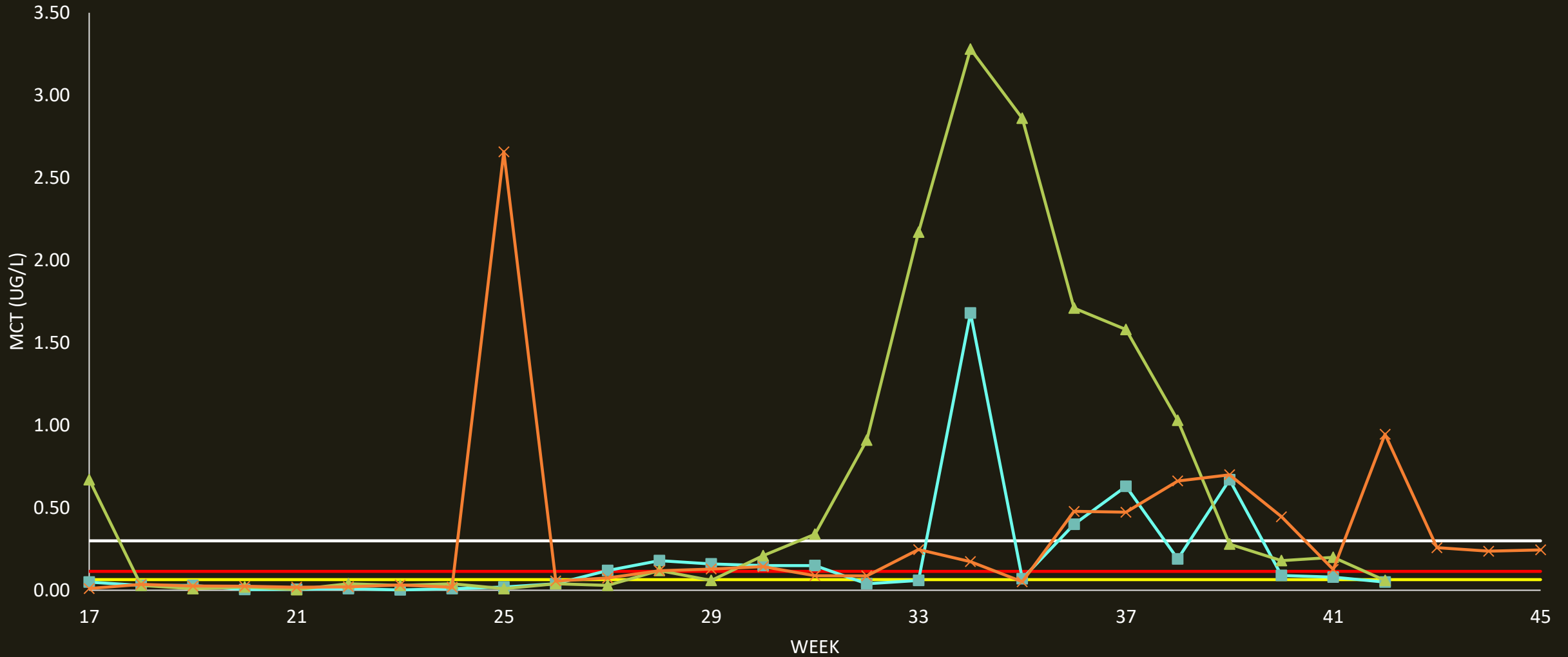


Detroit Reservoir, 29 September 2019

	2020	2021	2022	Total
≥MDL (0.05 µg/L)	47	56	21	124
≥MRL (0.10 µg/L)	1	2	0	3
≥HAL (0.7 µg/L)	0	0	0	0
Total Samples	689	708	743	2140

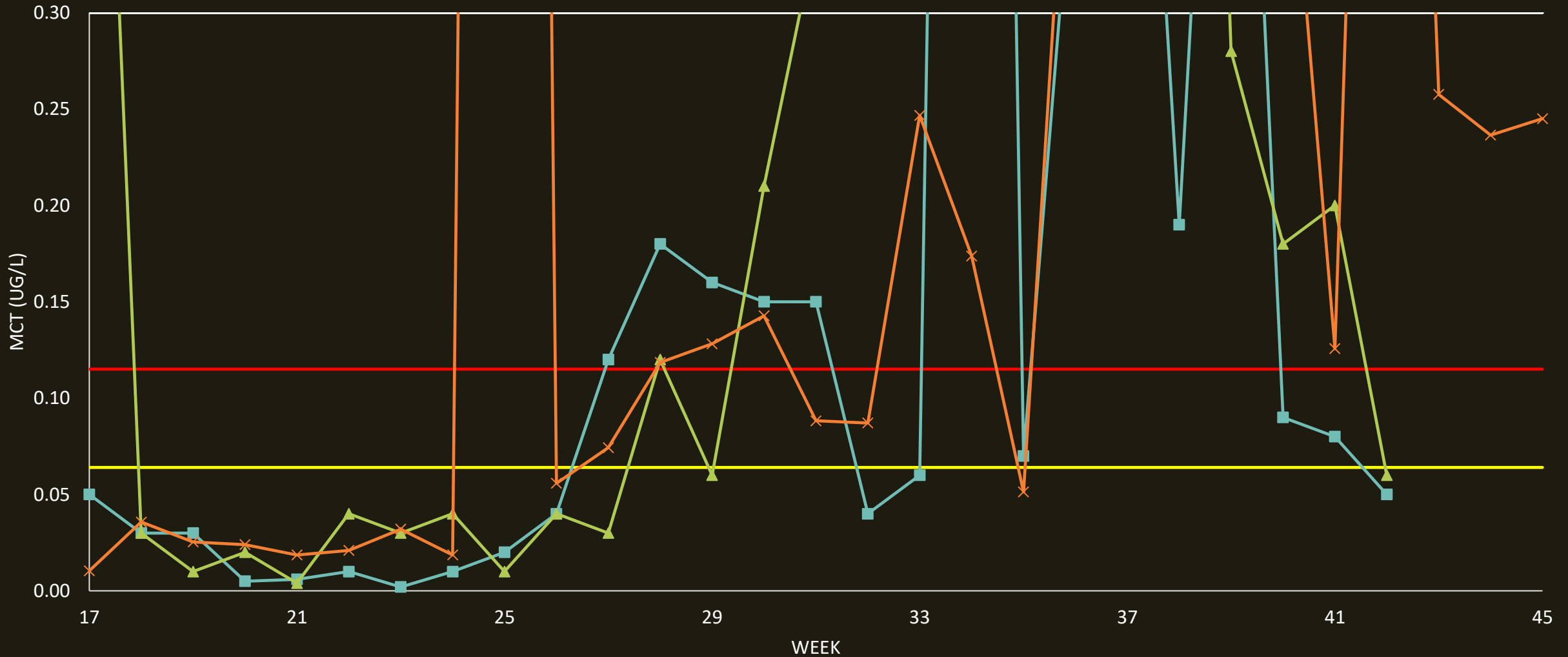
MICROCYSTINS IN OREGON SOURCE WATER, WEEKLY MAXIMUM

MDL (0.064 µg/L) MRL (0.115 µg/L) HAL (0.30 µg/L) 2020 2021 2022

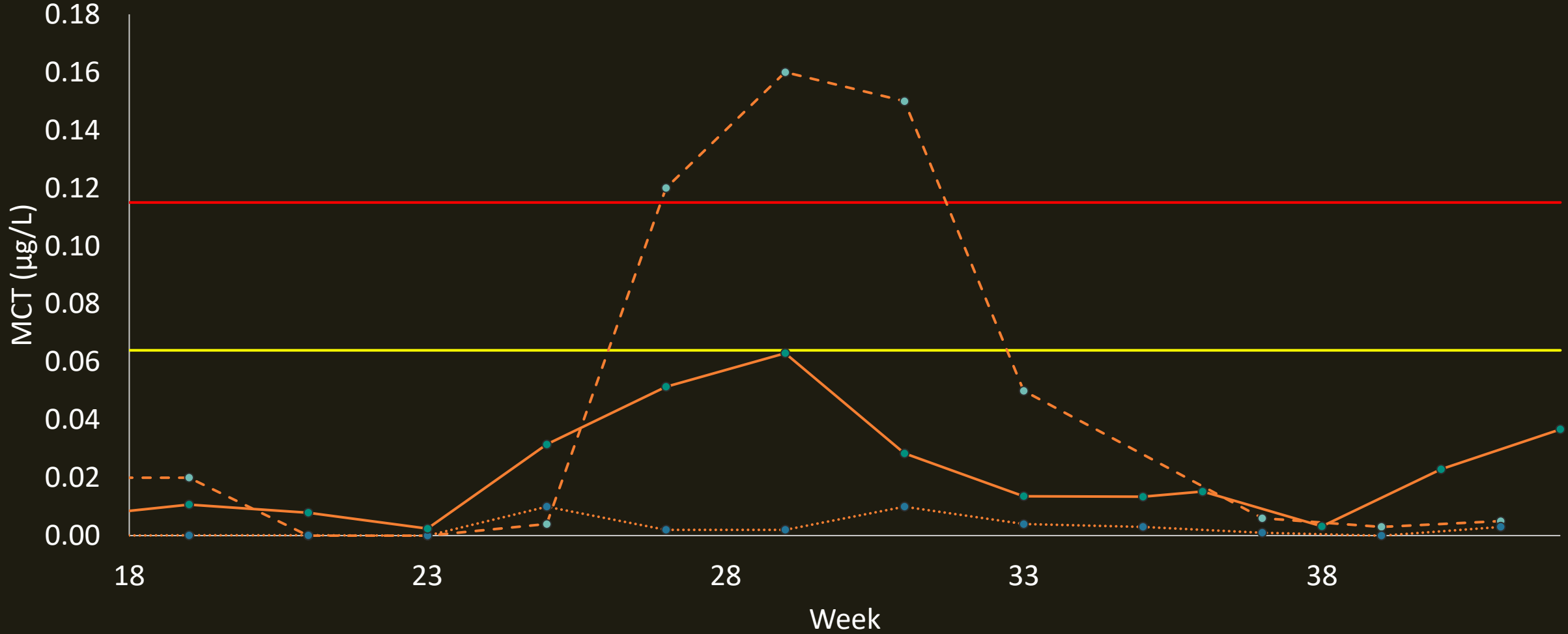


MICROCYSTINS IN OREGON SOURCE WATER, WEEKLY MAXIMUM

— MDL (0.064 µg/L) — MRL (0.115 µg/L) — HAL (0.30 µg/L) ■ 2020 ▲ 2021 × 2022

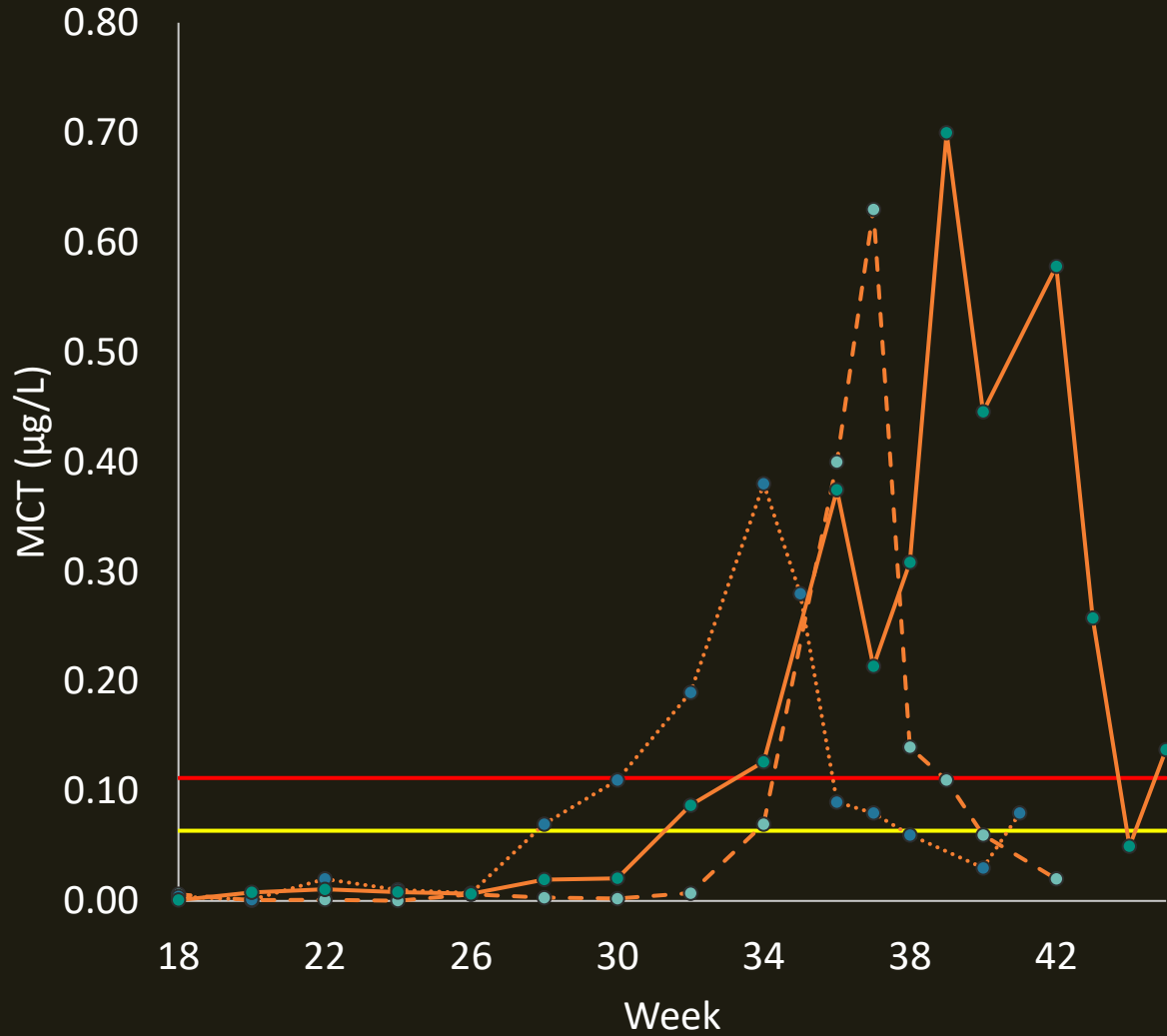


City of Gates – North Santiam River

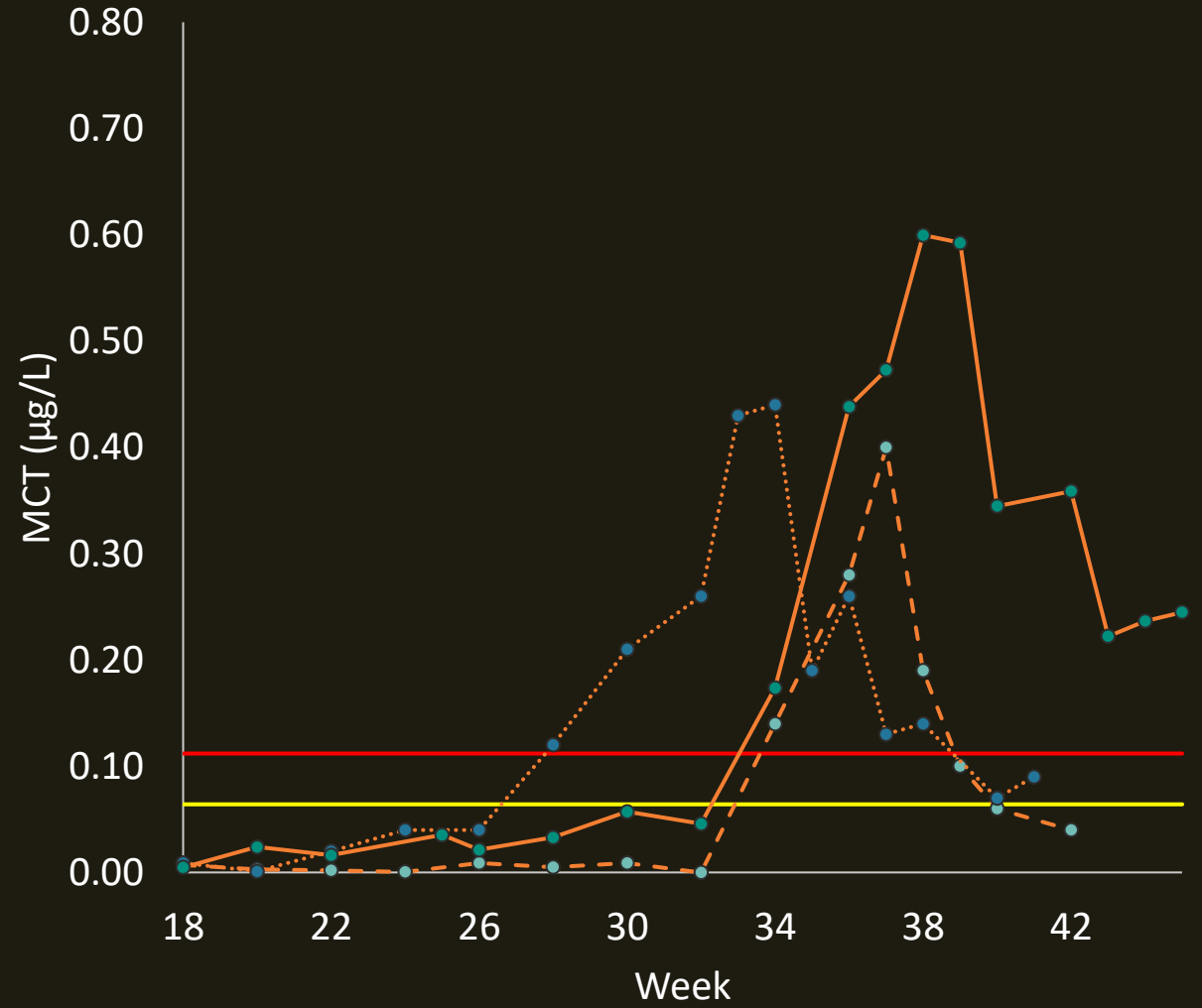


— MDL (0.064 µg/L) — MRL (0.115 µg/L) -•- 2020 ••• 2021 —•— 2022

Source Water (Lake Selmac #1, OR)



Source Water (Lake Selmac #2, OR)



— MDL (0.064 µg/L) — MRL (0.115 µg/L) —●— 2020 ●●● 2021 —●— 2022

Summary of 2020-2022

- SAES ELISA kit allows precision and accuracy well below health advisory level and, depending on system, should provide advance warning for response monitoring at higher frequency.
- Lower detection limit and qPCR give more confidence when confirming non-toxin producing HCBs.



Blue River Reservoir, 13 July 2020 (Credit: EWEB)

What about samples prior to the SAES kit?



2018 – Temporary Rule

Analyzed with ‘Low Sensitivity’ kit

- HAL=MRL=LCMRL
- 2018 DEQ MDL determined by 40 CFR Part 136B was 0.12 µg/L (99% Conf)
- EPA LCMRL 0.21 µg/L
- 8 PWS tested in 2018 had robust detections of MCT and were not added to permanent rule.

PWS	Source	Date	MCT(µg/L)
Adair Village	Willamette R.	7/17/18	0.19
Adair Village	Willamette R.	10/24/18	0.15
City of Carlton	Panther Ck.	8/21/18	0.15
City of Coquille	Coquille R.	7/17/18	0.16
Lakeside	Eel Lk.	9/26/18	0.26*
City of Lebanon	S. Santiam R.	10/15/18	0.20
City of Riddle	Cow Ck.	10/15/18	0.15
Sutherlin – SRC AA	Calapooya Ck.	10/16/18	0.18
Sutherlin – SRC BA	Cooper Ck.	10/2/18	0.18
Westfir	Willamette, N.Fk.	10/15/18	0.22*

“These data are provisional, and have not gone through Oregon DEQ’s quality assurance review. Final values are subject to revision and may change from what is reported here. The data are released on the condition that neither Oregon DEQ, nor the State of Oregon, may be held liable for any damages resulting from their authorized or unauthorized use.”

Thank You!

- Partners contributing to this work:
 - Oregon Health Authority
 - Oregon DEQ LEAD Lab
 - EPA Region 10
 - Oregon PWS Operators!



Cronemiller Pond, 28 July 2022

Title VI and alternate formats

DEQ does not discriminate on the basis of race, color, national origin, disability, age or sex in administration of its programs or activities.

Visit DEQ's [Civil Rights and Environmental Justice page](#).

[Español](#) | [한국어](#) | [繁體中文](#) | [Русский](#) | [Tiếng Việt](#) | [العربية](#)
Contact: 800-452-4011 | TTY: 711 | deqinfo@deq.state.or.us