Advanced technologies for analysis of poly-halogenated dioxins and furans in controlled burn samples

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Special Thanks To:

- Kari Organtini
- Gary Perdew
- Ross Hardison
Firefighting is a Dangerous Profession

- Fire fighters have more cancer deaths and cancer cases than the average person.
- This increase in cancer was primarily due to digestive, oral, respiratory, and urinary cancers.
  - NIOSH Fire Fighter Cancer Study, November 2013
- Excess bladder and prostate cancer incidence was found among firefighters
- Increased risk of Hodgkin lymphoma and cancers of the cervix and thyroid among women firefighters
Effect of large scale fires:

- **World Trade Center**
  - New York City (2001)
  - 50,000 first responders at scene
  - 19% increase in total cancer rates among World Trade Center exposed NYC firefighters
  - Prostate, thyroid, and multiple myeloma cancers most prominent

- **Plastimet Fire**
  - Hamilton, Ontario (1997)
  - 294 firefighters at scene

Dibenzo-p-dioxins/Dibenzofurans

- Persistent environmental pollutants
  - 17 congeners monitored by WHO
  - Part of Stockholm Convention “dirty dozen”
  - In USA, regulated as hazardous air pollutant
- Unintentional byproducts combustion
  - Municipal waste incinerators
- Many studies performed on polychloro’s (PCDD/Fs)
- Very few analytical and biochemical studies of the mixed halo congeners have been performed (PXDD/Fs and PBDD/Fs)
Narrowing the scope: “Dioxins” at the World Trade Center Site…

- USEPA declared Ground Zero air “safe” within days after the collapse of the towers
  - Based on analytical analysis looking only for 17 polychloro dioxins/furans
  - CALUX cell based tests indicated much higher levels of AhR activators
- Elevated serum levels of PCDDs and PCDFs were observed in firefighters
What about small scale fires?

What is the effect of small dose, long term/chronic exposure to fire debris?
What is something they all have in common?

Brominated Flame Retardants

Polybrominated diphenyl ether (PBDE)

Δ + source of chlorine

Br, Cl

Dibenzofuran

Br, Cl

Dibenzo-p-dioxin

Br, Cl
Why do we care about these compounds?

- TCDD misidentified as the “most toxic compound known to man”
  - Avid tumor promoter in rats
  - Human exposure
    - Chloracne, immune effects, birth defects
    - Does it cause cancer?
- Do PXDD/Fs follow similar chemistry as the polychloro analogs?
  - Structures are very similar
  - Long half lives → bioaccumulate
Potency studies in human liver cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of replicates</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
<td>21</td>
<td>1.00</td>
</tr>
<tr>
<td>2Br, 378Cl DD</td>
<td>18</td>
<td>0.99 ± .13</td>
</tr>
<tr>
<td>23Br, 78Cl DD</td>
<td>19</td>
<td>0.84 ± .10</td>
</tr>
<tr>
<td>2378-TBDD</td>
<td>17</td>
<td>0.77 ± .14</td>
</tr>
<tr>
<td>2378-TCDF</td>
<td>21</td>
<td>0.83 ± .07</td>
</tr>
<tr>
<td>3Br, 278Cl DF</td>
<td>20</td>
<td>0.97 ± .12</td>
</tr>
<tr>
<td>23Br, 78Cl DF</td>
<td>20</td>
<td>1.02 ± .15</td>
</tr>
<tr>
<td>2378-TBDF</td>
<td>21</td>
<td>0.86 ± .10</td>
</tr>
</tbody>
</table>

Manuscript submitted to Chemosphere
Further investigation is needed:

Analytical Characterization of Fire Debris Samples
## Simulated burn studies

<table>
<thead>
<tr>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Household Fire</strong></td>
<td><strong>Household Fire</strong></td>
</tr>
<tr>
<td>• Mattress</td>
<td>• 2 Sofa Chairs</td>
</tr>
<tr>
<td>• Sofa Chair</td>
<td>• Vinyl and Metal Chair</td>
</tr>
<tr>
<td>• Vinyl / Wood Chair</td>
<td>• 2 Carpets</td>
</tr>
<tr>
<td>• Carpet</td>
<td>• Pillows and Cushions</td>
</tr>
<tr>
<td>• Pillows</td>
<td>• Coffee Table</td>
</tr>
<tr>
<td>• Television</td>
<td>• Vinyl Kitchen Chairs</td>
</tr>
<tr>
<td><strong>Electronics Fire</strong></td>
<td><strong>Electronics Fire</strong></td>
</tr>
<tr>
<td>• Televisions</td>
<td>• Televisions</td>
</tr>
<tr>
<td>• Microwave</td>
<td>• Microwave</td>
</tr>
<tr>
<td>• Printers</td>
<td>• Computer Monitors</td>
</tr>
<tr>
<td>• Computer monitors</td>
<td>• Computer Towers</td>
</tr>
<tr>
<td>• Laptop</td>
<td>• VHS tapes</td>
</tr>
<tr>
<td>• Cables/Wires</td>
<td>• Keyboards</td>
</tr>
<tr>
<td></td>
<td>• Used Ink Cartridges</td>
</tr>
</tbody>
</table>
Simulated burn studies
Simulated burn studies
Sample Prep

- Soxhlet extraction
  - 24 hours
  - Solvents: Toluene, Hexane
- Two stages of clean-up
  - Acid/Base Silica column
  - Carbon/Silica phase
- Rotovap to 1.0 ml
Initial Analytical Approach

- Comprehensive two dimensional gas chromatography coupled to time of flight mass spectrometry
  - GCxGC-TOFMS

- Why use GCxGC?
  - “Targeted-discovery”
  - Complex matrix
  - Complex separation
    - 5000 possible PXDD/F, PCDD/F, and PBDD/F congeners
    - 421 are 2,3,7,8-substituted congeners

Dibenzo-p-dioxin

Dibenzo-furan
Household Fire Simulation

Masses: 404, 484, 562

Br$_5$ dibenzofuran
Br$_4$ dibenzofuran
Br$_3$ dibenzofuran

First Dimension RT (sec) → Second Dimension RT (sec)

Burnt Carpet

Organtini et al; Journal of Chromatography A, October 2014
Household Fire Simulation

Number of PBDF congeners

- Carpet
- Vinyl Chair
- Mattress
- Sofa Chair
- Wall Wipe

Organtini et al; Journal of Chromatography A, October 2014
Electronics Fire Simulation

Masses: 282, 316, 360, 394, 440, 474, 247, 324, 404, 484, 562

First Dimension RT (sec) ➔

Burnt Circuit Board

Organtini et al; Journal of Chromatography A, October 2014
Electronics FireSimulation

Burnt Circuit Board

Organtini et al; Journal of Chromatography A, October 2014
Electronics Fire Simulation

Burnt Circuit Board

Dibenzofurans

BrCl, BrCl₂, Br₂Cl, Br₃Cl, Br₄, Br₅, Br₂Cl₂, Br₃Cl₂

Organtini et al; Journal of Chromatography A, October 2014
Number of PBDF congeners:
- Br
- Br2
- Br3
- Br4
- Br5
- Br6

Number of PXDF congeners:
- BrCl
- BrCl2
- Br2Cl
- BrCl3
- BrCl4
- Br2Cl2
- Br3Cl
- Br3Cl2
- Br4Cl
- Br5Cl
Initial Conclusions

- GCxGC was a powerful first step
- Household fire generated a variety of PBDFs
- Electronics fire generated a variety of PBDFs and PXDFs
- No dioxin compounds identified
- Congener profiles are very heterogeneous between samples
- More sensitivity is likely needed as very few congeners are observed relative to what we expect
  - Some samples had very little detectable content
- What are our options?
Historically, dioxin analysis is performed using a high resolution mass spectrometer

- GC-HRMS utilizing magnetic sector based instruments
  - Considered the “gold standard” for decades
  - Validated methods based around HRMS
- Small range of target compounds
  - Only monitor for 17 most toxic poly-Cl dioxins
- Reference data based on HRMS system
- Sample prep methods to remove interferences
Downfalls of using a high resolution mass spectrometer for dioxin analysis

- GC-HRMS utilizing magnetic sector based instruments
  - Instruments are a bit more challenging to operate
- Small range of target compounds
  - 17 “regulated” dioxins not enough for a true toxicity determination
- In more modern samples, there is a greater chance of containing mixed halogen (Br and/or Cl) species
- Expanding HRMS methods will result in a decrease in sensitivity for all congeners and homolog groups
  - Homolog group overlap
We propose APGC-TQS as an alternative.
**Atmospheric Pressure Ionization Gas Chromatography (APGC)**

- **Benefits of APGC:**
  - Reduced fragmentation
    - Soft ionization process
      - Charge transfer vs. protonation
    - “See” molecular ion
    - High Sensitivity → fg level
  - Less restrictions
    - Can run at higher flow rates (GC) because no vacuum

- **Benefits of tandem MS**
  - Fast “switching” between ions monitored
  - High Sensitivity → MRM
  - One injection for analysis of ALL halogenated dioxins and furans?
Charge Transfer

- Corona discharge needle
- e\(^-\)
- 2e\(^-\)
- N\(_2\) → N\(_2\)\(^+\) → N\(_4\)\(^+\) → M\(^+\) → M
- “Dry” source conditions
- Favoured by relatively non-polar compounds
Application of APGC-MS/MS to dioxin analysis
Step 1: Can we qualify APGC-MS/MS for dioxin analysis?

- Collaboration between Penn State and the Ontario Ministry of Environment
  - Compare data on reference materials run on both the Autospec and Xevo APGC-TQS
APGC-TQS Method Conditions:

- 60m x 0.18 mm ID x 0.10 μm RTX-Dioxin2 column
  - 0.32mm ID deactivated stainless steel (Sulfinert) column at back end of column through heated transfer line
- Injector temperature = 290°C
- Restek uniliner
- Toluene pre/post injection washes
- Oven program:
  - 120°C hold 1 min
  - 35°C/min to 200°C
  - 4.50°C/min to 280°C hold 8 min
  - 20 °C/min to 330°C hold 10 min.
- Transfer line temperature = 360°C

**Samples and standards MUST be diluted accordingly prior to injection**
APGC-TQS Method

Conditions:
- Monitored –COCl loss
- Qualifier ion for each compound
- Five functions (tetra – octa) with 45 MRM transitions in a single method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent (m/z)</th>
<th>Product (m/z)</th>
<th>Fragment Lost</th>
<th>Dwell (s)</th>
<th>Cone (V)</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PentaCDF</td>
<td>339.9</td>
<td>276.9</td>
<td>-COCl</td>
<td>0.05</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>PentaCDF_q</td>
<td>337.9</td>
<td>274.9</td>
<td>-COCl</td>
<td>0.05</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>13C12-PentaCDF</td>
<td>351.9</td>
<td>287.9</td>
<td>-^{13}COCl</td>
<td>0.025</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>13C12-PentaCDF_q</td>
<td>349.9</td>
<td>285.9</td>
<td>-^{13}COCl</td>
<td>0.025</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>PentaCDD</td>
<td>355.9</td>
<td>292.9</td>
<td>-COCl</td>
<td>0.05</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>PentaCDD_q</td>
<td>353.9</td>
<td>290.9</td>
<td>-COCl</td>
<td>0.05</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>13C12-PentaCDD</td>
<td>367.9</td>
<td>303.9</td>
<td>-^{13}COCl</td>
<td>0.025</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>13C12-PentaCDD_q</td>
<td>365.9</td>
<td>301.9</td>
<td>-^{13}COCl</td>
<td>0.025</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Cl7DPO</td>
<td>409.8</td>
<td>339.8</td>
<td>-COCl</td>
<td>0.05</td>
<td>30</td>
<td>35</td>
</tr>
</tbody>
</table>
Sensitivity

Low level tetrachloro dioxin mix
Magnetic Sector vs. TQS
Sediment reference sample

NIST 1944 Waterway Sediment Reference Material Comparison

- APGC-MS/MS
- GC-HRMS
- Reference values
## Method Detection Limit (MDL)

### Xevo vs. Autospec

**Xevo MDLs are 2 – 20 times lower**

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

Conditions:
- 60m x .18 mm ID x .10 μm RTX-Dioxin2 column
  - .32mm ID deactivated stainless steel (Sulfinert) column at back end of column through heated transfer line
- Injector temperature = 290°C
- Restek uniliner
- Toluene pre/post injection washes
- Oven program:
  - 120°C hold 1 min
  - 35°C/min to 200°C
  - 4.50°C/min to 280°C hold 8 min
  - 20 °C/min to 330°C hold 10 min.
- Transfer line temperature = 360°C

**Samples and standards MUST be diluted accordingly prior to injection**
MRMs of PXDD/Fs

- MRMs were tested using a combination of the following common fragment losses:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fragments Monitored</th>
<th>Cone (V)</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br/Cl Dibenzo-</td>
<td>-COBr</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Dibenzofurans</td>
<td>-Br2</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>-COBr2</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>-COBrCl</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>-COBr2Cl</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>Br/Cl Dibenzo-p-</td>
<td>-COBr</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>dioxins</td>
<td>-COBr2</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>-Br2</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>-(CO)2 BrCl</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

- Current method contains approximately 149 MRM transitions in a single method
Tetra substituted dibenzofurans in electronics debris

- $\text{Cl}_4\text{DF}$
- $\text{BrCl}_3\text{DF}$
- $\text{Br}_2\text{Cl}_2\text{DF}$
- $\text{Br}_3\text{Cl}\text{DF}$
- $\text{Br}_4\text{DF}$
Electronics Fire
Dry Wall
Br₂Cl dibenzofuran (TIC)

PtP S:N = 233
PtP S:N = 178
PtP S:N = 134

Only one peak of this congener class detected on TOF
Household Fire
Helmet Wipe
BrCl dibenzofuran (TIC)

No peaks of this congener class detected on TOF

PtP S:N = 6.13
BrCl3 dioxins, not found with TOF

Br/Cl standard mix
BrCl3 dibenzo-p-dioxin (TIC)
2-Br 3,7,8-Cl

Electronics Fire
Plastic
BrCl3 dibenzo-p-dioxin (TIC)

Electronics Fire
Wires
BrCl3 dibenzo-p-dioxin (TIC)
BrCl$_3$ dioxins, not found with TOF

Br/Cl standard mix
BrCl$_3$ dibenzo-p-dioxin (TIC)
2-Br 3,7,8-Cl

Could the 2,3,7,8 congener be the largest in concentration?

Electronics Fire
Plastic
BrCl$_3$ dibenzo-p-dioxin (TIC)

Could the 2,3,7,8 congener be the largest in concentration?

Electronics Fire
Wires
BrCl$_3$ dibenzo-p-dioxin (TIC)
BrCl2 DF TICs of all the electronics fire samples, scaled

No peaks detected on GCxGC-TOF
Number of PXDF congeners

- BrCl
- BrCl2
- Br2Cl
- BrCl4
- Br2Cl2
- Br3Cl
- Br3Cl2
- Br4Cl
- Br5Cl
- Br4Cl2

GCxGC-TOFMS
APGC-TQS

Very conservative estimate!
Simulated burn studies
Simulated burn studies
Simulated burn studies
Firefighter helmet wipes
BrCl$_3$ DF
Firefighter helmet wipes
Br$_2$Cl$_2$ DF
Firefighter helmet wipes - Br₃Cl DF

Firefighter 1

Firefighter 2

Firefighter 3
Electronics Fire vs House Fire – \( \text{Br}_2\text{Cl}_2 \) DF

\( \text{Br}_2\text{Cl}_2 \) DF Standards

Electronics Fire Debris
Peak intensity = \( 5.30 \times 10^5 \)

House Fire Debris
Peak intensity = \( 3.63 \times 10^4 \)
2013 vs 2014 Fires – BrCl₃ DF

BrCl₃ DF Standards

2013 Fire

Peak intensities: 9.76e5

2014 Fire

Peak intensities: 4.42e5
2013 vs 2014 Fires – Br₄ DF

Br₄ DF Standards

2013 Fire

Peak intensities: 1.73e6

2014 Fire

Peak intensities: 5.17e7
Conclusions

- APGC-TQS allows for considerable improvement in sensitivity
  - 20-40 X versus Autospec
  - 500 X versus GCxGC
- Mixed-halo congeners can be quantified
  - But, congener identity is not possible…yet
- PXDDs and PXDFs are found in mostly all of the fire debris samples studied so far
  - APGC analysis confirmed GCxGC data
  - Allowed for identification of more compounds in samples
- Firefighters are being exposed to a complex mixture of PXDD/Fs through inhalation and contact
  - Increased regulation of these compounds needed
  - Reconsider fire fighter safety procedures and standards
These are essentially unmonitored compounds at this time!

- Additional method development and sample characterization
- Homolog group quantification?
  - Most groups do not have available 13C labels
- Congener ID?
- Other non-PCDD/F compounds?
- Propose additional compounds for TEQ determination?
Acknowledgements

- Penn State University
  - Biochemistry, Microbiology, and Molecular Biology Department
- Dorman Research Group
- FESTI
  - Brian Ross
- Ontario Ministry of the Environment
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  - Terry Kolic
  - Karl Jobst
- Waters Corporation
- Wellington Laboratories
- Restek Corporation
- Leco Corporation
Thank you for your attention!

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