Emergency Preparedness: Rapid Screening of Potentially Deleterious Organics in Unknown Environmental Samples

Dayue Shang, Brad McPherson and Michelle Lam
Pacific Environmental Science Centre
Environment Canada
North Vancouver, B.C.

Overview

1. What is the issue?
2. What are the objectives of the project?
3. What are our new approaches to the challenges?
4. What has been accomplished so far?
5. What is the take-home message?
The issue

• In an emergency situation, rapid identification of unknown chemicals is critical for the initial responders and decision makers.

• Currently, the typical approach to identification of unknown substances has been to “guess, then test”.

• A comprehensive, systematic, and rapid procedure is urgently needed to provide specific information on the identity of the adulterants or contaminants in the sample.

• The new procedure should also demonstrate scientific rigor and acceptability in legal cases.

Four Project Objectives

• To develop a rapid detection method that can be used to identify a wide range of organic compounds in various sample matrices.

• To be able to process up to 8 samples, i.e., extract, analyze and report results within a working day by one chemist.

• To build an LC/MS spectral library which covers a wide variety of deleterious chemicals.

• To apply the method to spiked blind “white” and “black” powder samples.
The Basic Idea: The Dose Makes the Poison!

“All Ding sind Gift, und nichts ohn Gift; allein die Dosis macht, daß ein Ding kein Gift ist.”

“All things are poison and nothing is without poison, only the dose permits something not to be poisonous.”

Paracelsus (1493-1541)
German chemical analyst, father of toxicology

New Approaches

- Regard all sample matrices as aqueous, e.g. water laden with particulate materials.

- For small molecular compounds, over 96% can be detected by either GC/MS or LC/MS after proper sample processing. Instead of “either/or”, the new method has to be GC/MS and LC/MS.

- As there is no established commercial spectral library available for LC/MS, an Excel based spectral library was built to help interpret the results from LC/MS runs.

- Universal extraction and clean-up, simultaneous LC/MS and GC/MS analysis, and specific LC/MS library are the key components of this screening method.
“Universal” extraction and clean-up

- Add to each of two 20 mL glass vials (Sample A1 and A2) 1 g of powder sample A, 5 mL of 1 ppm triclosan in water (the surrogate/internal standard) and 5 mL of DCM:Hex mixture (40:60; v:v). Sample A1 is acidified with 1 mL of formic acid and Sample A2 alkalized with 1 mL of NH₄OH.

- Vortex for 10 sec. Repeat twice with 1 min interval. Centrifuge at 3000 X g for 2 min until two layers are formed and particulate matter has settled. Freeze the sample at – 40 °C for 30 min.

- Decant the organic layer into pre-labeled glass vials. Aliquot 1 mL of the organic extract to a GC vial.

- Pipette 4 mL of 50% MeOH to the rest of the sample, vortex and evaporate the sample to 4 mL using EZ-2 centrifugal vacuum solvent removal device. Filter and add 1 mL sample to an LC sample vial.

Rapid Sample Processing for Screening of Unknown Compounds from Particulate Materials

1. Sample received
   - Check if it is organic or inorganic material following the flow chart of D. Shang 18/05/2007.
   - Organic
     - Add 1g of powder to a 20mL glass vial with 5mL 1 ppm triclosan and 5mL DCM/Hex (40/60). Add 1 mL of formic acid.
     - Vortex twice, each 30 sec with 1 min interval.
     - Centrifuge to settle down particulate and separate solution into two phases.
     - Store at -40 °C for 30 min. in an EZ-2 holder.
     - Put in the sample holder EZ-2 for 20 min. to thaw the aqueous sample. Temp 40-50°C.
     - Filter 1.5mL of sample into LC vial using 13mm GHP filter 0.2µM.
     - Filter GHP. Filter if necessary.
     - Add 1mL into GC vial. Filter with GHP. Filter if necessary.
     - GC/ MS scan and library search for major peaks.
     - Evaporate off organic layer. Vortex. Add 1.5mL to LC vial.
     - Based on GC/ MS, LC MS, and toxicity test results, write a report.
   - Micro-toxicity test

2. Run LC/ MS and do a spectral library check.
   - Add 4 mL 50% MeOH to the remaining organic solution. Vortex twice.
   - Filter 1.5mL of sample into LC vial using 13mm GHP filter 0.2µM.
   - Add 1mL into LC vial. Filter with GHP. Filter if necessary.
   - GC/ MS scan and library search for major peaks.
   - Store at -40°C for 30 min. in an EZ-2 holder.
   - Centrifuge to separate organic and aqueous solution.

Based on the results, write a report and determine the toxicity of the sample.
**GC/MS Operating Conditions**

**Instrumentation:** Agilent 6890/5973

**GC/MS Column:** Restex-5ms, 30 m, 0.25 mm ID, 0.25 μm film thickness.

**GC/MS Parameters**
- **Injection Volume:** 3 μL
- **Injection Type:** Splitless
- **Injector Liner:** 4 mm ID, Glass-wool packed
- **Injector Temperature:** 240 ºC
- **Transfer Temperature:** 280 ºC
- **Carrier Gas:** Helium, 1.2 mL/min
- **Oven Program:** Initial temperature 60 ºC hold for 1.5 min, followed by 20 ºC to 180 ºC, zero hold time, 3 ºC to 230 ºC, zero hold time, 30 ºC to 300 ºC, 8.5 min hold Time. Total run time is 35 minutes.

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**LC/MS Operating Conditions**

**Instrumentation:** Agilent 1100 LC/MSD

**Column:** Eclipse Plus C8, 3.5 μ, 2.1x150 mm.

**Flow rate:** 0.20 mL/min

**Injection Volume:** 20 μL

**Mobile Phase:** Water + 0.1 % FA (A), ACN + 0.1 % FA (B)

**Mass Range:** 150 to 400 amu, both positive and negative

**Vcap:** 3500 v (positive) 4250 V (negative)

**Fragmentor:** 140 v (positive) 120 V (negative)

**Gradient Time Table:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solvent B</th>
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<tbody>
<tr>
<td>0.00</td>
<td>5.00</td>
</tr>
<tr>
<td>16.00</td>
<td>95.0</td>
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<tr>
<td>24.00</td>
<td>95.0</td>
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<tr>
<td>24.10</td>
<td>5.00</td>
</tr>
<tr>
<td>30.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>
QA and QC Considerations

- Surrogate standard for GC/MS and LC/MS: triclosan
- Internal standard for GC/MS: Anthracene D-10
- Reagent blank, method blank and matrix standards to avoid and identify cross contamination.
- Signal intensity of triclosan to check extraction recovery, instrument performance and method repeatability.
- Blind samples to verify the applicability and accuracy of the method.

Application: Mysterious “White” and “Black” Powder Samples

- Two white and one black powder samples were delivered at PESC sample reception area with a note attached asking for chemical compound identification.

  ![Image of three powdered samples labeled A, B, and C]

- Initial water solubility and visual examination indicated that the base materials of one white powder (Sample A) was some kind of silicate material, the second white powder (Sample B) was most likely sugar based, and the black powder (Sample C) appeared to be some sort of sandy soil.
Vortex Sample Mixed with Water and DCM/Hexane Solvent

Freeze Aqueous Layer and Decant Organic Solvent
Add LC Compatible Solvent (4 mL of 50% MeOH)

Remove Organic Solvent with EZ-2 Evaporator
Filter Sample to LC Vial

GC/MS Results of White Powder Sample A
LC/MS Results of White Powder Sample A

- atrazine
- triclosan

GC/MS Results of White Powder Sample B

- quintozene
- triclosan
LC/MS Results of White Powder Sample B

benomyl

triclosan

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GC/MS Results of Black Powder Sample C

triclosan

m/z→ Abundance

Scan 2945 (18.536 min): 0801008.D\data.ms

40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360

0 1000 2000 3000 4000 5000 6000 7000 8000

m/z→ Abundance

#110635: Triclosan

5/2/2008
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LC/MS Results of Black Powder Sample C

Deleterious Organic Chemical Spectral Library V 1.1

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>Category</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Type</th>
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<tbody>
<tr>
<td>cyprazine</td>
<td>PEST</td>
<td>212</td>
<td>227</td>
<td>170</td>
<td>58</td>
<td>GC</td>
</tr>
<tr>
<td>disulfoton-sulfoxide</td>
<td>PEST</td>
<td>212</td>
<td>153</td>
<td>184</td>
<td>61</td>
<td>GC</td>
</tr>
<tr>
<td>rabenzazole</td>
<td>PEST</td>
<td>212</td>
<td>195</td>
<td>170</td>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>aldicarb</td>
<td>PEST</td>
<td>213</td>
<td>208</td>
<td>192</td>
<td>116</td>
<td>LC</td>
</tr>
<tr>
<td>chlorpropham</td>
<td>PEST</td>
<td>213</td>
<td>171</td>
<td>153</td>
<td>127</td>
<td>GC</td>
</tr>
<tr>
<td>clofibric acid</td>
<td>PPCP</td>
<td>213</td>
<td>85</td>
<td></td>
<td></td>
<td>LC</td>
</tr>
</tbody>
</table>
Results and Discussion

• Both GC/MS and LC/MS correctly identified Atrazine in Sample A, it significantly increased the confidence level of positive identification.

• LC/MS results indicated that Sample B containing Benomyl, a pesticide. But same sample analyzed by GC/MS only found Sample B another pesticide, Quintozene.

For Sample C, GC/MS technique could not find any spilled chemicals while LC/MS identified two very toxic chemicals, i.e. Aldicarb and Indomethacin.

Do not rely on one single technique: you may run risk of no reporting of some very toxic chemicals!

CSI Report of “White” and “Black” Samples

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Spiked (unknown to the chemist)</td>
<td>Atrazine</td>
<td>Quintozene</td>
<td>Aldicarb Indomethacin</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Atrazine</td>
<td>Quintozene</td>
<td>ND</td>
</tr>
<tr>
<td>LC/MS</td>
<td>Atrazine</td>
<td>Benomyl</td>
<td>Aldicarb Indomethacin</td>
</tr>
</tbody>
</table>
LC/MS and Spectral Library to Fill the Gap

- GC/MS scan failed to find two very toxic compounds in black powder sample C.

- Both Aldicarb and Indomethacin were identified by LC/MS and spectral library search.

- Toxicity of identified chemical compounds (high values are less toxic):

<table>
<thead>
<tr>
<th>Name</th>
<th>Oral LD_{50} (mg/kg)</th>
<th>Dermal LD_{50} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>12</td>
<td>Unknown</td>
</tr>
<tr>
<td>DDT</td>
<td>87</td>
<td>1931</td>
</tr>
<tr>
<td>Caffeine</td>
<td>127</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Summary

- A deleterious organic chemical screening method was developed, and was successfully applied to the identification of several unknown toxic compounds in two “white” powder and one “black” powder samples.

- The sample processing is based on a “universal” sample extraction procedure with pH adjustment, binary solvent L-L extraction and centrifugal vacuum solvent removal.

- An in-house prepared spreadsheet based spectral library is a great tool to interpret the LC/MS and GC/MS results and indentify unknown chemicals.

- Take Home Message: a good screening method must use both GC/MS and LC/MS together.
Future Work

• Expand the current list of deleterious compounds from about 1000 to 2000 by literature search and by contributions from colleagues.

• Prepare a deleterious chemical inventory and generate spectral information under defined conditions for both GC/MS and LC/MS.

• Build a software searchable LC/MS spectral library to cover the whole range of organic compounds.

• Develop a computer based comprehensive and systematic procedure for the efficient and accurate identification of unknown and possibly hazardous substances.

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