Analytical tools for comprehensive micropollutant analysis

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Workshop on Constituents of Emerging Concern  
May 2017
Study Setup – Storm Driven Sampling

- rain event Jan 2016
- rain event March 2016
- grab samples for chemical analysis every day

Research Question: Do organic contaminants contribute to a decline in fish-prey?
Comparing Target and Nontarget Analysis

• **Target Analysis** (e.g., LC-MS/MS/MS)
  - Select target constituents and best ions to monitor (one parent ion and multiple ions produced in collision cell)
  - Advantages: selective, sensitive, good quantitation
  - Disadvantages: only find what you know to look for—possible to miss key constituents, especially byproducts

• **Nontarget Analysis** (e.g., LC-QTOF-MS)
  - Use high resolution capability of TOF-MS to determine accurate mass of ions (<5 ppm=±0.001 amu @ m/z=200) to produce short list of possible molecular formulas
  - Further narrow identification using MS/MS and databases
  - Advantages: can find unknown unknowns
  - Disadvantages: recovery and detection of non-target constituents uncertain; definitive compound identification challenging without standards
Overview of Hybrid Approach

- **polar chemicals**
  - Filtration: only water analysis
  - SPE: multilayer cartridge (Oasis, anion & cation exchanger)
  - adapted from Eawag, Switzerland
  - Analysis: Agilent LC-QTOF-MS/MS
  - All-Ions ESI pos, ESI neg

- **non-polar chemicals**
  - Filtration: separate analysis water and filter
  - Water: SPE Oasis
  - Filter: sonication extraction
  - adapted from USGS, CA
  - Analysis: Agilent GC-QTOF-MS
  - NCI mode, RT-locked EI mode

27 targets LC-QTOF
21 targets GC-QTOF
A) Search for exact masses in Chromatogram

- Peak found for mass 330.1100
- Isotope pattern match $\text{C}_{19}\text{H}_{14}\text{F}_3\text{NO}$ (score 98)

$\rightarrow$ 1 database match: Fluridone
$\rightarrow$ confirmation of fragments with library spectra
Suspect Confirmation LC-QTOF

- Example Herbicide Fluridone

- Library spectra

- 4 fragments confirmed

Detected in all samples of March event, no samples in Jan event

- Confirmed by reference standard
Suspect Screening GC-QTOF-MS

- Retention time locked Agilent GC-EI accurate mass pesticide library
- cis- and trans-propiconazole (Fungicide) identified

RT library:
26.16 min + 26.38 min

Retention Time (min)

![Graph showing retention times and mass spectra](Image)
Target and Suspect Results

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>Targets Detected</th>
<th>Suspects Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-QTOF-MS</td>
<td>21</td>
<td>57*</td>
</tr>
<tr>
<td>GC-QTOF-MS</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>90**</td>
</tr>
</tbody>
</table>

* 18 of 21 confirmed with reference standard  
** 10 in both GC-MS and LC-MS, 25 not confirmed with MS/MS

→ 15-25 targets in every sample

**GC-QTOF-MS Targets**
- 7 Pyrethroids, e.g. Cyhalothrin, Bifenthrin, Cypermethrin, Chlorpyrifos
- Fipronil and degradates

**LC-QTOF-MS Targets**
- Insecticides: e.g. Methoxyfenozide, Imidacloprid, Dimethoate
- Fungicides: e.g. Azoxylostrobin, Boscalid, Cyprodinil
- Herbicides: e.g. Diuron, 2,4-D, Hexazinone
- Biocides: e.g. Triclosan, DEET

**GC-QTOF-MS Suspects**
- Dacthal, 2,6-Dichlorobenzamide (BAM), Bromacil, Oxadizone, Propiconazole, Kinoprene, Diazinone

**LC-QTOF-MS Suspects**
- Propiconazole, Norflurazone, Triclopyr, Fluridone, Quinclorac, Diethofencarb
Overview of Spatial/Temporal Trends

**January Rain Event**

**March Rain Event**

- Graphs showing precipitation (mm/hr) over time for the January and March rain events.
- Concentration graphs for different months showing the sum of concentration (ng/L).
- Legend includes indicators for UB, C1, C2, C3, C4, and Li.
Acute Toxicity and Pyrethroids

January Event

March Event

% survival (comp. to Li)

C4 C3 C2 C1

% survival (comp. to Li)

C4 C3 C2 C1

Prevailing flow direction

Cyfluthrin + Bifenthrin

Sum of pyrethroids (ng/L)

Permethrin

C4 C3 C2 C1

Sum of pyrethroids (ng/L)

Permethrin

Cypermethrin

Bifenthrin, Cyhalothrin, Cypermethrin, Deltamethrin

Prevailing flow direction
Finding Transformation Products

- Used EAWAG-PPS to predict 1409 transformation products (3 generations) for 76 detected pesticides
- Theoretically ionizable TPs (1338) entered into database
- All 51 samples screened for TPs using LC-QTOF-MS in ESI+/ESI- with MassHunter Qual Find-by-Formula
- Manual screening of all compounds with score >70 and >5 detections
- Plausible candidates re-run in targeted MS/MS mode
- MS/MS spectra predicted using Molecular Structure Correlator (Agilent) and CFM-ID (http://cfmid.wishartlab.com/predict)
- Further prioritization based on comparing spatial/temporal similarity of TP to parent compound
Nontarget TP Detection Example

- Insecticide Dimethoate and two TPs
  - Omethoate found in All-Ions Workflow and confirmed with standard
  - O-desmethyl dimethoate- no reference standard available but plausible MS/MS fragments
- 7 TPs detected via this workflow
Nontarget TP Detection Example

[Diagram showing ESI Product Ion (rt: 7.750-7.779 min, 2 scans) Frag=110.0V CID@20.0 (213.9767[z=1] -> ***) 160706_neg_tMSMS_Mar_D5-C1_TP78.e]
Nontarget Analysis Supports Source ID

212 patterns like 2,4-D
similarity > 0.75

235 patterns like sucralose
similarity > 0.75

* using Agilent MPP software
Nontarget: Ethoxylated Surfactants

Procedure: LC QTOF ESI+, EnviHomolog, Repeat Unit Range: 44.0242-44.0262, mztol=5 ppm
Hyallela Toxicity vs. Passive Sampling

Log2 normalized intensity

Chemcatcher® Passive Sampler

Location

Blank Li C3 C2 C1

H. azteca toxicity (%)

Hyallela azteca

UC DAVIS UNIVERSITY OF CALIFORNIA
## Assessing Significance

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Compound Class</th>
<th>Work-flow</th>
<th>Instrument</th>
<th>Max RQ</th>
<th>Max MEC</th>
<th># Det.</th>
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<tbody>
<tr>
<td>Cypermethrin</td>
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</table>
Conclusion and Outlook

• Toxicity towards *H. azteca* \(\rightarrow\) pesticide exposure possible cause for decline of fish prey
• With over 100 detected pesticides from varied classes, mixture toxicity likely important
• Broad scope suspect/non-target screening finds many more compounds than those on a typical target list
• Nontarget workflow finds ubiquitous pesticide TPs
• Statistical analysis can group molecular features to provide information regarding contaminant sources, similar fate processes—currently coupling results with hydrologic models
• Significance of non-target analytes being confirmed by toxicity correlations and genomic profiling (S. Hasenbein and H. Poynton)
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