ASMS Environmental Applications Working Group – Workshop Report 2015

The Environmental Working Group's workshop this year featured scientists early in their careers. Each participant spoke five minutes about their work and the challenges they are having or have had. The mix included two post docs Whitney Stutts (FDA, CFSAN), Veronica Termpoli (University of Urbino) and three PhD candidates Hannah Liberatore (University of South Carolina), Kari Organtini (Penn State) and Jeremy Koelmel (University of Florida). After each participant discussed their research, time was allowed for questions and discussion with the working group. The question and discussion period provided a good opportunity for the exchange of ideas and information for both the attendees and speakers. Topics covered included microcystins contamination of dietary supplements (W. Stutts), the discovery of new disinfectant byproducts (H.Liberatore), PFOS and a possible link to exposure and obesity (J. Kolmel), health risks to firefighters from fire debris (K. Organtini), and advancements in LC-Direct EI interfacing (V. Termopoli). Overall, the workshop seemed quite successful, and lively discussions continued after its completion at the annual post workshop dinner. As an additional summary of the activities, pdf's for each participant's discussion slides are provided (with their permission) on the working group webpage.

Sincerely,

Chris Gill and Marc Engel,
Co-Chairs, ASMS Environmental Working Group

Challenges in the Identification of New Toxic Iodinated Disinfection By-Products Using Mass Spectrometry

Hannah K. Liberatore[1], Yang Yang[3], Yukako Komaki[2,], Susana Y. Kimura[1], Hong-Ying Hu[3], Elizabeth D. Wagner[2], Michael J. Plewa[2], and Susan D. Richardson[1]

[1]University of South Carolina, Columbia, SC

[2]University of Illinois, Urbana, IL

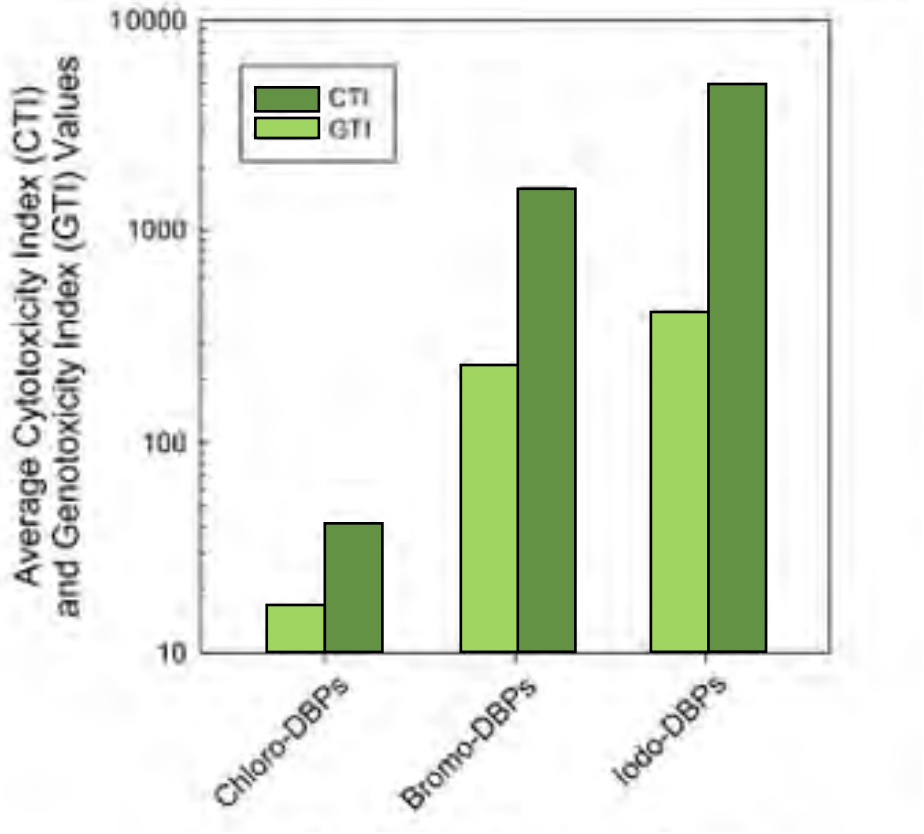
[3]Tsinghua University, Beijing, China

Disinfection By-Products (DBPs)

NOM
+ Br^-/I^-

DISINFECTANT

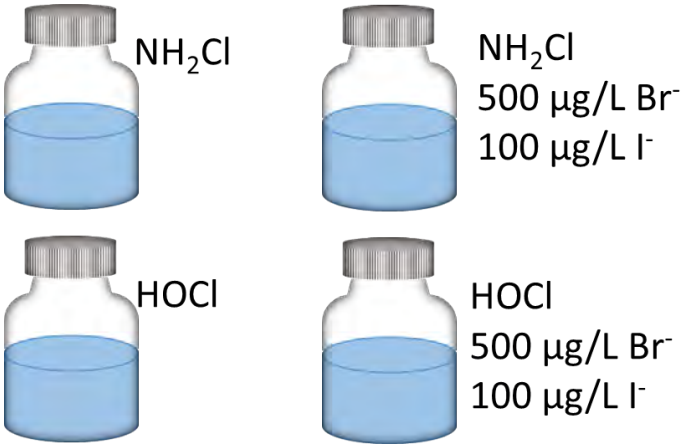
Bromo/Iodo-
DBPs



Halogenated DBP
Relative Toxicity:

I > Br >> Cl

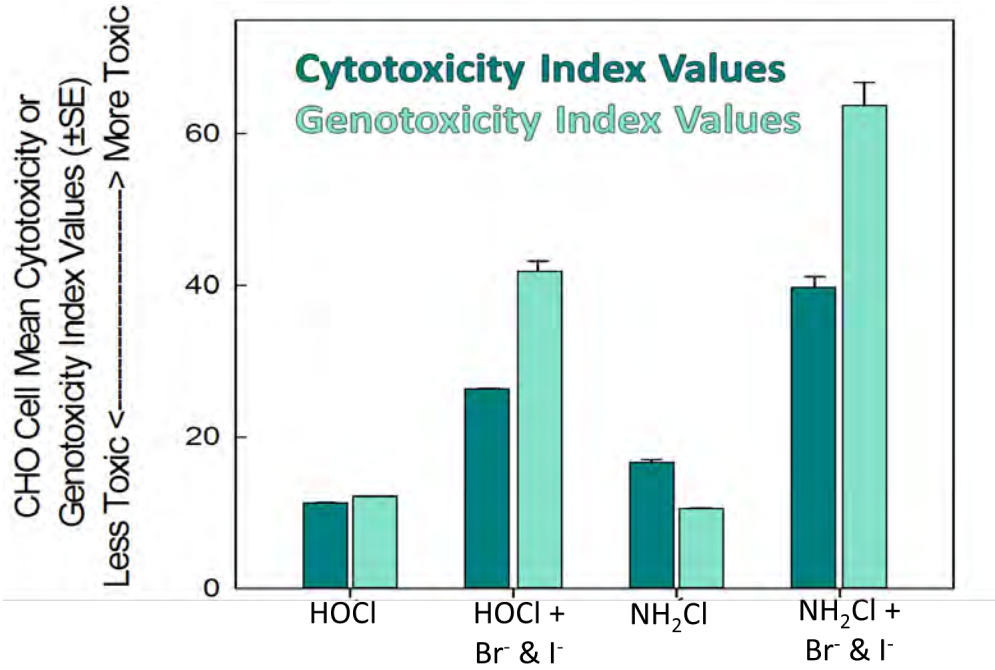
Disinfection Scenarios Investigated:



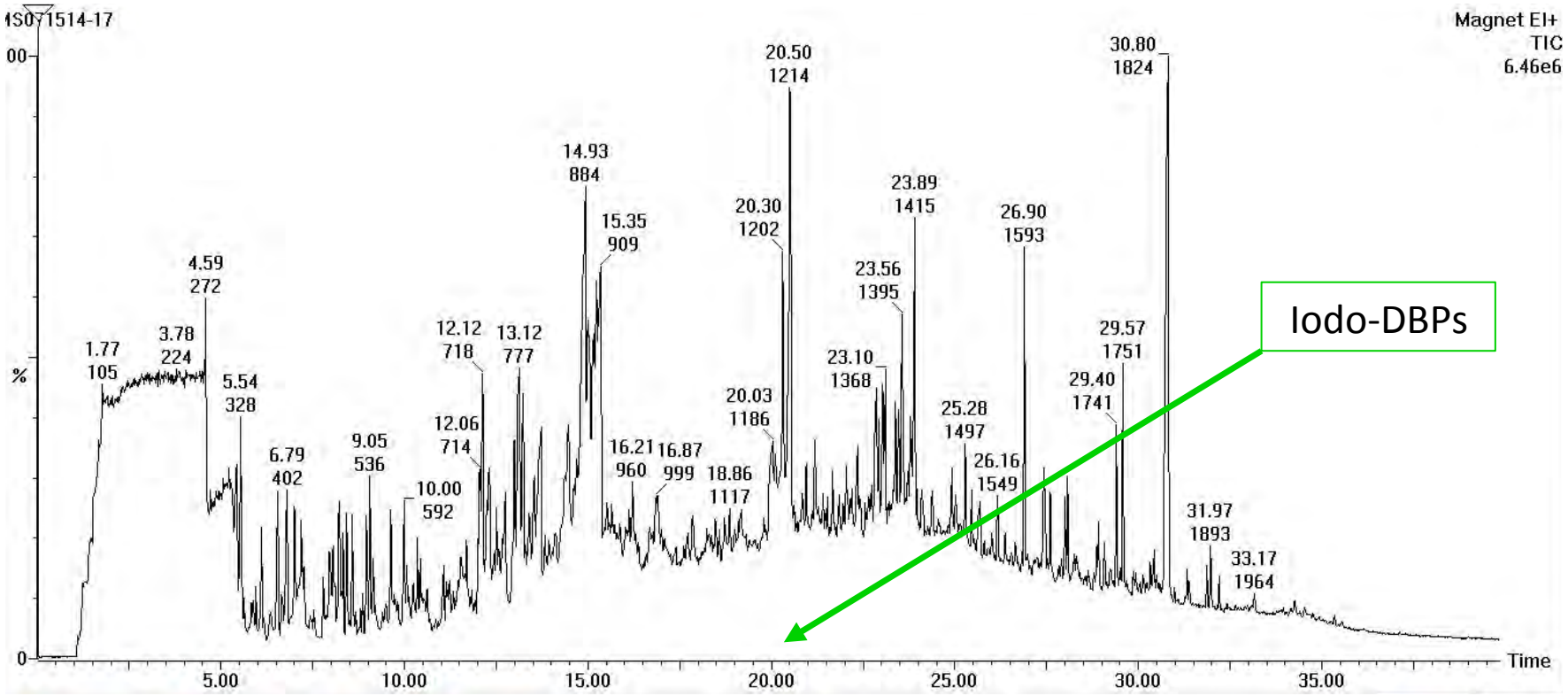
Toxicity of both disinfection processes increased with the addition of bromide and iodide

Chloraminated water with **Br^- and I^-** spike was the most toxic of the scenarios tested

High and low resolution GC/MS used to identify DBPs contributing to this elevated toxicity



Finding a Needle in a Haystack:
DBP Identification By GC/MS



> 1000 compounds per sample – most unidentified

Toxic Iodo-DBPs of particular concern

Challenges of Iodo-DBP Identification

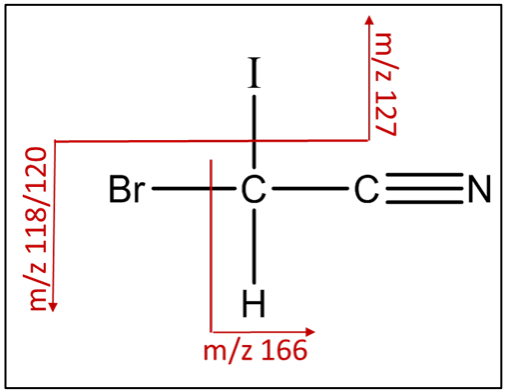
- Not many iodo-DBPs have been identified
- No telltale isotopic patterns indicating iodine
- XIC m/z 127 – I^+ fragment
- 127 not unique to I-containing compounds

Solution: High resolution & accurate mass

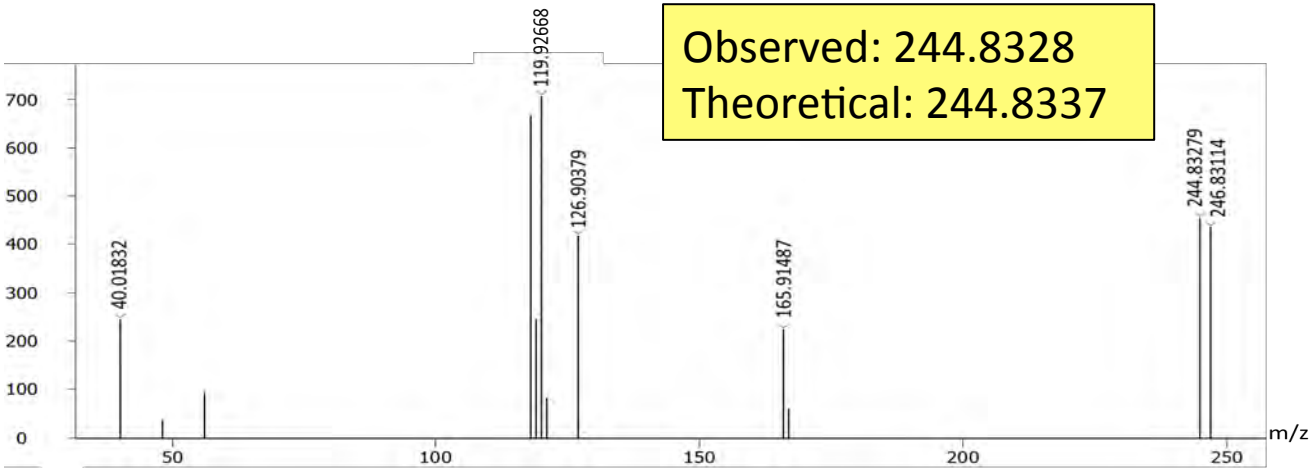
Fragment	Exact Mass	Resolution Needed
$CHClBr^+$	126.8950	13,421
I^+	126.9045	
$C_9H_{19}^+$	127.1487	520

Discovery of New Iodo-DBP

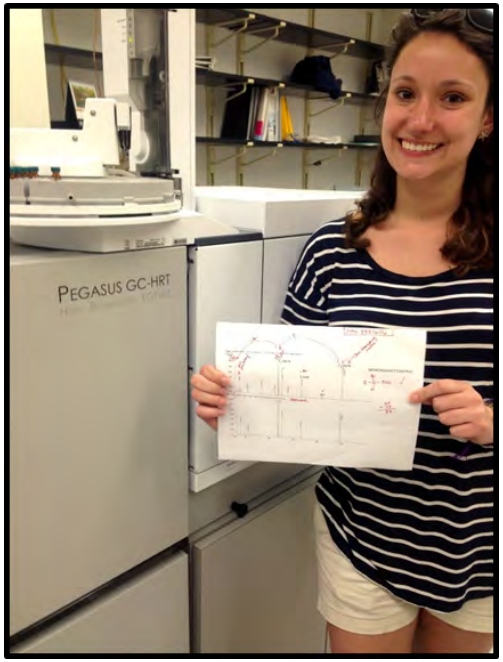
- LECO Pegasus GC-HRT time-of-flight mass spectrometer
- High resolution: 25,000
  - Maximum: 50,000
- Accurate mass
- XIC m/z 126.904
- Continuing data analysis for iodoacetonitriles and other new iodo-DBPs



Bromoiodoacetonitrile

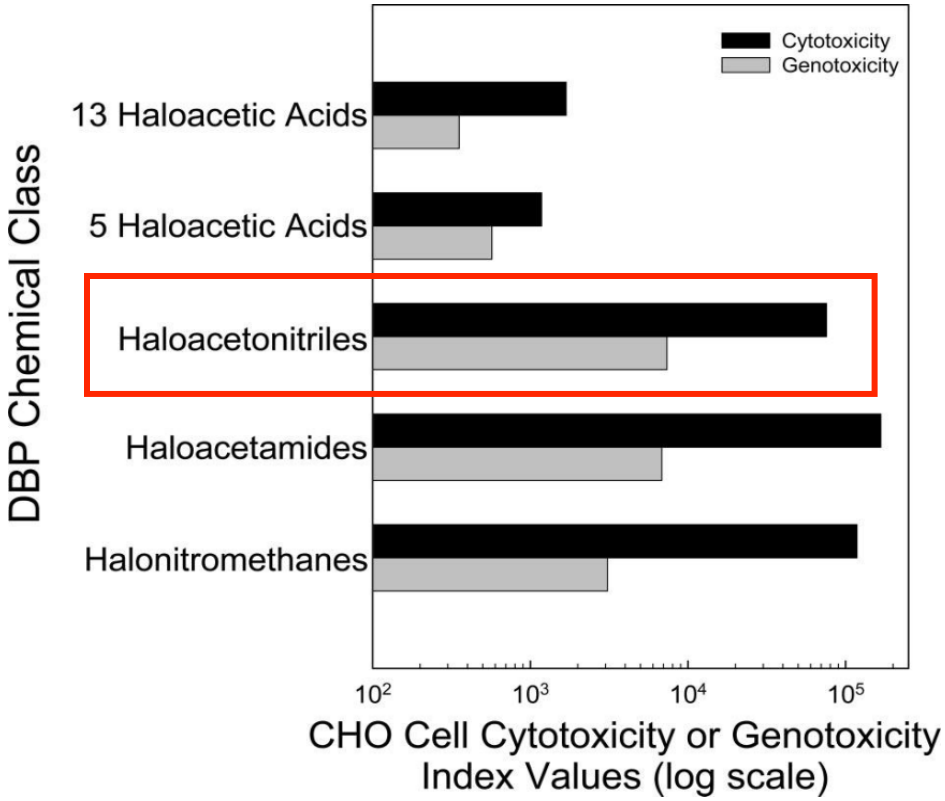


Observed: 244.8328
Theoretical: 244.8337



Haloacetonitriles are one of the most toxic classes of DBPs

Bromoiodoacetonitrile is likely to be the ***most toxic*** haloacetonitrile identified to date



Acknowledgments

- NSF WaterCAMPWS (Award CTS-0120978)
- U.S. EPA STAR grant R834867
- U.S. Department of Education's GAANN Fellowship through the University of South Carolina's Department of Chemistry & Biochemistry

Michael Plewa

Susan Richardson
Susana Kimura-Hara
Richardson Group

Jonathan Byer

ADVANCEMENTS IN LC-DIRECT EI-MS INTERFACING: NEW STRATEGIES TO BOOST SENSITIVITY AND SPECIFICITY.

- **Veronica Termopoli**, Laura Magrini, Giorgio Famiglini, Pierangela Palma and Achille Cappiello.
- LC-MS Laboratory, University of Urbino, Urbino,Italy.

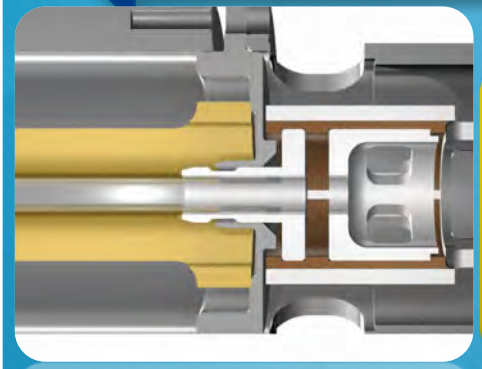
DIRECT EI INTERFACE: HOW DOES IT WORK?

TRIPLE QUAD

EI SOURCE

VACUUM HEATED REGION

UHPLC FLOW RATE



M^+

4 - Analyte ionization

3 – Solute vaporization

2 - Solvent evaporation

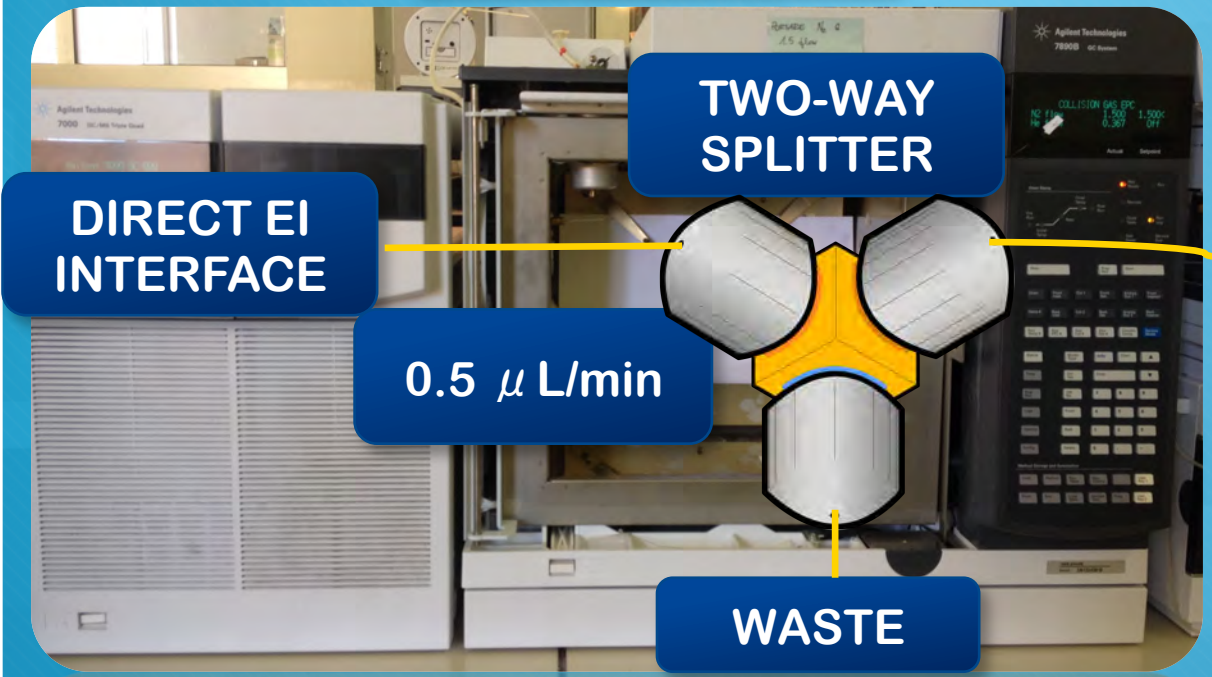
1 - Aerosol formation

Cappiello et al., *Anal. Chem.*, 2008, *80* (23), pp 9343–9348.
Cappiello et al., *Anal. Chem.*, 2007, *79* (14), pp 5364–5372.

LC- DIRECT ELECTRON IONIZATION MASS SPECTROMETRY

Agilent UHPLC 1290 Infinity

Agilent 7000 QqQ



TWO-WAY SPLITTER

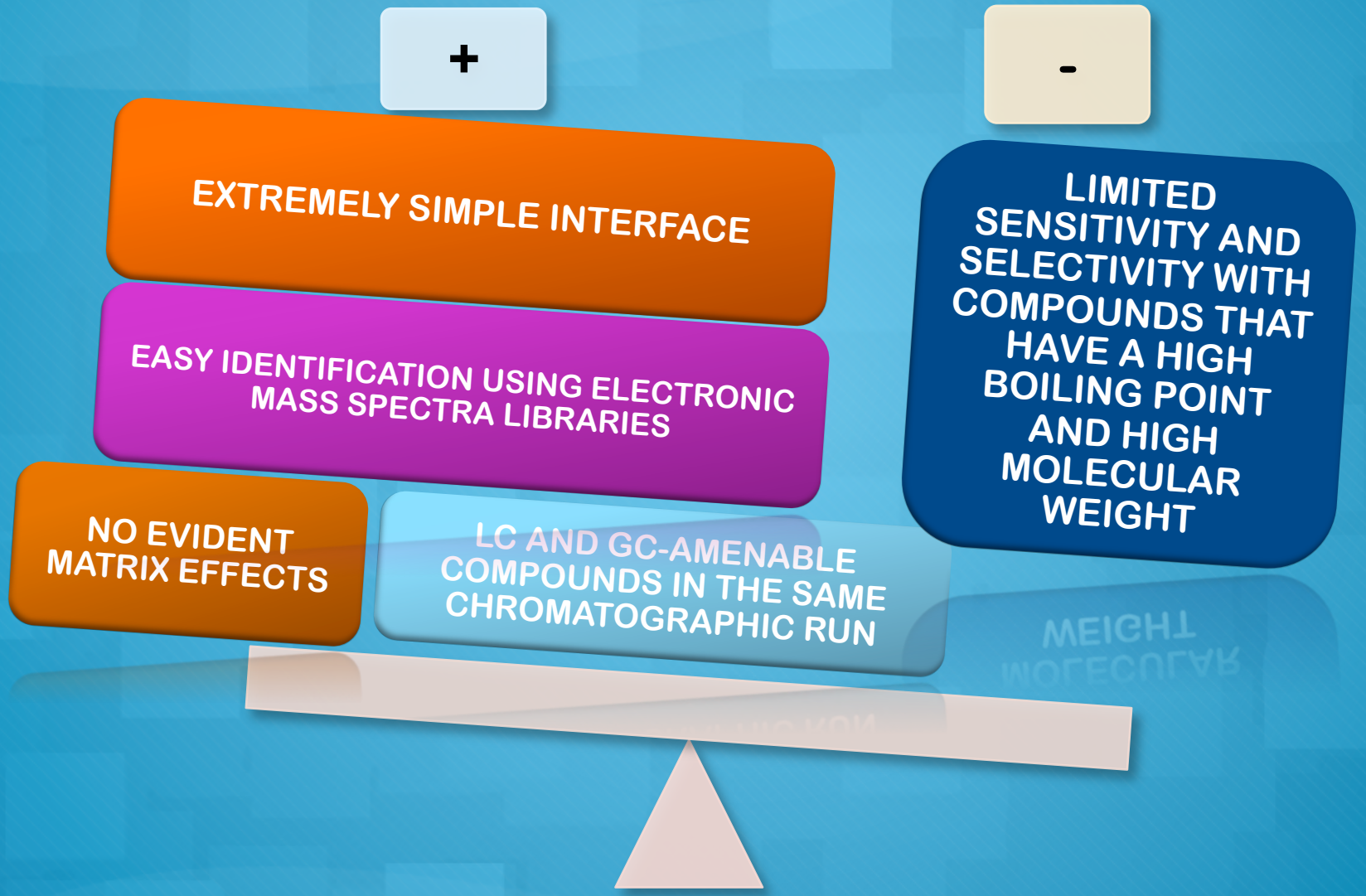
DIRECT EI INTERFACE

100 μ L/min

0.5 μ L/min

WASTE

ADVANTAGES…
DISADVANTAGES…



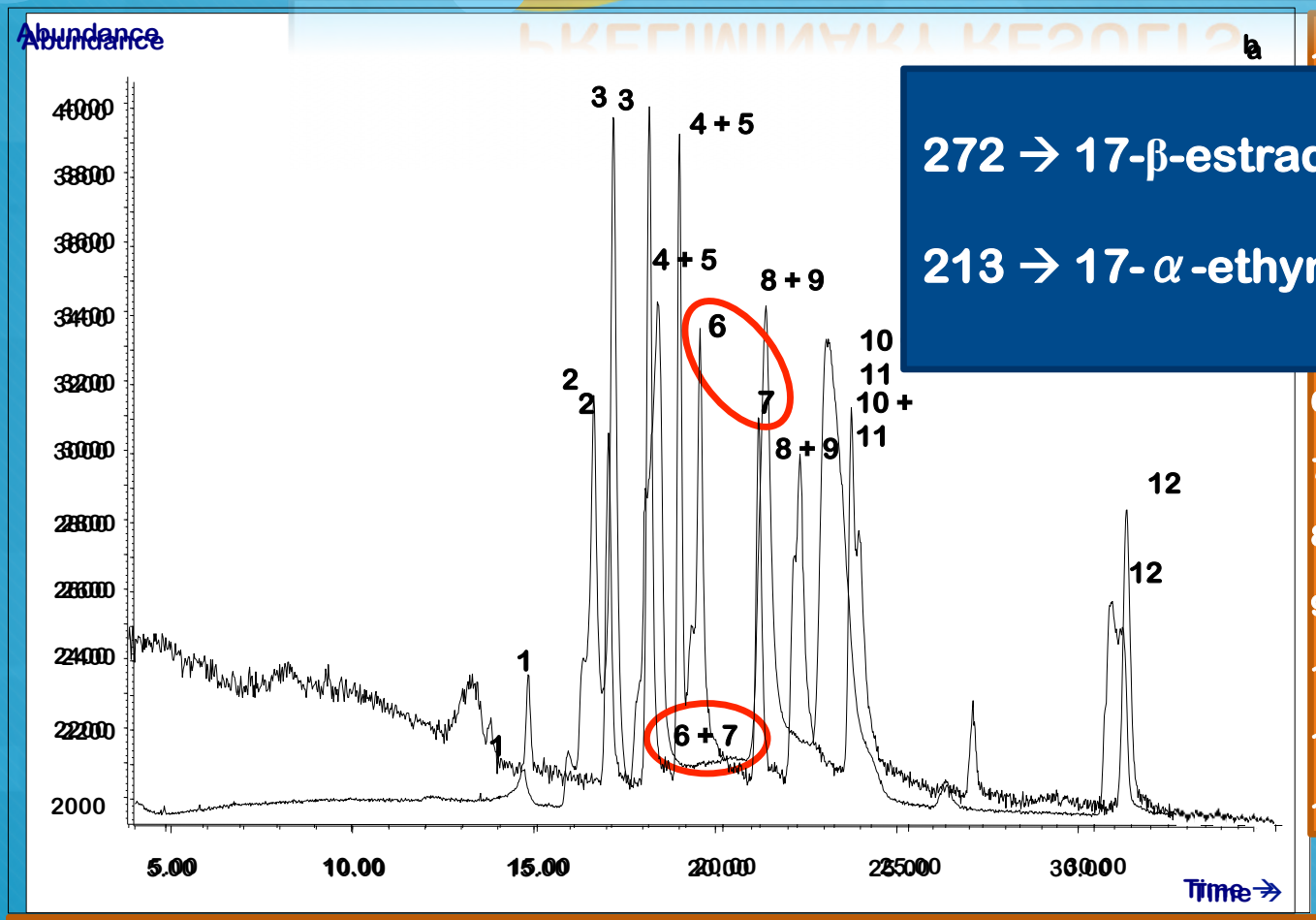
HOW TO BOOST SENSITIVITY AND SPECIFICITY?

MS/MS

NEW VAPORIZATION SURFACE

CERAMIC COATED ION SOURCE

METAL Vs CERAMIC COATING: PRELIMINARY RESULTS



272 → 17-β-estradiol

213 → 17-α-ethynilestradiol

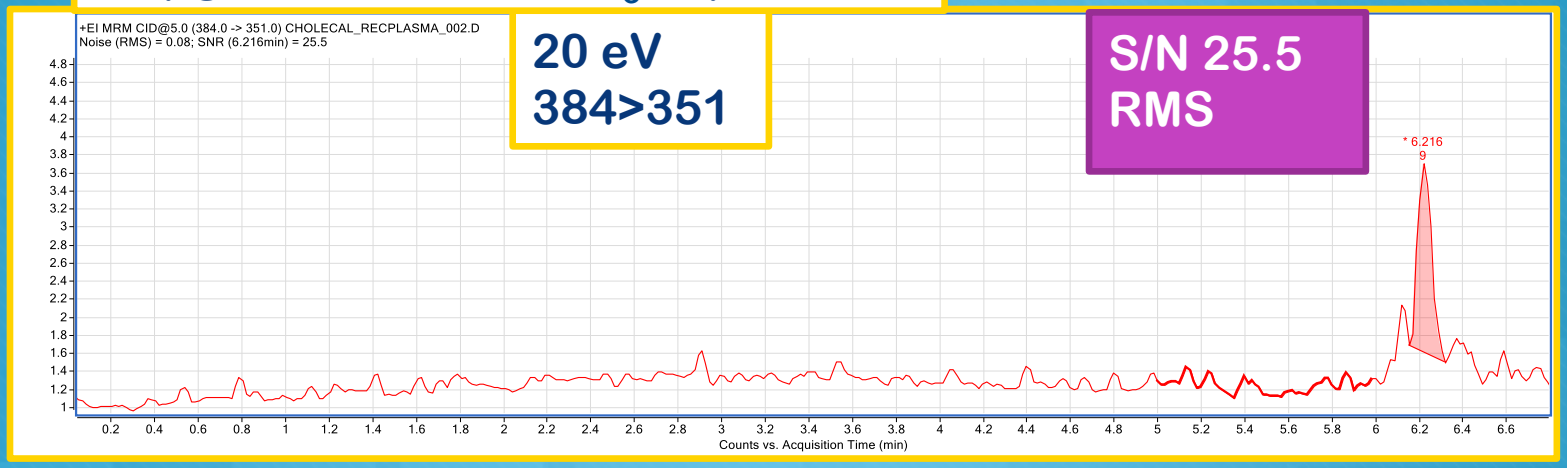
1. 115→ aldicarb
6. 272 → 17-β-estradiol
7. 213 → 17-α-ethynilestradiol
8. 162 → 2,4 DB
9. 142 → MCPB
10. 268 → diethylstilbestrol
11. 196 → silvex
12. 227 → mestranol

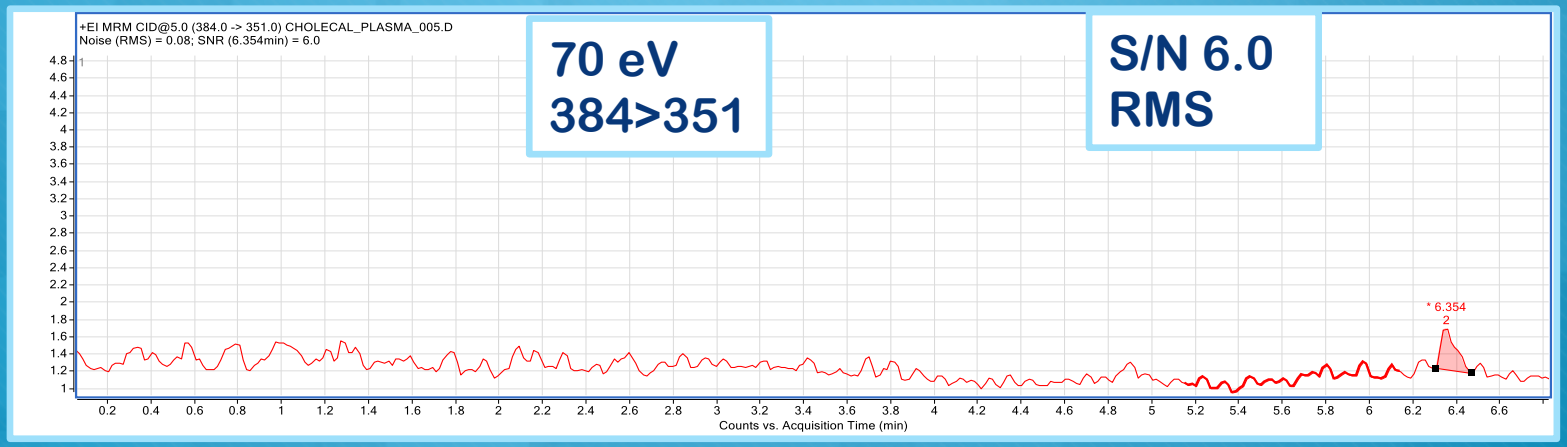
Mix 12 50/50 MeOH/EtOH; flow rate: 500 nL/min; mobile phase: water (A), acetonitrile (B), both acidified with 0,1% of TFA. Elution gradient : 0% B → 40 % B in 5 min, 40% B → 80% B in 35 min. Injection volume : 60 nL;
SIM one group : dwell time : 40 millis, 0,86 cycle/s; Temperature: 350°C

Cappiello et al., submitted to JASMS, may 2015.

….PLAY WITH DIFFERENT IONIZATION ENERGIES!!!!

40 pg/μL of Vitamin D_3 in plasma…





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DI URBINO
CARLO BO

Thanks.....

Agilent Technologies

Paraffina
SUPBOARDS

Utilization of Atmospheric Pressure Ionization Coupled to Triple Quadrupole Mass Spectrometry for the Analysis of Mixed-Halogenated Dioxins and Furans

Kari Organtini, Eric Reiner, Karl Jobst, Anne Myers, Adam Ladak, Doug Stevens, and Frank Dorman

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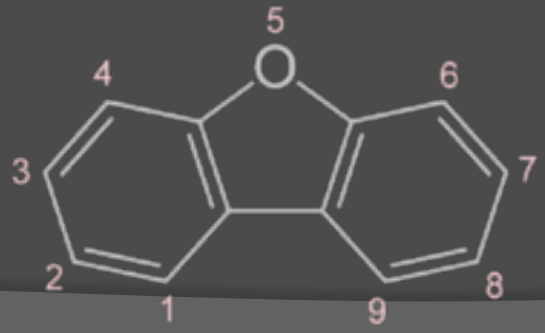
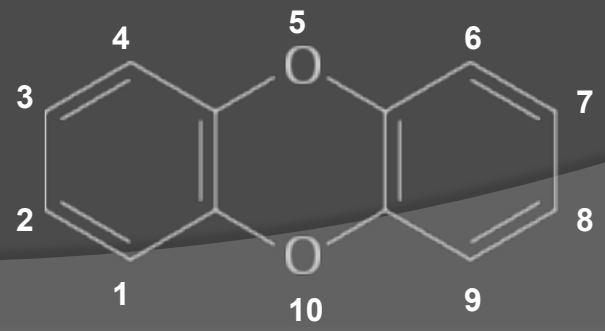
Dibenzo-p-dioxin/Dibenzofuran in fire debris

- Persistent environmental pollutants
  - 17 polychloro- congeners monitored by WHO
- Unintentional combustion byproducts
  - Municipal waste incinerators
  - Generation from brominated flame retardants during fires?
- Many studies performed on polychloro's (PCDD/Fs)
- Few analytical and biochemical studies of the mixed halo congeners have been performed (PXDD/Fs and PBDD/Fs)





Analytical Challenges:

- Complex matrix
- Complex separation
  - 5000 possible PXDD/F, PCDD/F, and PBDD/F congeners
    - 421 2,3,7,8-substituted congeners
- Limited availability of commercially available standards
- Trace level concentrations

GCxGC-TOFMS

APGC-MS/MS

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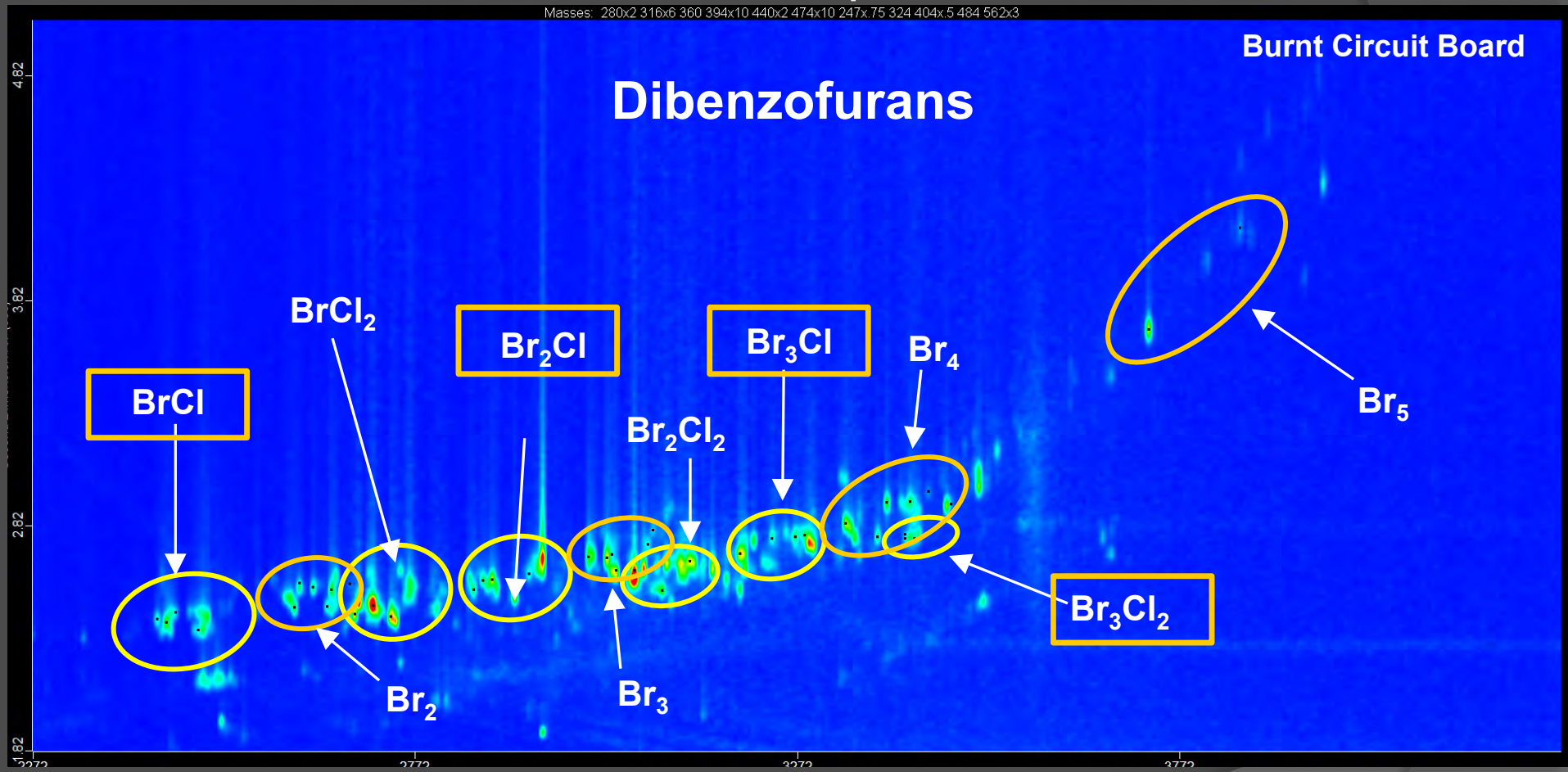
GCxGC-TOFMS Analysis

Electronics Fire Simulation Sample

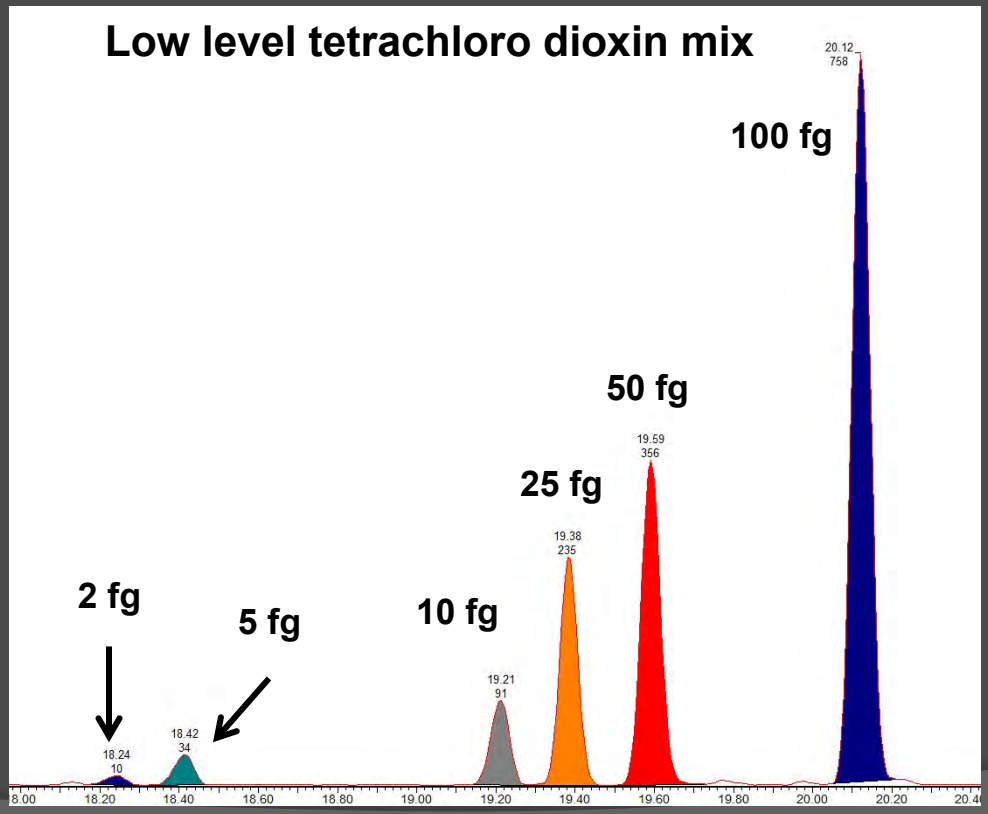


- Household fire generated a variety of PBDFs
- Electronics fire generated a variety of PBDFs and PXDFs
- No dioxin compounds identified
- Congener profiles very heterogeneous between samples

Organtini et al; Journal of Chromatography A, October 2014

APGC-MS/MS Analysis

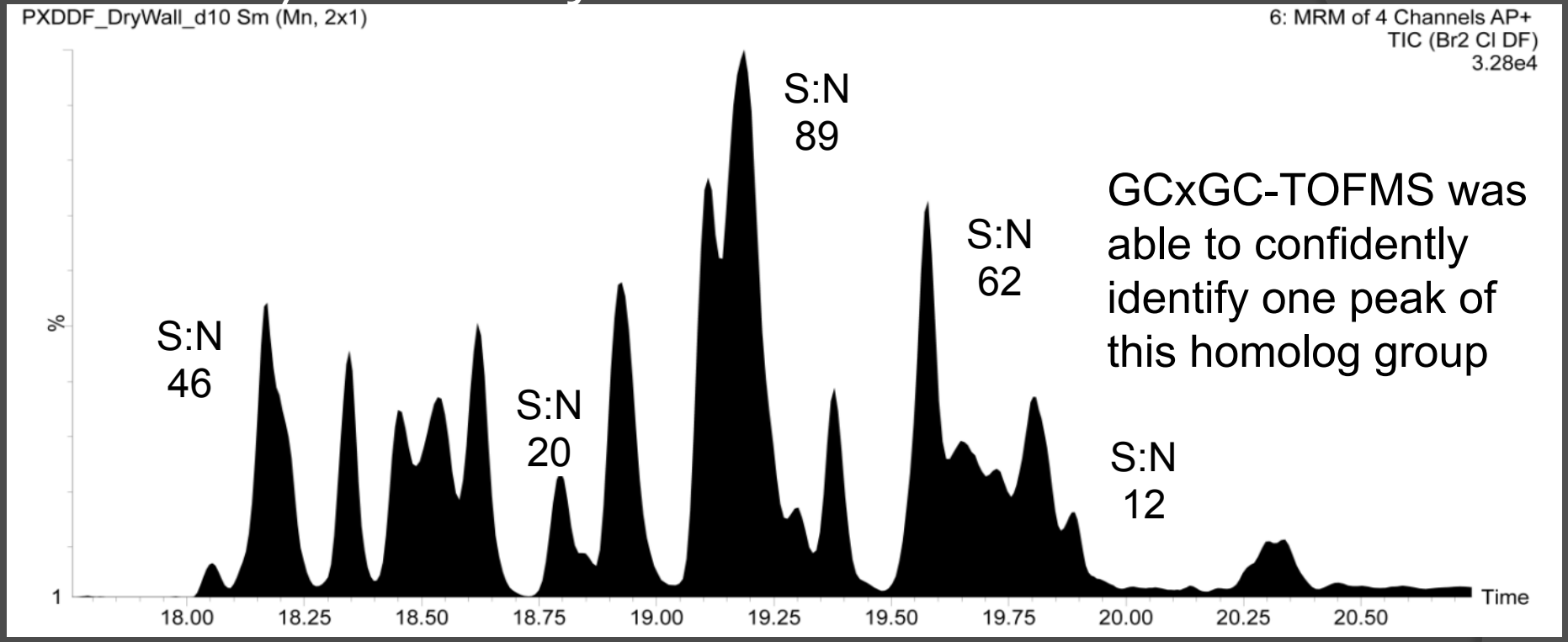
- Instrumentation is highly sensitive (fg level)
- We qualified APGC-MS/MS as a dioxin instrument
  - Historically GC-HRMS is used
- APGC-MS/MS MDLs are 2-20 times lower than GC-HRMS in multiple matrices for dioxins



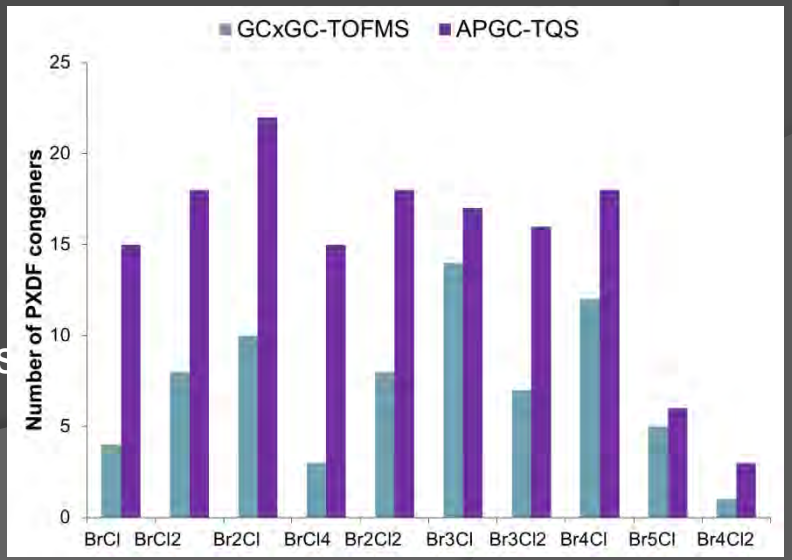
		Soil matrix (n=10)	
Compound	units	APGC-MS/MS MDL	GC-HRMS MDL
2,3,7,8-TCDF	pg/g	0.17	0.68
2,3,7,8-TCDD	pg/g	0.15	0.80
1,2,3,7,8-PeCDF	pg/g	1.32	2.64
2,3,4,7,8-PeCDF	pg/g	0.48	2.22
1,2,3,7,8-PeCDD	pg/g	0.39	3.85
1,2,3,4,7,8-HxCDF	pg/g	0.78	2.28
1,2,3,6,7,8-HxCDF	pg/g	0.54	1.01
1,2,3,7,8,9-HxCDF	pg/g	0.41	2.21
2,3,4,6,7,8-HxCDF	pg/g	0.37	2.30
1,2,3,4,7,8-HxCDD	pg/g	0.62	3.79
1,2,3,6,7,8-HxCDD	pg/g	0.40	3.01
1,2,3,7,8,9-HxCDD	pg/g	0.35	4.28
1,2,3,4,6,7,8-HpCDF	pg/g	0.28	3.36
1,2,3,4,7,8,9-HpCDF	pg/g	0.56	4.87
1,2,3,4,6,7,8-HpCDD	pg/g	0.41	1.62
OCDF	pg/g	0.74	4.85
OCDD	pg/g	1.42	4.49

Manuscript submitted for publication

APGC-MS/MS Analysis



- APGC-MS/MS was considerably more sensitive than both GCxGC-TOFMS and GC-HRMS
- APGC-MS/MS analysis confirmed the GCxGC data but identified many more compounds in the samples
- Polyhalogenated dioxins were identified in samples
- Congener profiles were more homogeneous between samples



Overall Conclusions and other studies

- Multiple analytical approaches have identified the generation of PXDD/Fs in fire debris
  - Semi-quantification has been completed to determine homolog group concentrations
  - Congener identity is not possible…yet
    - Ion mobility mass spectrometry
- Initial toxicity studies have shown these compounds behave similarly to 2,3,7,8-TCDD
  - Using a human cell culture system
- Fire fighters are being exposed to a complex mixture of PXDD/Fs through inhalation and contact
  - Better/increased regulation needed
  - Reconsider fire fighter safety procedures and standards

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Thank you for your attention!

klo5013@psu.edu

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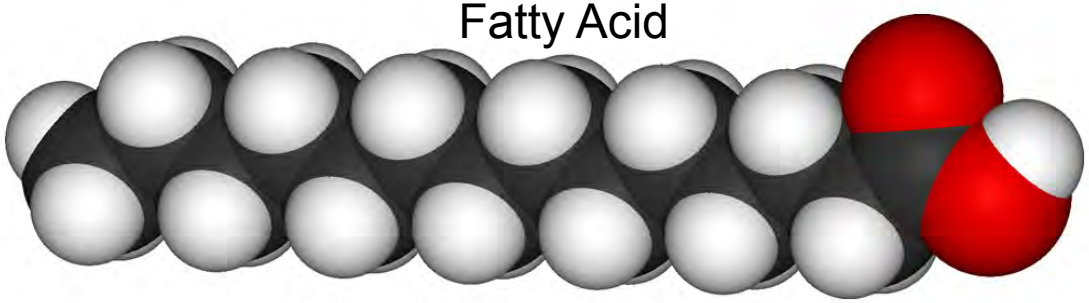
Waters THE SCIENCE OF WHAT'S POSSIBLE.™

UF Southeast Center *for* Integrated Metabolomics
Clinical and Translational Science Institute

NIST

Fatty Acid



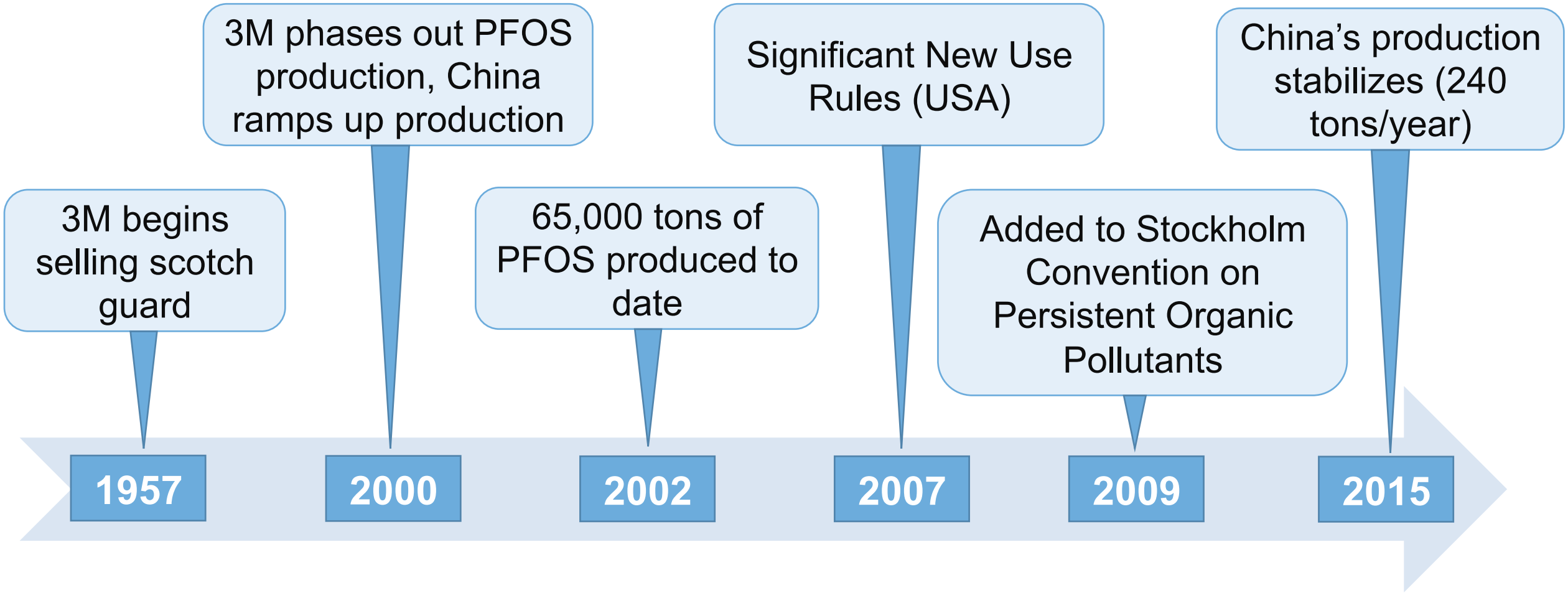
Lipidomics for Elucidating Biomarkers and Mechanisms of Perfluorooctanesulfonic acid (PFOS) Toxicity

Jeremy P. Koelmel[1], John A. Bowden[2], Timothy J. Garrett[1], Louis J. Guillette[3], and Richard A. Yost[1]

PFOS

1: University of Florida, SECIM
2: NIST
3: Medical University of South Carolina

PFOS: Production, Fate, and Transport



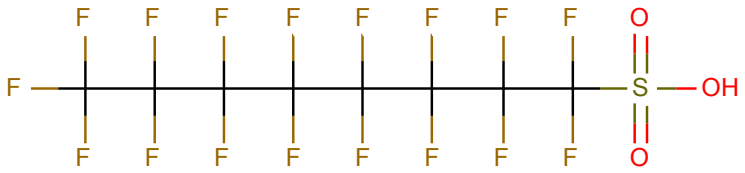
Environmental fate and transport:

Degradation Water: 41 years (do not partition to sediment)

Atmospheric half life: 114 days (arctic)

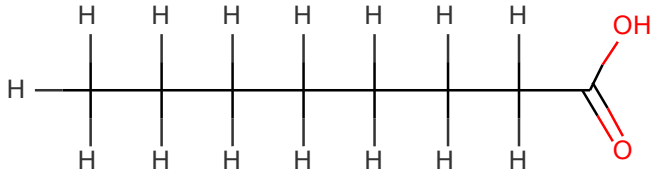
PFOS: Biology, the Case for Lipidomics

Perfluorooctanesulfonic acid



PFOS

Octanoic Acid

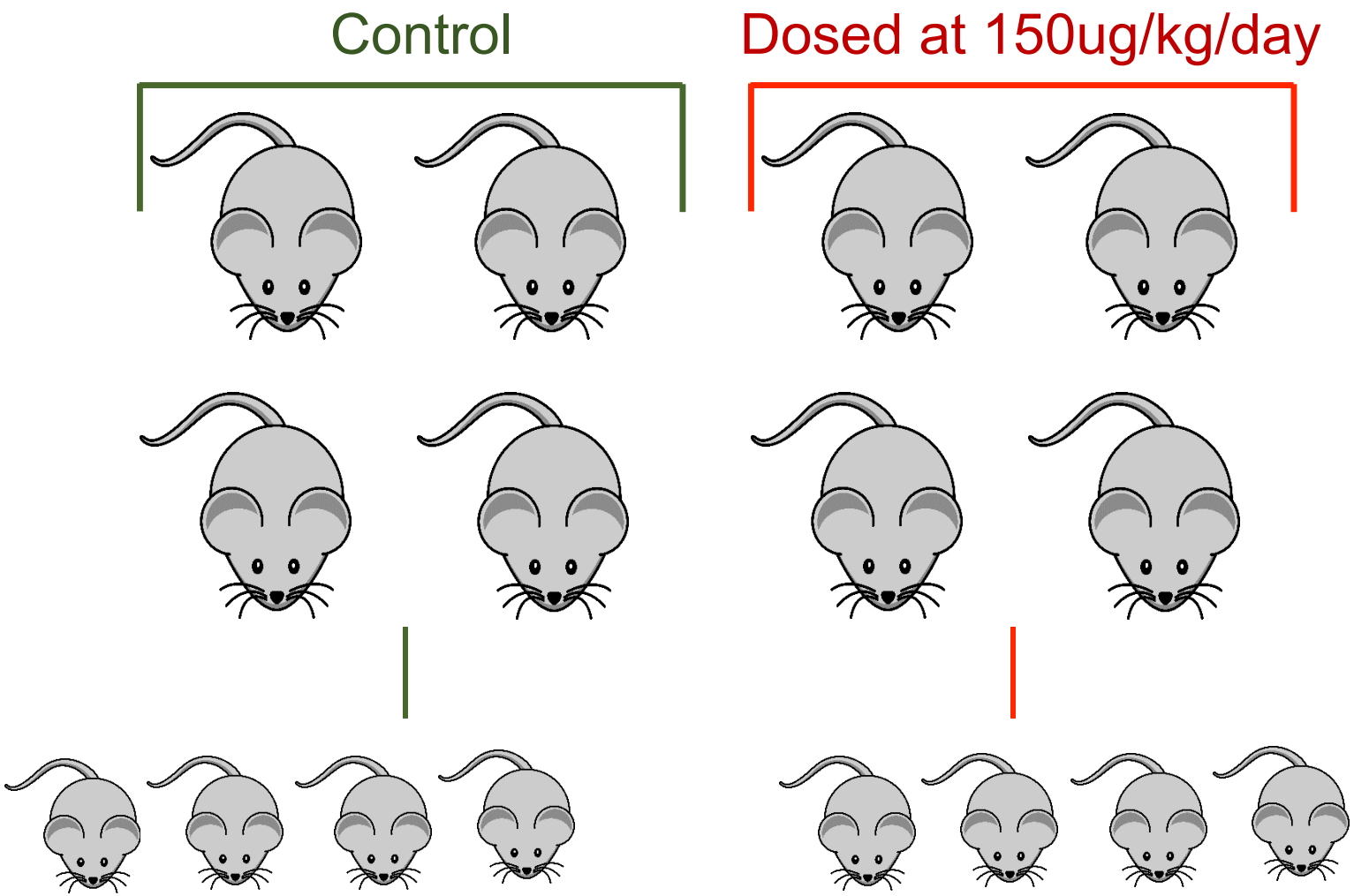


FFA(8:0)

Obesogenic:
PPARα inhibition: ↑**secretion cholesterol** and lipoproteins in liver, ↓**β-oxidation of fatty acids**, ↑**lipid droplets**

Increased cholesterol. No true lipidomics study.

Lipidomics: Workflow



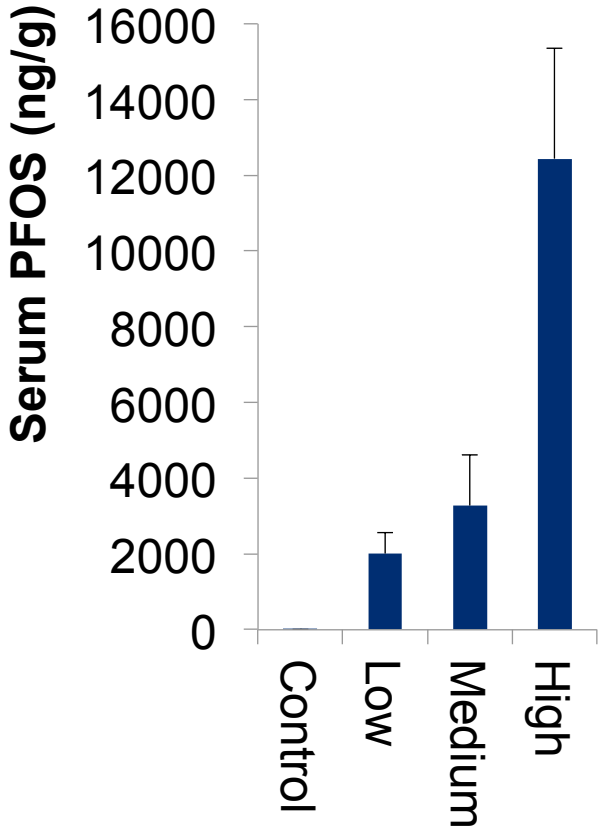
After gestation and weaning (18 weeks)
Daughter liver, fat and brain

- Extraction
- Acquisition
- Feature Detection
- Feature Identification
- Statistics
- Biology

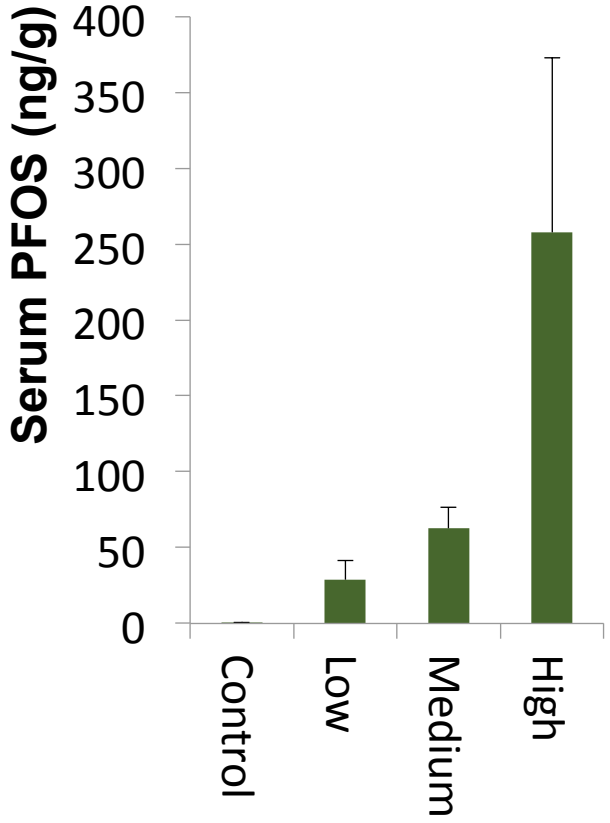
PFOS Measurements

Serum PFOS

Mothers

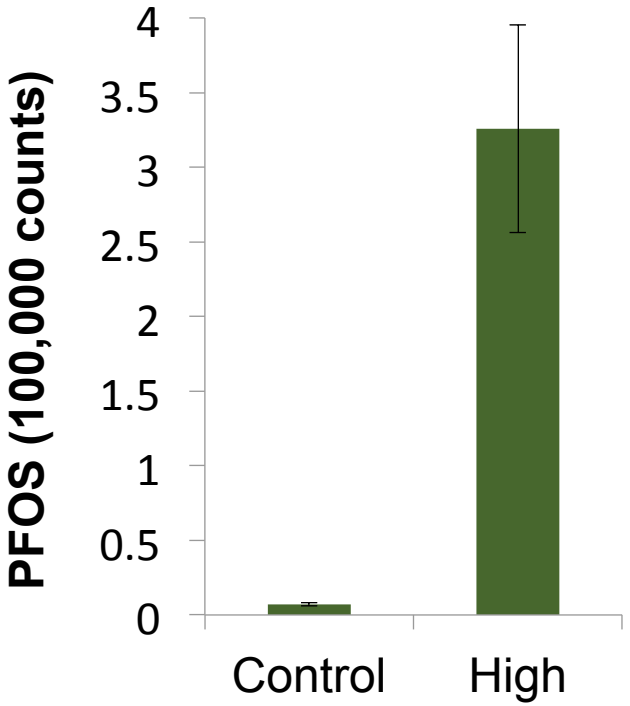


Daughters (18 weeks)



Liver PFOS

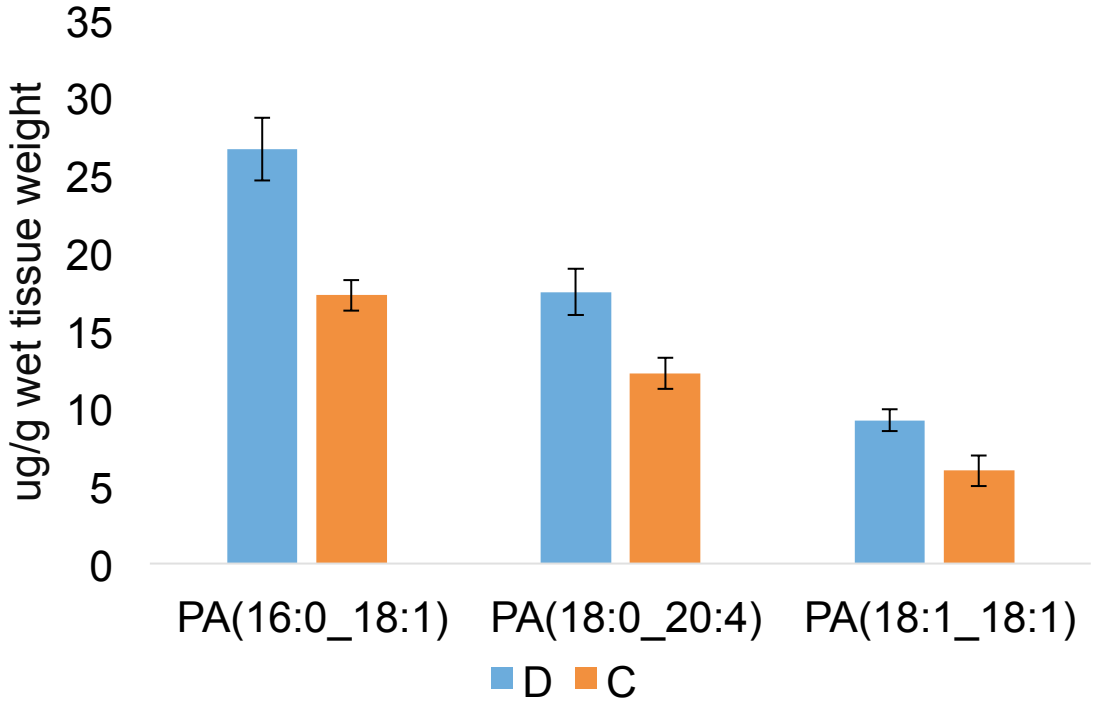
Daughters (18 weeks)

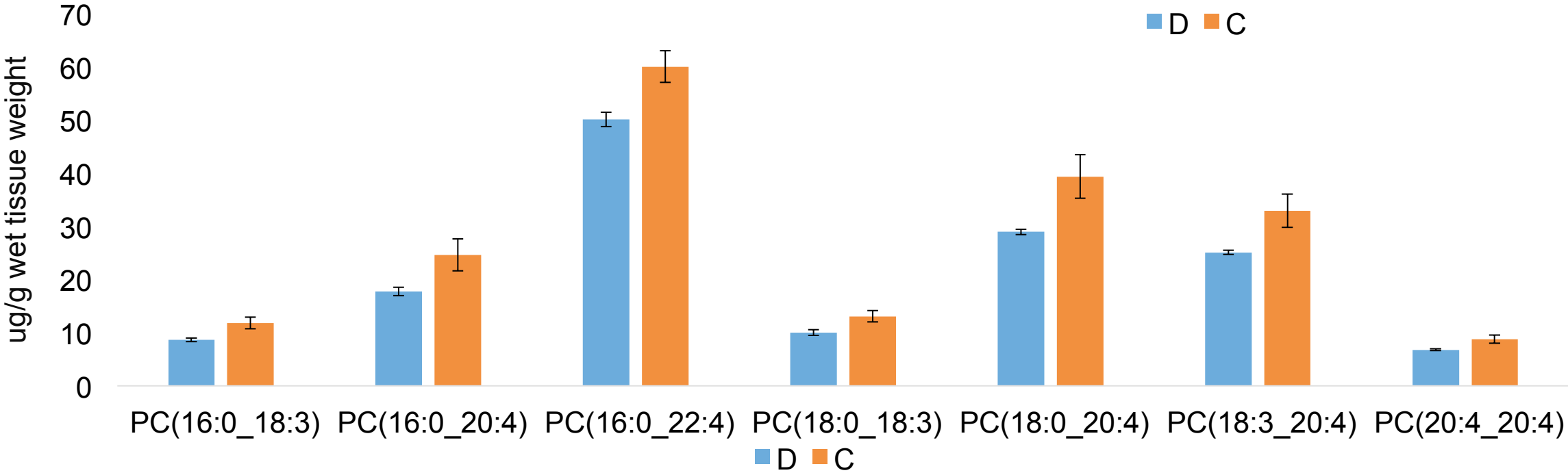


*Brain and fat:
no increase in PFOS

Lipid Measurements

<u>Brain</u>: 112 Lipids Confirmed
3 out of 3 PAs upregulated dosed
11 out of 36 PCs downregulated dosed
4 out of 11 Cers upregulated dosed
4 out of 9 PSs upregulated dosed





Development of an LC-MS/MS Method for the Quantification of Microcystins in Blue-Green Algal Dietary Supplements

Whitney L. Stutts, Christine H. Parker, and Stacey L. Degrasse

ASMS Environmental Working Group

June 3, 2015

Blue-Green Algal Dietary Supplements & Microcystins

- **Problem:**

  The cyanobacterium *Aphanizomenon flos-aquae* (AFA), which is harvested from natural lakes and commercially distributed as blue-green algal (BGA) dietary supplements, may be contaminated with toxic microcystins produced by co-occurring *Microcystis aeruginosa.*

- **Regulation:**

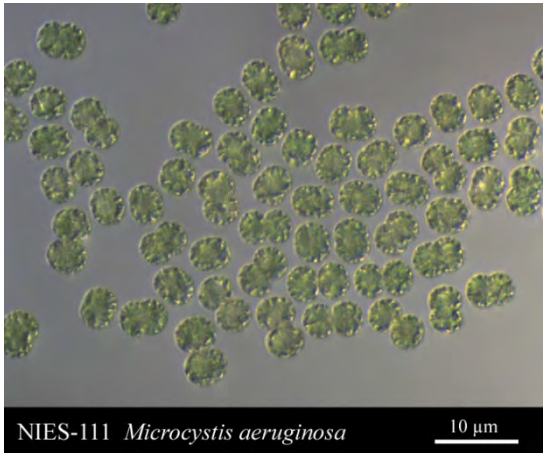
  – No regulatory action level for microcystins (MC) in dietary supplements

  – Oregon Health Division and Oregon Department of Agriculture: state guidance value of 1 µg MC-LR_{eq}/g for microcystins in BGA products

- **Research Goal:**

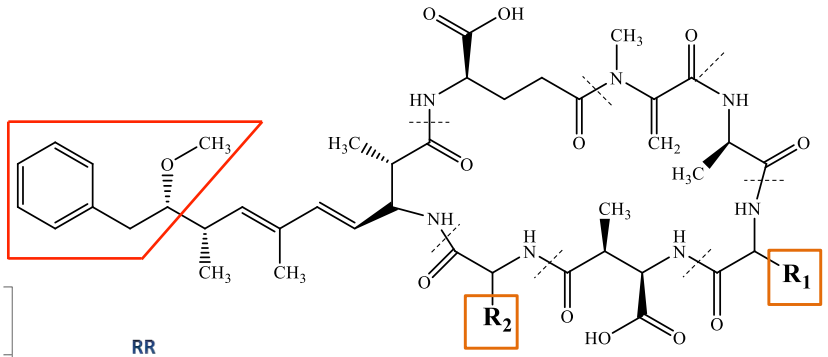
  Develop and validate a selective LC-MS/MS method for the simultaneous detection and quantification of 7 MC congeners in AFA dietary supplements

http://www.oregonwild.org/waters/klamath/the-klamath-river/klamath-river-water-quality

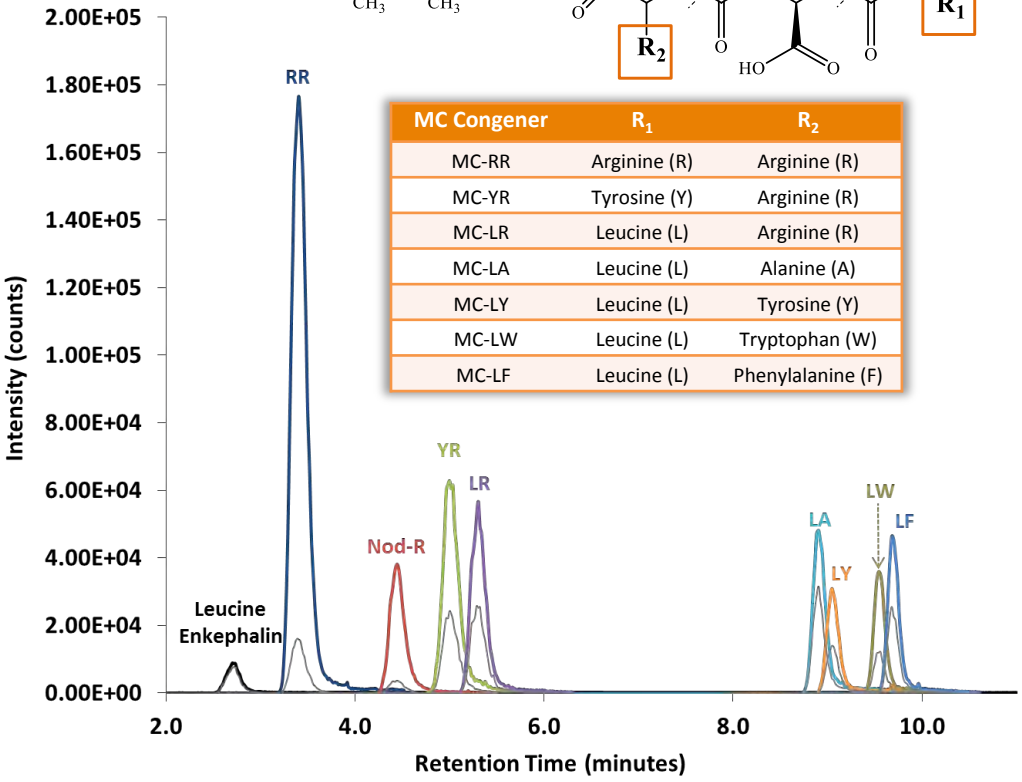


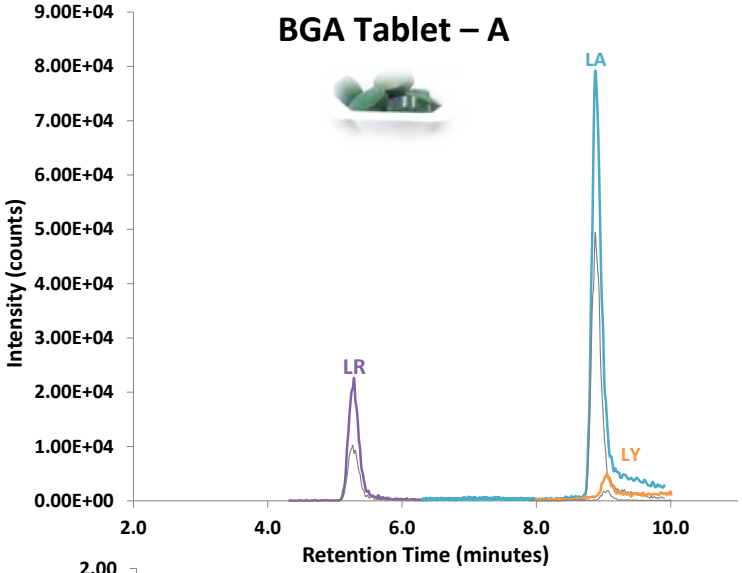
http://www.shigen.nig.ac.jp/algae/strainDetailAction.do?stockNo=NIES-111

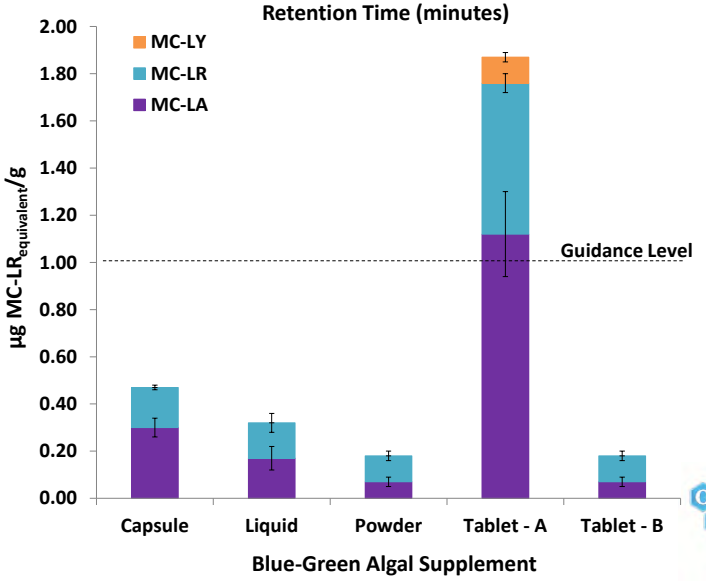
Quantitative LC-MS/MS Method Development



MC Congener	R_1	R_2
MC-RR	Arginine (R)	Arginine (R)
MC-YR	Tyrosine (Y)	Arginine (R)
MC-LR	Leucine (L)	Arginine (R)
MC-LA	Leucine (L)	Alanine (A)
MC-LY	Leucine (L)	Tyrosine (Y)
MC-LW	Leucine (L)	Tryptophan (W)
MC-LF	Leucine (L)	Phenylalanine (F)







Waters ACQUITY UPLC coupled with an AB Sciex 5500 QTRAP
ACQUITY UPLC analytical column (150 mm × 1 mm ID), 1.7 µm C18 BEH particles (130 Å)

OFFICE OF REGULATORY SCIENCE

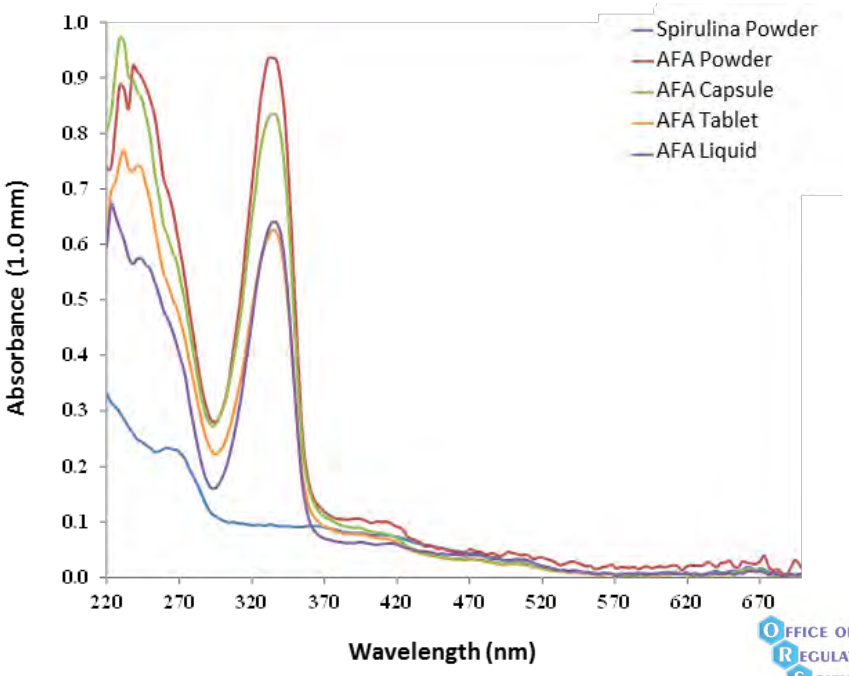
The Challenge of Finding a Matrix Blank

- **BGA supplements**
  - AFA-based: detectable concentrations of MC in all supplements tested
  - Spirulina-based: free of MC contamination, but not a suitable matrix blank

Congener	z	% Recovery† AFA	% Recovery† Spirulina
MC-RR	2	65.1 ± 0.6	53.2 ± 1.0
Nod-R	1	72.2 ± 1.7	56.9 ± 4.0
MC-YR	1	54.9 ± 1.5	17.2 ± 0.4
MC-YR	2	59.8 ± 1.8	76.7 ± 2.7
MC-LR	1	67.7 ± 1.7	31.2 ± 1.6
MC-LR	2	71.7 ± 1.4	61.9 ± 1.1
MC-LA	1	77.2 ± 1.4	57.0 ± 2.2
MC-LY	1	63.3 ± 1.4	43.3 ± 1.5
MC-LW	1	50.0 ± 1.4	25.0 ± 1.4
MC-LF	1	60.1 ± 1.4	42.7 ± 2.7
Average		**64.2 ± 8.3**	**46.5 ± 18.2**
%RSD		**13.0%**	**39.2%**

†Peak areas were compared for biological replicates of pre- and post-fortified sample extracts at a 1 µg/g spike concentration.

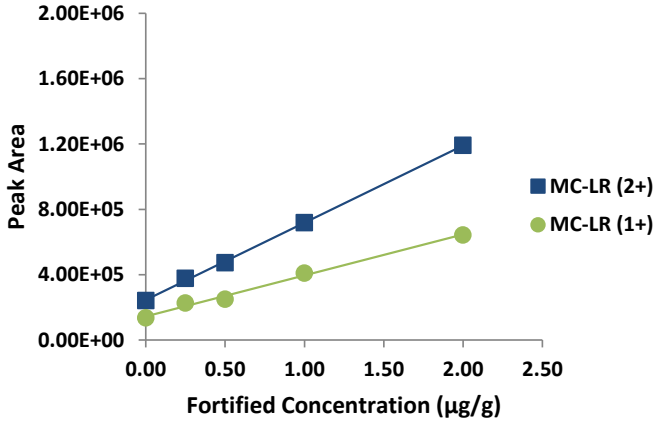
- **Problems with Spirulina**
  - Lower sample processing recoveries
  - Possibly due to the absence of planar Mycosporine-like amino acids (MAAs)—accessory pigment molecules produced in cyanobacteria under high UV radiation



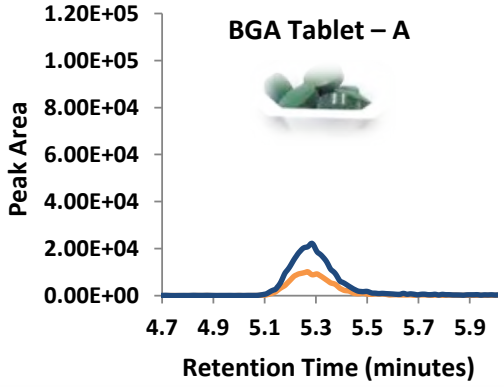
Solutions for Quantitation

Fortified Concentration (µg/g) MC-LR	Standard Addition % Recovery	Matrix-Corrected Neat Calibration %Recovery
2.00	100.1 ± 3.7	97.4 ± 3.6
1.00	100.1 ± 1.0	97.9 ± 1.4
0.50	95.9 ± 6.0	94.8 ± 6.1
0.25	111.3 ± 1.6	111.6 ± 4.3

Standard Addition

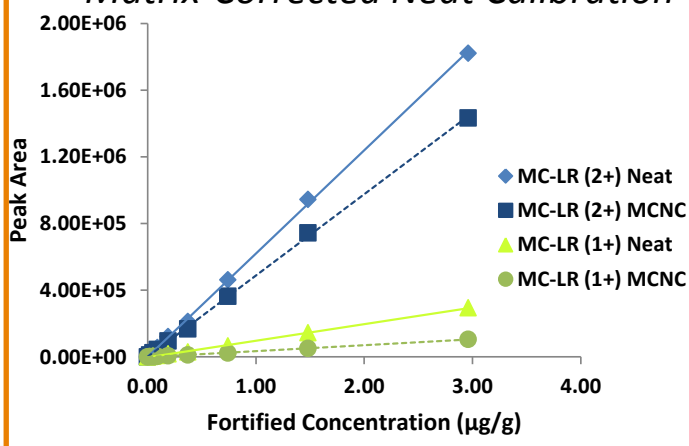


Endogenous MC-LR



Standard Addition	Matrix-Corrected Neat Calibration
0.52 ± 0.02 µg/g	0.49 ± 0.01 µg/g

Matrix-Corrected Neat Calibration



Conclusions & Ongoing Challenges

Microcystin Congener	AFA Capsule (µg/g)	AFA Liquid (µg/g)	AFA Powder (µg/g)	AFA Tablet Lot A (µg/g)	AFA Tablet Lot B (µg/g)
MC-LR	0.17 ± 0.01	0.15 ± 0.04	0.11 ± 0.02	0.64 ± 0.04	0.11 ± 0.02
MC-LA	0.30 ± 0.04	0.17 ± 0.05	0.07 ± 0.02	1.12. ± 0.18	0.07± 0.01
MC-LY	ND	ND	ND	0.11 ± 0.02	ND
Total µg MC-LR_{eq}/g	**0.47 ± 0.04**	**0.32 ± 0.06**	**0.18 ± 0.03**	**1.87 ± 0.19**	**0.18 ± 0.02**

ND = Not Detected

- **94 MC variants reported**
  - Toxicities for all MC variants have not been determined
  - Chronic effects of exposure are unknown
  - Risk assessment data is needed before a regulatory level(s?) can be established
- **Lot-to-lot variability poses a challenge for screening and regulation**
- **Isotopically labeled internal standards are not yet available, making accurate quantitation more challenging**

Experimental

- Instrumentation: AB Sciex QTrap 5500 equipped with a Turbo V ionization source and a Waters Acquity UPLC system
- LC Parameters:

Acquity UPLC Column	BEH C18 (1.7 µm, 1.0 mm × 150 mm)
Column Temperature	40 °C
Injection Volume	2 µL

- MS Parameters:

Source Temperature	400 °C
IonSpray Voltage	5000 V
Curtain Gas	20 psi
Gas 1	40 psi
Gas 2	30 psi