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. Koelmel), health risks to firefighters from fire debris (K. Organtini), and advancements in LC-Direct EI interfacing (V. Termpoli).

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. Liberatore¹, Yang Yang³, Yukako Komaki², Susana Y. Kimura¹, Hong-Ying Hu³, Elizabeth D. Wagner², Michael J. Plewa², and Susan D. Richardson¹

¹University of South Carolina, Columbia, SC
²University of Illinois, Urbana, IL
³Tsinghua University, Beijing, China
Disinfection By-Products (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**

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Exact Mass</th>
<th>Resolution Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHClBr⁺</td>
<td>126.8950</td>
<td>13,421</td>
</tr>
<tr>
<td>I⁺</td>
<td><strong>126.9045</strong></td>
<td></td>
</tr>
<tr>
<td>C₉H₁₉⁺</td>
<td>127.1487</td>
<td>520</td>
</tr>
</tbody>
</table>
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
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.
1 - Aerosol formation
2 - Solvent evaporation
3 - Solute vaporization
4 - Analyte ionization

DIRECT EI INTERFACE: HOW DOES IT WORK?

TRIPLE QUAD
VACUUM HEATED REGION
EI SOURCE
UHPLC FLOW RATE

M⁺

4 - Analyte ionization
3 - Solute vaporization
2 - Solvent evaporation
1 - Aerosol formation

LC- DIRECT ELECTRON IONIZATION MASS SPECTROMETRY

Agilent UHPLC 1290 Infinity

Agilent 7000 QqQ

TWO-WAY SPLITTER

DIRECT EI INTERFACE

WASTE

100 μL/min

0.5 μL/min
ADVANTAGES...

+ EXTREMELY SIMPLE INTERFACE
+ EASY IDENTIFICATION USING ELECTRONIC MASS SPECTRA LIBRARIES
+ NO EVIDENT MATRIX EFFECTS

- LC AND GC-AMENABLE COMPOUNDS IN THE SAME CHROMATOGRAPHIC RUN
- LIMITED SENSITIVITY AND SELECTIVITY WITH COMPOUNDS THAT HAVE A HIGH BOILING POINT AND HIGH MOLECULAR WEIGHT

DISADVANTAGES...
HOW TO BOOST SENSITIVITY AND SPECIFICITY?

MS/MS

NEW VAPORIZATION SURFACE

CERAMIC COATED ION SOURCE
METAL Vs CERAMIC COATING: PRELIMINARY RESULTS

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 D₃ in plasma...

20 eV
384>351
S/N 25.5 RMS

70 eV
384>351
S/N 6.0 RMS
Thanks.....

Agilent Technologies
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
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 congener 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 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

**Low level tetrachloro dioxin mix**

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

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
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.

GCxGC-TOFMS was able to confidently identify one peak of this homolog group.
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
Thank you for your attention!

klo5013@psu.edu
Lipidomics for Elucidating Biomarkers and Mechanisms of Perfluorooctanesulfonic acid (PFOS) Toxicity

Jeremy P. Koelmel¹, John A. Bowden², Timothy J. Garrett¹, Louis J. Guillette³, and Richard A. Yost¹

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)
Perfluorooctanesulfonic acid

Octanoic Acid

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

Increased cholesterol. No true lipidomics study.
Lipidomics: Workflow

Control

Dosed at 150ug/kg/day

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

Extraction

Acquisition

Feature Detection

Feature Identification

Statistics

Biology
PFOS Measurements

**Serum PFOS**

- **Mothers**
  - Control
  - Low
  - Medium
  - High

- **Daughters (18 weeks)**
  - Control
  - Low
  - Medium
  - High

**Liver PFOS**

- **Control**
- **High**

*Brain and fat: no increase in PFOS*
Lipid Measurements

Brain: 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<sub>eq</sub>/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
Quantitative LC-MS/MS Method Development

<table>
<thead>
<tr>
<th>MC Congener</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-RR</td>
<td>Arginine (R)</td>
<td>Arginine (R)</td>
</tr>
<tr>
<td>MC-YR</td>
<td>Tyrosine (Y)</td>
<td>Arginine (R)</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Leucine (L)</td>
<td>Arginine (R)</td>
</tr>
<tr>
<td>MC-LA</td>
<td>Leucine (L)</td>
<td>Alanine (A)</td>
</tr>
<tr>
<td>MC-LY</td>
<td>Leucine (L)</td>
<td>Tyrosine (Y)</td>
</tr>
<tr>
<td>MC-LW</td>
<td>Leucine (L)</td>
<td>Tryptophan (W)</td>
</tr>
<tr>
<td>MC-LF</td>
<td>Leucine (L)</td>
<td>Phenylalanine (F)</td>
</tr>
</tbody>
</table>

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)
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

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

†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

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

**Standard Addition**

Endogenous MC-LR

**Matrix-Corrected Neat Calibration**

### Endogenous MC-LR

**BGA Tablet – A**

<table>
<thead>
<tr>
<th>Standard Addition</th>
<th>Matrix-Corrected Neat Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.52 ± 0.02 µg/g</td>
<td>0.49 ± 0.01 µg/g</td>
</tr>
</tbody>
</table>

---

[OFFICE OF REGULATORY SCIENCE](www.fda.gov)
Conclusions & Ongoing Challenges

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

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